

Characterization of the sensory profile of sea urchin aiming at the selection of premium gonads

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Diretivas Legais

No cumprimento do disposto no Decreto-Lei no 204/2018 de 23 de Outubro, declara-se que o autor desta Tese participou na conceção e na execução do trabalho experimental que esteve na origem dos resultados apresentados, bem como na sua interpretação e na redação dos respetivos manuscritos.

Nesta tese incluem-se ainda seis artigos científicos publicados em revistas internacionais resultantes de uma parte dos resultados obtidos no trabalho experimental, referenciados como:

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- Baião, L. F., Moura, A. P., Rocha, C., Valente, L. M. P., & Cunha, L. M. (2021). Dimensions for the valorisation of sea urchin (*Paracentrotus lividus*) gonads production

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Legal Details

In compliance with what is stated in Decree-Law no. 204/2018 of 23 October 23, it is hereby declared that the author of this thesis participated in the creation and execution of the experimental work leading to the results shown, as well as in their interpretation and the writing of respective manuscripts.

This thesis includes six scientific papers published in international journals from part of the results obtained in the experimental work, referenced to as:

- Baião, L. F., Rocha, F., Costa, M., Sá, T., Oliveira, A., Maia, M. R. G., Fonseca, A. J. M., Pintado, M., & Valente, L. M. P. (2019). Effect of protein and lipid levels in diets for adult sea urchin *Paracentrotus lividus* (Lamarck, 1816). *Aquaculture*, 506, 127-138. <https://doi.org/https://doi.org/10.1016/j.aquaculture.2019.03.005>

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“Science knows no country, because
knowledge belongs to humanity, and
is the torch which illuminates the world”

Louis Pasteur

To my parents
To my sister
To my wife
To my daughter

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“No man ever did or can do a great work alone” by Elbert Hubbard

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Table of Contents

Diretivas Legais	iii
Legal Details	v
Acknowledgments	ix
List of Tables	xii
List of Figures	xiii
List of Abbreviations	xiv
Resumo	xvi
Abstract	xx
Thesis structure	xxiv
Chapter I	1
Sea urchin	1
Biogeography, ecology and reproduction	2
Economic importance	7
Gastronomy and markets	13
Sea urchin sensory profile	17
Purchase and consumption drivers	17
Quality and sensory attributes	19
Echinoculture	27
Global production	27
Nutritional composition of the diets and roe quality	28
Objectives of this thesis	34
References	37

Chapter II. The effect of sex, season and gametogenic cycle on gonad yield, biochemical composition and quality traits of <i>Paracentrotus lividus</i> along the North Atlantic coast of Portugal	56
Chapter III. Survey into the sea urchin consumption profile in the Portuguese population	70
Chapter IV. Dimensions for the valorisation of sea urchin (<i>Paracentrotus lividus</i>) gonads production through the eyes of experienced chefs	83
Chapter V. Analysis of volatile compounds in <i>Paracentrotus lividus</i> by HS-SPME/GS-MS related to its sensorial properties.....	110
Chapter VI. Sensory profiling, liking and acceptance of sea urchin gonads from the North Atlantic coast of Portugal, aiming future aquaculture applications	120
Chapter VII. Development of a Rate-All-That-Apply (RATA) ballot for sensory profiling of sea urchin (<i>Paracentrotus lividus</i>) gonads	131
Chapter VIII. Effect of protein and lipid levels in diets for adult sea urchin <i>Paracentrotus lividus</i>	155
Chapter IX. Sensory profiling, liking and gonad composition of sea urchin gonads fed synthetic or natural sources of β -carotene enriched diets	168
Chapter X. General discussion, conclusions and final considerations	201
General discussion	202
Conclusions and final considerations	209
References	211

List of Tables

Table 1 – Global imports and exports volumes (tonnes), trade values (USD 1000) and average price (USD) per kilo of sea urchin products, in 2017 (FAO, 2019).	8
Table 2 – Volume of imports and exports (tonnes), volume value (USD 1000) and average price (USD) per kilogram of sea urchin (live, fresh, chilled, frozen, dried, salted or in brine; fermented, smoked), taking into account the most important global sea urchin markets in each category, in 2017 (FAO, 2019).	9
Table 3 - Volume of imports and exports (tonnes), volume value (USD 1000) and estimated value (USD) per kilogram of sea urchin (live, fresh, chilled, frozen, dried, salted or in brine; fermented, smoked), taking into account the most important European sea urchin markets in each category, in 2017 (FAO, 2019).....	11
Table 4 - General summary about sensory studies in sea urchin, published during the last 18 years (2002–2020).	24
Table 5 - General summary about <i>P. lividus</i> gonads colour enhancement studies.....	33

List of Figures

Figure 1 - Sea urchin, <i>Paracentrotus lividus</i>	2
Figure 2 - <i>P. lividus</i> (two right photos shared through “Instagram” networking service by @submpolvo).	3
Figure 3 - <i>Paracentrotus lividus</i> . Histology of testes: Stage IV - mature testis; Stage V – partly spawned testis; Stage VI – spent testis devoid of contents; and of ovaries: Stage IV - mature ovary; Stage V – partly spawned ovary (except for the spaces between ova, ovary is similar to stage IV); Stage VI – spent ovary containing unspawned ova.	4
Figure 4 - Scheme representative of the gonadosomatic index (GSI) variation during the year (<i>Paracentrotus lividus</i> , Ireland). Adapted from Unuma (2002).	5
Figure 6 - <i>P. lividus</i> gonads.	12
Figure 7 – Sea urchin gonads market presentation (photos from Shiki Singapore).	14
Figure 8 - Polpa di Riccio.	15
Figure 9 – Different ways of presenting <i>P. lividus</i> gonads to the consumer.	19
Figure 10 – Recirculation aquaculture system (from CIIMAR, Porto, Portugal).	31
Figure 11 – Overview of methodological approaches	36

List of Abbreviations

<i>a</i> *	Redness
ABW	Average body weigh
ALA	Linolenic acid
AIC	Algarve chefs
ANOVA	Analysis of variance
ARA	Arachidonic acid
<i>b</i> *	Yellowness
<i>C</i> *	Chroma
CA	Correspondence analysis
CATA	Check-all-that-apply
CTRL	Control diet
DHA	Docosahexaenoic acid
DM	Dry matter
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid
EriC	Ericeira chefs
FA	Fatty acid
FACT	Food action rating scale
FAME	Fatty acid methyl esters
FBW	Final body weigh
FCR	Feed conversion ratio
FNS	Food neophobia scale
GC-MS	Gas chromatography coupled to mass spectrometry
GSI	Gonad somatic index
<i>h</i> *	Hue angle
HCA	Hierarchical cluster analyses
HIGH	High shellfish consumption
HIGO	High gourmet shellfish consumption
HL	High lipid
HP	High protein
HPAP	High incorporation of natural β -carotene source (250 mg kg ⁻¹)
HS-SPME	Headspace solid-phase microextraction

HSYN	High incorporation of synthetic β -carotene source (250 mg kg ⁻¹)
HVS	High variety seekers
IBW	Initial body weigh
IPMA	Instituto Português do Mar e da Atmosfera
<i>L</i> *	Brightness/Lightness
LA	Linoleic acid
LC-PUFA	Long chain-polyunsaturated fatty acids
LL	Low lipid
LOW	Low shellfish consumption
LP	Low protein
LPAP	Low incorporation of natural β -carotene source (100 mg kg ⁻¹)
LSD	Least significant difference
LSYN	Low incorporation of synthetic β -carotene source (100 mg kg ⁻¹)
LVS	Low variety seekers
MDA	Multi-dimensional alignment
MFA	Multiple factor analysis
MsC	Michelin-star Chefs
MUFA	Monounsaturated fatty acids
MVS	Medium variety seekers
NIST	National Institute of Standards and Technology
NOTO	Neither one nor other
PAP	Natural β -carotene source - Paprika
PCA	Principal component analysis
PER	Protein efficiency ratio
PM	Projective mapping
PUFA	Polyunsaturated fatty acids
RAS	Recirculating aquaculture system
RATA	Rate-all-that-apply
RI	Kovats retention indices
SFA	Saturated fatty acids
SGR	Specific growth rate
SYN	Synthetic β -carotene source
UFP	Ultra-flash profiling
VARSEEK	Variety seeking scale
WW	Wet weigh

Resumo

As gónadas de ouriço-do-mar (*Paracentrotus lividus*) têm sido consideradas um produto de marisco apreciado e uma iguaria na cozinha mediterrânea, devido ao seu sabor e textura. A crescente procura do mercado por ovas de ouriço-do-mar resultou na sobre-exploração desta espécie e, conseqüentemente, no esgotamento dos stocks selvagens. Por esta razão, o interesse pela aquacultura de ouriços do mar tem aumentado nas últimas décadas. Embora a aquacultura possa acelerar a produção de ouriços-do-mar de tamanho comercial, ainda não pode assegurar uma elevada qualidade de gónadas, o que afeta o valor de mercado e a aceitabilidade do consumidor. A primeira abordagem desta tese foi a identificação das propriedades organoléticas das gónadas de ouriços-do-mar que determinam a sua comercialização, aceitação e decisão de compra por parte dos consumidores, o que permitirá a valorização deste produto. Numa segunda fase, foi avaliada a capacidade das dietas formuladas modularem e melhorarem a qualidade das gónadas, sempre com o objetivo de alcançar uma gónada *premium* de alta qualidade e grande valor comercial.

No presente trabalho, a população de ouriços-do-mar recolhidos na costa Atlântica do Norte de Portugal foi caracterizada em termos de crescimento das gónadas, composição nutricional e outras importantes características relacionadas com a sua qualidade, ao longo de um ciclo reprodutivo. A maioria dos atributos de qualidade mostrou uma variação sazonal fortemente dependente do sexo do ouriço-do-mar, apresentando níveis mais elevados durante as fases de recuperação e crescimento. Durante o período de crescimento e maturação precoce, as gónadas eram mais avermelhadas, amareladas e mais brilhantes, bem como mais firmes, independentemente do sexo. Tendo em conta todas as alterações sazonais de qualidade das gónadas, o período entre Novembro e Fevereiro demonstrou ser o mais adequado para colher gónadas de alta qualidade na costa Atlântica Portuguesa (Capítulo II).

O seguinte estudo teve como objetivo explorar e compreender as perceções e comportamentos dos consumidores relativamente ao consumo de marisco, nomeadamente o ouriço-do-mar, aplicando uma metodologia mista aos questionários. O consumo de marisco e de ouriço-do-mar foi afetado por muitos fatores, nomeadamente os associados ao cultivo, saúde e propriedades sensoriais. Por exemplo, para os consumidores com um elevado perfil de consumo de ouriço-do-mar, *Emoções* foi a categoria mais importante, enquanto que aqueles com baixo consumo realçaram o

Desconhecido como a categoria principal. Isto sugere que os participantes com maior consumo tinham um elevado nível de familiaridade com o ouriço-do-mar, enquanto os consumidores com baixos níveis de consumo tinham pouco conhecimento sobre este produto gourmet. Além disso, os participantes com elevado consumo de marisco gourmet foram fortemente associados à categoria *Métodos de Preparação*, o que pode sugerir um conhecimento profundo sobre o produto, enquanto que os participantes com menor consumo foram associados à *Exclusividade* do produto, principalmente devido aos preços elevados (Capítulo III).

As percepções dos Chefes em relação às características sensoriais, produção e utilização das gónadas de ouriço-do-mar, com ênfase na aquacultura, também foram exploradas. Os Chefes expuseram que existe alguma apreensão em relação ao consumo de ouriços-do-mar principalmente devido à sua aparência. Além disso, a existência de preconceitos e suspeitas sobre os produtos da aquacultura e a sua aceitação deve ser contrariada, uma vez que a disponibilidade futura destes produtos gourmet e outros produtos do mar de alta qualidade dependerá da sua produção em sistemas de cultivo sustentáveis. Estes resultados sublinham a necessidade de estratégias de comunicação e marketing através de locais, Chefes e eventos gastronómicos, centrados na promoção da consciência dos consumidores sobre a exclusividade deste produto e a importância da aquacultura para o futuro do mercado de produtos do mar. A avaliação sensorial realizada pelos Chefes de cozinha deu origem a uma lista preliminar de atributos sensoriais, a ser usado nos capítulos seguintes (Capítulo IV).

As características sensoriais e o perfil de compostos orgânicos voláteis (COV) de *P. lividus* também foram estudados, tendo em consideração o sexo e o local de recolha do ouriço-do-mar. As características sensoriais do ouriço-do-mar foram principalmente influenciadas pelo sexo, embora o seu perfil volátil foi afetado tanto pelo sexo como pelo local da colheita. Os machos tendiam a ser mais doces, mais macios e com uma cor amarelo-pálida, e as fêmeas revelavam um intenso sabor tropical doce e uma cor alaranjada brilhante (Capítulo V). Os dados deste estudo e do Capítulo IV contribuíram para a caracterização do perfil sensorial das gónadas de ouriço-do-mar, visando o desenvolvimento de um boletim sensorial preliminar.

Além disso, foi construído um perfil sensorial de gónadas de ouriço-do-mar (*P. lividus*) para avaliar as diferenças entre sexo, local de colheita, bem como o impacto da apresentação na aceitação do consumidor. No Capítulo VI, as gónadas foram separadas em relação ao método de apresentação: o fundo branco da taça parecia realçar a cor laranja das fêmeas; a apresentação gourmet favoreceu as fêmeas em relação aos machos, principalmente porque a cor da gónada masculina e o exsudado de cor branca foram realçados pelo fundo da carapaça do ouriço-do-mar. Em ambos os estudos, o perfil

sensorial separa claramente as gónadas de acordo com o sexo, sendo as fêmeas mais atrativas do que os machos. Estes dois estudos também concluíram que um ouriço-do-mar de cor laranja, com sabor tropical intenso, doce e agradável e um odor agradável, fresco e tropical são mais atrativos e aceites entre os consumidores. Isto pode contribuir com informação valiosa para futuros estudos de nutrição visando a valorização dos atributos das gónadas de ouriço-do-mar e, subsequentemente, a sua valorização no mercado. Esta caracterização minuciosa do *P. lividus* selvagem, incluindo o seu perfil sensorial, ajudou a definir as exigências de qualidade dos consumidores com vista à seleção de gónadas de qualidade superior, permitindo focar os dois últimos estudos desta tese no crescimento e melhoria da qualidade das gónadas de ouriço-do-mar. De facto, a aquacultura pode desempenhar um papel importante no fornecimento de animais de alta qualidade. No entanto, o sucesso desta atividade depende em grande parte do desenvolvimento de dietas nutricionalmente equilibradas capazes de produzir excelentes gónadas.

Após uma avaliação minuciosa do perfil sensorial do ouriço-do-mar, este trabalho focou-se na preparação e avaliação de quatro dietas formuladas e extrudidas com níveis baixos ou altos de proteína (30 e 50%, matéria seca (MS)) e lípidos (6 e 11% MS) foram dadas a indivíduos selvagens durante 15 semanas. Todas as dietas foram eficazes na promoção do crescimento das gónadas, mas a dieta com o nível mais baixo de proteínas (30% MS) e de lípidos (6% MS) resultou no maior crescimento de gónadas em ambos os sexos. O índice gónado-somático das gónadas variou entre os sexos, mas foi semelhante entre os tratamentos (19-23%) e foi superior aos valores observados em indivíduos selvagens (6-7%). Os tratamentos e o sexo não afetaram a textura das gónadas (firmeza e resiliência), nem os parâmetros de cor (brilho, avermelhado, amarelado, intensidade ou tonalidade), mas afetaram a composição das gónadas. As fêmeas tinham concentrações de carotenoides significativamente mais elevada do que os machos, independentemente dos tratamentos. Os indivíduos selvagens tinham níveis mais elevados de equinenona, β -criptoxantina e β -caroteno e apresentavam valores mais elevados da cor avermelhada e firmeza quando comparados com a indivíduos alimentados com uma dieta formulada, independentemente do sexo. Este estudo mostrou claramente que é possível aumentar a produção de gónadas de ouriço-do-mar em relação a animais selvagens num Sistema de Recirculação em Aquacultura a 18 °C, mas não conseguiu melhorar as características sensoriais e de qualidade (Capítulo VIII).

Por fim, na etapa seguinte, foram também produzidas gónadas com cor aceitável com a incorporação de diferentes fontes de β -caroteno na melhor dieta utilizada no Capítulo VIII. O conteúdo e perfil carotenoides, o brilho (L^*) e a textura das gónadas não variaram significativamente entre os tratamentos. A dieta com menor concentração de β -caroteno sintético (L_SYN) mostrou valores mais elevados da cor avermelhada (a^*) e

amarelada (b^*) e valores mais elevados de aceitação entre os consumidores. Além disso, as fêmeas apresentaram maiores taxas de aceitação do que os machos, provavelmente devido ao facto de as fêmeas serem mais cor-de-laranja, atraentes e agradáveis em comparação com os machos que são amarelos, granulados e menos atraentes. Os machos alimentados com a dieta L_SYN exibiram uma cor e teor de carotenoides semelhantes aos animais selvagens na mesma fase de maturação das gónadas (fase III), mas as fêmeas apresentavam diferenças significativas; contudo, nenhum dos sexos demonstrou diferenças de aceitação dos consumidores em relação aos seus homólogos selvagens (Capítulo IX).

Esta tese foi realizada num ambiente industrial, sob a parceria da empresa Sense Test, Lda, e centrou-se na caracterização sensorial do ouriço-do-mar, bem como na melhoria e formulação de uma dieta para ouriço-do-mar. A combinação dos resultados obtidos nos diferentes capítulos proporciona uma contribuição significativa para o desenvolvimento contínuo de uma dieta adequada de *P. lividus*. Este trabalho propõe uma dieta capaz de aumentar significativamente o tamanho das gónadas e, mais amplamente, contribuirá para superar as restrições de fornecimento que atualmente impedem uma utilização mais extensiva deste equinoide. É também patente que a cor das gónadas não é exclusivamente modulada pela suplementação nutricional com carotenoides, e que pode ser afetada por outros fatores nutricionais ou metabólicos dependentes do sexo, o que requer mais estudos.

Abstract

In Mediterranean cuisine, sea urchin (*Paracentrotus lividus*) gonads have been considered a prized seafood product and a delicacy due to their flavour and texture. The increasing market demand for sea urchin roe has resulted in the overexploitation of this species and consequently the depletion of wild stocks. For this reason, the interest in sea urchin aquaculture has widened over the last decades. Although aquaculture may accelerate the production of market-sized sea urchins, it cannot yet assure high gonad quality, which affects market value and consumer acceptability. The first approach of this thesis was the identification of the organoleptic properties of sea urchin gonads that determine the marketability, consumers' acceptance and purchase decision, which will allow for the valorisation of this caviar-like product. In a second phase, the ability of formulated diets to induce high-quality gonads was evaluated.

In the present work, a sea urchin population harvested in the North Atlantic coast of Portugal was characterized in terms of gonad yield, nutritional composition and important market-related traits, over one reproductive cycle. Most of the quality attributes showed a seasonal variation strongly dependent on sea urchin sex, presenting highest levels during the gametogenic stages of recovery and growing. During the growing and early maturation period, gonads appeared more reddish, yellowish and brighter, as well as more firm, irrespective of the sex. Taking in account all gonad quality seasonal changes, the period between November and February seemed to be the most suitable to harvest high quality gonads in the Portuguese Atlantic coast (Chapter II).

The following study aimed to explore and understand consumers' perceptions and behaviours regarding consumption of shellfish, namely sea urchin, applying a mixed methodology to web-based questionnaires. The consumption of shellfish and sea urchin was affected by many factors, namely those associated with culture, health and sensory properties. For example, consumers with a high sea urchin consumption profile, *Emotions* was the most important category, while those with low consumption emphasised the *Unknown* as the main category. This suggested that participants with high consumption patterns had a high level of familiarity with sea urchin, while low consumers had poor knowledge concerning this gourmet product. Moreover, participants with high gourmet shellfish consumption patterns were strongly associated with the category *Preparation Methods* suggesting a deep knowledge of the product, while the ones with low consumption were associated with *Exclusivity* of the product, mainly due to the high prices (Chapter III).

Meanwhile, the Chefs' perceptions regarding sea urchin gonads sensorial features, production and use, with an emphasis on echinoculture, was also explored. The Chefs exposed that there is some apprehension regarding the consumption of sea urchin mainly due to its appearance. Also, the existent preconception and suspicion about aquaculture products and their acceptance should be countered, since the future availability of these gourmet products and other seafood commodities with high-quality will be dependent on their production in sustainable culture systems. These results emphasize the need for communication and marketing strategies through locals, chefs and gastronomic events, focused on the promotion of consumer awareness about the exclusivity of this product and the importance of aquaculture for the future of the seafood market. The sensory evaluation performed by the Chefs originated a preliminary sensory attributes list (Chapter IV).

The sensory features and odour active volatile organic compounds (VOC) profile of *P. lividus* was also studied, taking into consideration the sex and harvest location. Sea urchin gonads' sensory features were mainly influenced by sex, while their volatile profile was affected both by sex and harvest location. Males tended to be sweeter, softer and with a pale-yellow colour, while females revealed an intense sweet tropical taste and a bright-orange colour. Data from this study and Chapter IV contributed to the sea urchin gonads sensory profile characterization, aiming for the development of a preliminary sensory ballot (Chapter V).

Moreover, a sensory profile of sea urchin (*P. lividus*) gonads was also built, to evaluate differences between sex, harvest location, as well as the impact of presentation on consumer acceptance. In Chapter VI, gonads were separated in relation to the presentation method: the white background of the bowl seemed to highlight the orange colour of females; the gourmet presentation favoured the females in relation to the males, mainly because the male gonad colour and white-colour exudate were emphasized by the background of the sea urchin test. In both studies, sensory profiling clearly separates gonads according to sex, with females being more appealing than males. These two studies also concluded that a sea urchin with an orange colour, intense, sweet and pleasant tropical taste and a pleasant, fresh and tropical odour is more appealing and accepted among panellists, which may contribute valuable information for future nutritional aquaculture studies aiming for the enhancement of sea urchin gonads attributes and, subsequently, their market valorisation. This thorough characterization of the quality of wild harvest *P. lividus*, including its sensory profile, helped define the consumers' quality demands aiming the selection of premium gonads, which allowed to focus the last two studies in the growth and quality enhancement of sea urchin gonads, respectively. In fact, aquaculture can play an important role in supplying high-quality animals. Nevertheless, the success of this activity

largely depends on the development of nutritionally balanced diets capable of producing excellent gonads.

After thorough examination of the sensory profile of the sea urchin, the current study focused on the preparation and evaluation of four extruded diets formulated with low or high protein (30 and 50% dry matter (DM)) and lipid (6 and 11% DM) levels, which were fed to wild-caught individuals for 15 weeks. All diets were effective in promoting gonad growth, but the diet with the lowest protein (30% DM) and lowest lipid (6% DM) level resulted in the highest gonad yield in both sexes. The gonad somatic index varied between sex but was similar among dietary treatments (19–23%) and was higher than values observed in wild specimens (6–7%). Dietary treatments and sex did not affect gonad texture (firmness and resilience) or colour parameters (brightness, redness, yellowness, chroma or hue angle), but affected gonad composition. Females had significantly higher total concentration of carotenoids than males, irrespective of the dietary treatments. Wild individuals had higher levels of echinenone, β -cryptoxanthin and β -carotene, and showed higher redness and firmness values when compared to the LP/LL diet, irrespective of sex. This study clearly showed that it is possible to enhance sea urchin gonad yield in relation to wild animals in a Recirculating Aquaculture System at 18 °C, but failed to improve sensory and quality characteristics (Chapter VIII).

Finally, in a subsequent stage, gonads with acceptable colour with the incorporation of different sources of β -carotene in the LP/LL diet used in the Chapter VIII were also produced. Total carotenoid content and profile, lightness (L^*) and texture of gonads did not vary significantly among dietary treatments. The diet with lower concentration of synthetic β -carotene (L_SYN) showed highest values of redness (a^*) and yellowness (b^*) and higher values of acceptance among the naïve consumers. Moreover, females displayed higher acceptance rates than males, probably due to the fact that females were more orange, appealing and pleasant compared with the yellow, grainy and unappealing males. Males fed the L_SYN diet exhibited similar colour and carotenoid content compared with wild animals in the same stage of gonad maturation (stage III), but females differed significantly; however, neither of the sexes demonstrated acceptance differences in relation to their wild counterparts (Chapter IX).

This thesis was performed in an industrial setting, under the partnership of the company Sense Test, Lda, and focused on sea urchin sensory characterization, as well as improving sea urchin feed formulation. The combination of the results obtained in the different Chapters provides a significant contribution to the ongoing development of a *P. lividus* suitable diet. It clearly proposes a diet able to significantly increase gonads size and, more widely, it will contribute to overcome the supply bottlenecks currently preventing a more extensive utilisation of these echinoids. It is also patent that gonad colour is not

exclusively modulated by dietary carotenoid supplementation and that it may be affected by other sex-dependent nutritional or metabolic factors that need further studies.

Thesis structure

This thesis is organised into ten chapters. The first chapter includes the state-of-the-art, reflecting sea urchin ecological and economic factors, methodological considerations, and aims of the thesis. Chapters two to nine include all practical work presented in the form of manuscripts, using a paper-based structure, which resulted from this PhD project:

Chapter II. The effect of sex, season and gametogenic cycle on gonad yield, biochemical composition and quality traits of *Paracentrotus lividus* along the North Atlantic coast of Portugal.

Chapter III. Survey into the sea urchin consumption profile of the Portuguese population

Chapter IV. Dimensions for the valorisation of sea urchin (*Paracentrotus lividus*) gonads production through the eyes of experienced chefs

Chapter V. Analysis of volatile compounds in *Paracentrotus lividus* by HS-SPME/GS-MS related to its sensorial properties

Chapter VI. Sensory profiling, liking and acceptance of sea urchin gonads from the North Atlantic coast of Portugal, aiming for future aquaculture applications

Chapter VII. Development of a Rate-All-That-Apply (RATA) ballot for sensory profiling of sea urchin (*Paracentrotus lividus*) gonads

Chapter VIII. Effect of protein and lipid levels in diets for adult sea urchin *Paracentrotus lividus*

Chapter IX. Sensory profiling, liking and gonad composition of sea urchin gonads fed synthetic or natural sources of β -carotene enriched diets

The last chapter of the thesis provides an overall discussion and main conclusions of this PhD thesis and final considerations.

Chapter I.

Sea urchin

Biogeography, ecology and reproduction

Sea urchins are marine and free-living animals that belong to the class Echinoidea of the Phylum Echinodermata. Most edible sea urchins are small, spiny, globular animals, adapted for life in benthic surfaces. Sea urchins tend to live in shallow waters, usually from mid to low intertidal zones. Their round and spiny test typically varies from 3 to 10 cm and the common colours include black, green, brown and purple, depending on the species, geographic zone and diet (Lawrence, 2020). A common feature of all echinoids is the Aristotle's lantern associated with the mouth. This complex structure consists of more than 40 interconnected ossicles and powerful muscles that allow protraction and retraction of the teeth. The digestive tract of sea urchins is long and coiled, consisting of a pharynx, oesophagus, stomach, intestine, and rectum, which opens through the anus within the periproct at the apex of the test. The stomach comprises the greatest length of the gut, forming five pouches hanging from the body wall in an almost complete circuit. Most sea urchins preferentially feed on kelp and other macroalgae and can have distinct preferences for certain species, although availability is also a key factor (Boudouresque *et al.*, 2020). Despite all echinoids could be considered edible (Lawrence, 2020), only a few species are subject to harvest. The most worldwide harvested species of sea urchin are *Loxechinus albus* and *Strongylocentrotus* spp., while *Paracentrotus lividus* (Figure 1) is the most exploited species in Mediterranean coasts. Despite its popular name "purple sea urchin", *P. lividus* colour is highly variable: black-purple, purple, red-brown, dark brown, yellow-brown, light brown or olive green. This species is distributed throughout the Mediterranean Sea and in the Northeast Atlantic, from Scotland and Ireland to southern Morocco and the Canary Islands, including Azores.



Figure 1 - Sea urchin, *Paracentrotus lividus*

Temperature is the principal feature that influences the geographic distribution: *P. lividus* is more abundant in Portugal and the Biscayne Bay, where sea water temperatures can range between 10-15 °C in Winter and 18-25 °C during the Summer (Boudouresque *et*

al., 2020). *P. lividus* is typically a subtidal species, living from the mean low water mark down to 10-20 m, and in intertidal rock pools (Crook *et al.*, 2000); habitats formed by rocks or boulders, where individuals can find shelter by digging cavities in the substratum avoiding contact with waves and predation, being the preferred ones (Boudouresque *et al.*, 2020). Nonetheless, *P. lividus* populations can also be found in the open sea, mainly on solid rocks, boulders and in meadows of the seagrasses *Posidonia oceanica* and *Zostera marina*. This species is described as being mainly herbivorous in its natural habitat, consuming macroalgae and aquatic plants detritus captured over the seabed (Boudouresque *et al.*, 2020). However, *P. lividus* can also demonstrate omnivorous and opportunistic habits, by feeding on molluscs and another small animal (Cook *et al.*, 2007).



Figure 2 - *P. lividus* (two right photos shared through "Instagram" networking service by @submpolvo).

All echinoids are dioecious, and in sea urchins of both sexes the reproductive system consists of five separate gonads (Figure 2), each connected to the upper aboral surface by individual gonopores. Sea urchin gonads have a dual role, functioning as both reproductive organs and nutrient stores (Hughes *et al.*, 2006). Gonads increase in volume during the annual reproductive cycle because the size and number of germinal cells increase during gametogenesis, and also because somatic cells within the gonads store nutrients before gametogenesis (Walker *et al.*, 2020). Moreover, histological studies of *P. lividus* gonads indicate one or two seasonal peaks in the gametogenic cycle, which can differ between the locations and environmental conditions to which populations are subjected to (Byrne, 1990; Lozano *et al.*, 1995; Spirlet *et al.*, 1998). During the annual gametogenic cycle, gonads of both sexes of the sea urchin follow a predictable seasonal pattern characterized by six gametogenic stages and undergo some structural changes (Walker *et al.*, 2005). According to Byrne (1990), recovery (stage I) is constituted with primary gametes and nutritive phagocytes; growing (stage II) with clusters of primary

gametes and packed nutritive phagocytes; premature (stage III) with gametes at all stages of development and reduced amount of nutritive phagocytes; mature (stage IV) with mature gametes and few nutritive phagocytes; partly spawned (stage V) with loosely packed gametes and depletion of nutritive phagocytes; and spent (stage VI) with gonads empty of gametes (Figure 3).

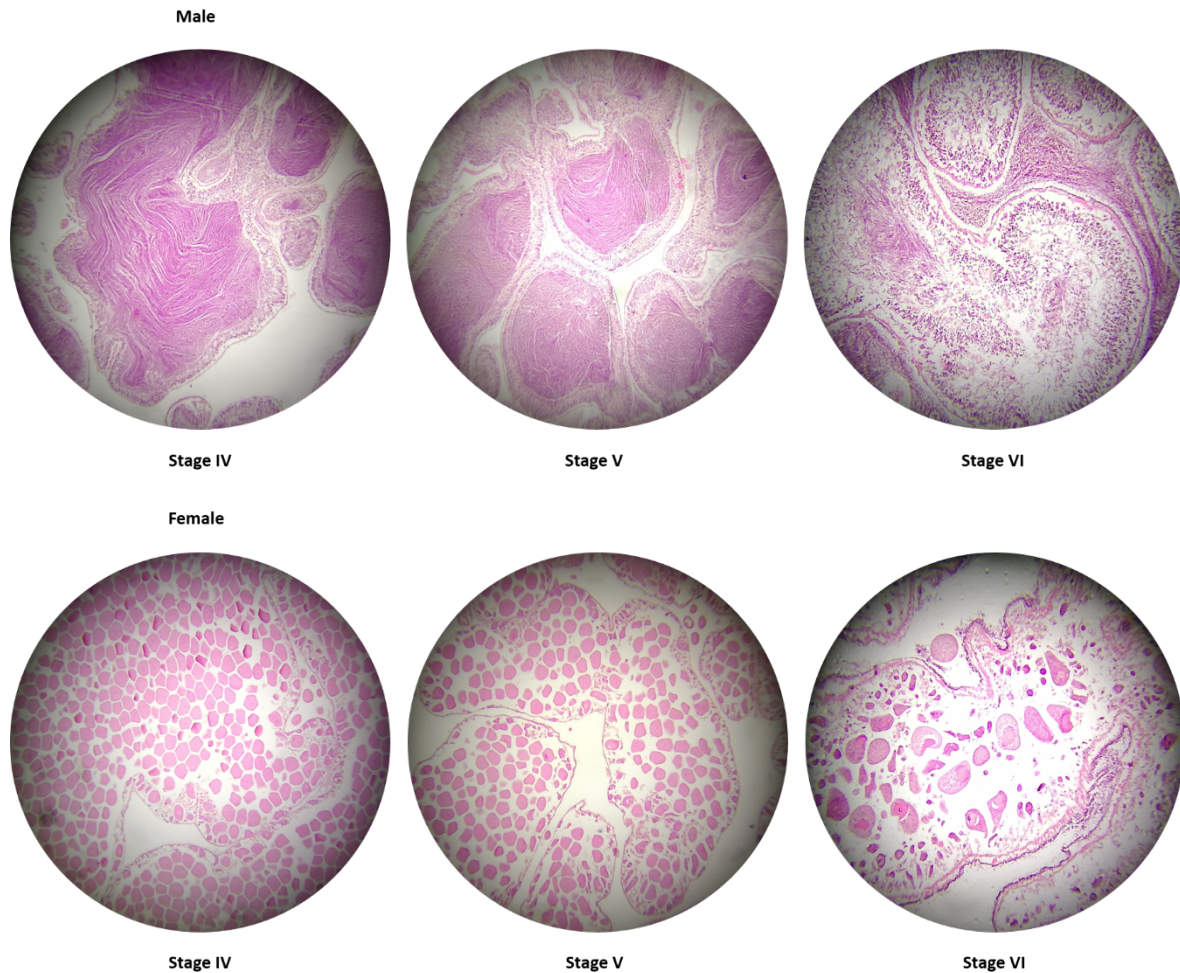


Figure 3 - *Paracentrotus lividus*. Histology of testes: Stage IV - mature testis; Stage V – partly spawned testis; Stage VI – spent testis devoid of contents; and of ovaries: Stage IV - mature ovary; Stage V – partly spawned ovary (except for the spaces between ova, ovary is similar to stage IV); Stage VI – spent ovary containing unspawned ova.

The gonadal growth and quality of *P. lividus*, along the annual reproductive cycle in their habitat, appears to be related to water temperature, photoperiod and density (Boudouresque *et al.*, 2020), food availability (Livore *et al.*, 2012) and food quality (Gago *et al.*, 2003), and also the gonads' maturation stage (Walker *et al.*, 2020). Water temperature and photoperiod, either alone or in combination, are responsible for the initiation of vitellogenesis and gametogenesis in many sea urchin species (Walker *et al.*, 2020). For many sea urchin species, from temperate zones such as *P. miliaris* and *S. droebachiensis*, a combination of low temperature followed by lengthening days is required for completion of gametogenesis (Scheibling *et al.*, 2020; Suckling *et al.*, 2020a). For other species, such as *P. lividus*, *P. depressus* and *H. pulcherrimus*, temperature is more important than

photoperiod for gonadal maturation (Agatsuma, 2020; Boudouresque *et al.*, 2020). Indeed, temperatures between 18 and 22 °C enhance *P. lividus* gonadal development and gametogenesis (Shpigel *et al.*, 2004), whilst temperatures lower than 13 °C may inhibit their growth (Byrne, 1990). Latitudinal patterns can also affect the echinoids' reproductive cycles and, consequently, gonadal growth. For example, the gonad index of *P. lividus* showed a clear geographical trend, with the Atlantic populations reaching higher gonadosomatic index (GSI) than the Mediterranean populations (Spirlet *et al.*, 1998). The food availability and quality also play an important role, since well-fed sea urchins produce larger and more productive gonads, whereas nutritionally deprived individuals may fail to develop sufficient gonads for spawning (Fabbrocini *et al.*, 2010; Garrido *et al.*, 2001). Furthermore, the sea urchin gonads' maturation stage, that varies along the reproductive cycle, affects the gonads' organoleptic and quality features - for instance gonad taste, colour and size - mainly due to transformations that occur during the reproductive cycle, and consequently their market acceptance and value (Figure 4) (Walker *et al.*, 2020). Their commercial value increases during the premature season, which presents the highest nutritional and quality organoleptic features. During this growing phase the nutritive phagocytes are full (Walker *et al.*, 2015) and accumulate high levels of protein, lipid, carbohydrates and pigments (Powell *et al.*, 2020), also reaching their maximum size, quality and more intense colour (Sartori *et al.*, 2016a). In fact, during this growing phase, just before the onset of gametogenesis, sea urchin gonads present the best organoleptic features, which leaves a narrow time window for commercially viable exploitation of this product. Afterwards, the changes in the biochemical content of gonads due to the maturation of gonads can impair their organoleptic features, becoming bitter, softer and overall unpleasant, leading to a loss of their economic value (McBride *et al.*, 2004; Walker *et al.*, 2015).

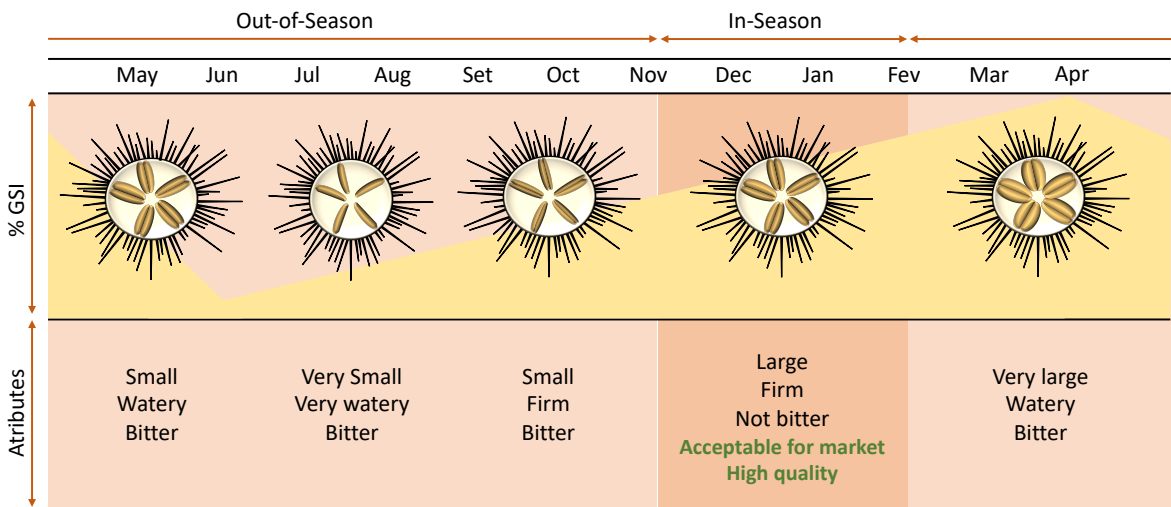


Figure 4 - Scheme representative of the gonadosomatic index (GSI) variation during the year (*Paracentrotus lividus*, Ireland). Adapted from Unuma (2002).

Thus, increasing gonad size, improving its quality and delaying gametogenesis hence extending harvesting season are all crucial appraised prerequisites to add economic value to this product. However, the physiological and chemical fluctuations that *P. lividus* suffers along the seasonal reproductive cycle in the Atlantic shores of the Portuguese coast, or the way they interact with their biotic and abiotic environment are still poorly understood.

Economic importance

At first glance, the hard shell and long spines of the sea urchin suggest that it is not something people would be overly interested in eating. However, sea urchin, or sea urchin gonads to be more specific, have proven to be a culinary delicacy prized in many European countries, as well as in Chile, North America, Asia, and especially in Japan, which accounts for around 80% of worldwide demand of sea urchin products (fresh, chilled, frozen, dried, salted or brined sea urchins). FAO (2020b) reported that worldwide capture production of sea urchin has decreased by almost 50% from the peak in 1995 (110,000 tonnes – live weight), reaching 58,528 tonnes (live weight) in 2018 (Figure 5) (FAO, 2020a). Live and

Global capture production

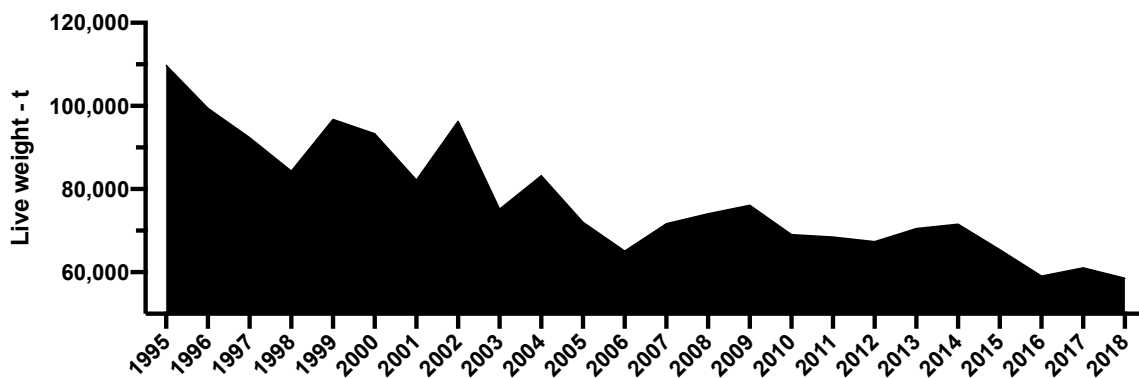


Figure 5 - Global sea urchin capture production 1995-2018 (FishstatJ).

fresh sea urchins dominate the global trades volume with an average price per kilo of US\$11 (€9)¹, followed by frozen sea urchin gonads that in 2017 had the average price of US\$36 (€30) (Table 1). Nowadays, sea urchin gonads are one of the most valuable seafood products, mainly in Japan where market demand for this delicacy has been growing for decades (Stefánsson *et al.*, 2017). Fresh gonads exported to the Japanese market range between US\$40-70 (€33-57)/kg (Unuma *et al.*, 2015), while the price of live/fresh sea urchin rounds US\$11-14 (€9-11)/kg (Table 1). Local fresh gonads served in sushi bars can frequently exceed US\$50 (€41)/kg, and top-quality gonads, served in expensive sushi bars, can even be sold for over US\$100 (€82)/kg (Sun *et al.*, 2015). From a commercial standpoint, high quality sea urchin gonads are large-sized and with a number of sensory attributes preferred by consumers (i.e., taste, texture, colour, and firmness). The most desirable combination of these features can differ between markets, making some species of sea urchin more profitable than others. Each species differs greatly in terms of gonad size, palatability, flavour, and other sensory attributes, that depend upon the interaction of many factors including environmental conditions (e.g. temperature and photoperiod),

¹ Currency conversion: 1USD = 0.82EUR
16/12/2020, 10:14UTC, Coinbase

annual reproductive cycle, sex (female or male; some cultures prefer one over the other) and the nutritional input (feed availability and quality). For instance, in Japan, a high-priced gonad is represented by a bright yellowish colour, as well as sweet ocean taste and lingering aftertaste, while a poorer quality urchin tends to be bitterer and/or have brownish coloration (Barker, 2015; Stefánsson *et al.*, 2017).

Table 1 – Global imports and exports volumes (tonnes), trade values (USD 1000) and average price (USD) per kilo of sea urchin products, in 2017 (FAO, 2019).

	Imports	Value	Value/kg	Exports	Value	Value/kg
Sea-urchin, live, fresh or chilled	12017	158834	13.2	24193	269890	11.2
Sea-urchin, frozen	2236	77609	34.7	2034	72461	35.6
Sea-urchin, dried, salted or in brine; fermented, smoked	214	8668	40.5	235	4476	19.1
Sea-urchin, prepared or preserved	190	5713	30.1	203	5750	28.3
Others	8	258	32.3	30	662	22.1

As Japanese urchin stocks were exploited and overfished, leading to the collapse of native stocks in the early 1970s, the continued growth of gonad consumption in Japan was increasingly met by other countries (Unuma *et al.*, 2015). In fact, in Japan the total landings of sea urchin were 7100 tonnes (FAO, 2019) and the total sea urchin imports were 11233 tonnes (Table 2), however still insufficient to cover the Japanese sea urchin market demand of around 50,000 tonnes, which is worth approximately 300 million USD (€246 million) (Sun & Chiang, 2015). The Japanese market is the major driver of global sea urchin prices. The major supplier of whole, live sea urchins is Russia, while Chile delivers the bulk of frozen sea urchin gonads and the United States and Canada provide the majority of fresh, processed sea urchin gonads for the Japanese market. Russia has the lowest live sea urchin price and shipping costs, allowing for it to outcompete other countries and thereby dominates this market category in Japan. The total landings of sea urchins, mainly of *Strongylocentrotus* sp. (*S. intermedius*, *S. franciscanus*, and *S. droebachiensis*), for the Russian Federation in 2018 were 8140 tonnes (FAO, 2020a). Chilean sea urchins (*Loxechinus albus*) harvests account for approximately 50% of global landings (30446 tonnes in 2018) (FAO, 2020a), however, *L. albus* usually presents lower gonads' quality and is mainly exported frozen to the Japanese markets, where its value is around US\$20-40 (€16-33)/kg (Sun *et al.*, 2015) (Table 2). Besides, the transport from Chile to Tokyo involves a considerable distance, which makes the transportation of live/fresh sea urchin difficult and expensive. The Chilean frozen gonads are also imported into Canada and the U.S. (Sun *et al.*, 2015). More recently, sea urchin gonads have also become popular in the United States, mainly on the west coast, and Canada. The total landings of sea urchins in U.S. and Canada were 3145 and 4474 tonnes in 2018, respectively (FAO, 2020a). Fresh and chilled *S. droebachiensis* and *S. franciscanus* gonads are imported from North America

and Canada to Japan, being known to be of high quality relative to Japanese and Chilean domestic products. For instance, they are highly prized when packaged in 120g wooden trays with prices ranging from US\$10–30 (€8-25) (Eddy *et al.*, 2015). Australia is another country that has seen increased interest in sea urchin gonads, namely in purple sea urchin, *Heliocidaris erythrogramma*, considered the highest quality local sea urchin. Following a similar pattern to the United States, Australian sea urchins are often exported to the lucrative Japanese market. Moreover, as in the United States, the development of an export market to Japan was followed by the growth of a domestic market (Williamson, 2015). The current demand for Australian sea urchins, particularly from Hong Kong, exceeds at least four times the supply (Williamson, 2015), however neither landings nor trades were reported in the last few years (FAO, 2019, 2020a).

Table 2 – Volume of imports and exports (tonnes), volume value (USD 1000) and average price (USD) per kilogram of sea urchin (live, fresh, chilled, frozen, dried, salted or in brine; fermented, smoked), taking into account the most important global sea urchin markets in each category, in 2017 (FAO, 2019).

Imports			Exports		
	Volume	Value/kg		Volume	Value/kg
Live, fresh or chilled			Live, fresh or chilled		
Japan	9008	115776	Russia	7984	19432
USA	2186	14336	Canada	2418	19463
France	192	2016	USA	641	26939
China	137	8050	China	183	12205
United Kingdom	72	757	Spain	182	2026
China	70	2558	Iceland	132	1141
Frozen			Frozen		
Japan	2009	72304	Chile	1874	67596
USA	103	1317	China	52	2580
Taiwan	35	853	Netherlands	27	232
Italy	30	673	Canada	19	233
China	13	482	Spain	13	435
South Korea	12	474	USA	11	62
Dried, salted or in brine; fermented, smoked			Dried, salted or in brine; fermented, smoked		
Japan	123	7510	India	124	288
Bangladesh	36	79	South Korea	39	3255
Italy	17	575	Russia	36	485
France	13	167	Malaysia	14	103
Lesotho	10	4	USA	13	59
Malaysia	5	71	Netherlands	2	104
Prepared or preserved			Prepared or preserved		
Japan	93	2660	Chile	121	3429
Italy	32	1498	Iceland	27	116
USA	18	56	Spain	16	1426
Belgium	12	83	Greece	15	76
France	10	452	Japan	8	239
China	7	474	USA	5	120

Salted, dried or brined sea urchin gonads, which are the least consumed of the four major product forms, are mainly imported from South Korea, being the unit price of this product

over US\$60 (€49)/kg (Table 2). However, prices are lower when urchin is imported from other countries, but they also have inferior quality.

In Europe, in most of its geographical range, there are several species of urchins, but the most economically important and appreciated species is *P. lividus*, being intensely harvested in France, Ireland, Spain, Portugal, Italy, Croatia and Greece (Boudouresque *et al.*, 2020; Guidetti *et al.*, 2004). The overall market in Europe is not large, estimated to be about 3,000-3,500 tonnes of sea urchin in live weight. Spain is the European country with highest captures in 2018 (755 tonnes) (Ministerio de Agricultura, 2019) followed by Iceland with 378 (FAO, 2020a), Portugal with 170 tonnes (INE, 2018), Croatia with 136 tonnes, France and Italy with 103 and 78 tonnes, respectively (FAO, 2020a). *P. lividus* gonads are considered a prized and gourmet seafood delicacy, due to their unique flavour, making their commercialization highly profitable, mainly in Mediterranean countries. A set of desirable features can be found in this species: the bittersweet and slightly saline flavour, unique and delicate texture, reddish-orange colour and high nutritional content (Sartori *et al.*, 2016a) (Figure 6). The gonadal yield should be at least 10-15% to be suitable for processing and marketing (Stefánsson *et al.*, 2017). Nowadays, the consumption of *P. lividus* is mainly limited to France, Italy and Spain, and to a lesser extent to Greece; France is the main consumer of sea urchin in Europe, being the second largest worldwide consumer after Japan (Stefánsson *et al.*, 2017; Sun *et al.*, 2015). In 2017, France had officially imported 205 tonnes, where the price of live, fresh sea urchin was around US\$11 (€9)/kg and the dried/salted gonads around US\$13 (11€)/kg (Table 3), however only 229 tonnes of sea urchins in total were sold in the French market (Stefánsson *et al.*, 2017). This is contradictory with the fact that France has been reported as the main European consumer of sea urchin. It is possible that there may be either some unreported imports of sea urchins, or that there is significant unlicensed and illegal fishing making real harvest difficult to estimate. It is also likely that the local demand for the product (*P. lividus*) might have decreased due to lack of supply or due to the increased price of imported sea urchins. Italy seems to be the second most matured sea urchin market in Europe with imports of 143 tonnes. Italy mainly imports live, fresh or chilled sea urchin which prices rounding US\$11 (€9)/kg. Spain is the main European exporter of sea urchin (213 tonnes), mainly due to excess from the harvest, and only imports 10 tonnes of frozen urchins. Nevertheless, there is limited published information regarding the sea urchin total landings and market in European countries, and it has been proven difficult to find reliable information about these markets.

Portugal is one of the European Union countries with higher annual average seafood consumption per capita (55.9 kg), corresponding to a 160 g meal of seafood per day (FAO, 2020b). Moreover, Portugal has an Exclusive Economic Zone of around 1,700,000 km² and

a coastline of 942 km, which gives extremely high prominence to fishery and fish products consumption. However, in Portugal, sea urchin gonads are still under-consumed mainly because they are not part of the national gastronomy culture. Nevertheless, commercial harvesting of sea urchin is increasing in Portugal as observed by the raise in landings from 60 to 170 tonnes, between 2016 and 2018, mainly for exportation to Spain, Italy and France (FAO, 2020a). The landings were mainly concentrated in the Northwest coast (Viana do Castelo) that reached around 83 tonnes, while in Centre (Ericeira) and South of Portugal (Algarve), with a long-standing tradition on culinary exploitation of this delicacy, 1 and 3 tonnes of sea urchins were harvested respectively (INE, 2018). Additionally, the Portuguese population is mostly concentrated in coastal regions, so there is a strong correlation between human activity and the exploitation of marine resources through fishing, seafood harvesting and others. Indeed, the potential to explore marine resources are vast, namely exotic and prized species like sea urchins. Also, a consumer shift towards seafood as key sources of healthy nutrients might represent an opportunity for this species as a high-valued product (Rocha *et al.*, 2019). Most likely, new emerging markets outside the traditional ones will appear in the next few years, as sea urchin is being seen as a novel and trendy product, in which interest has been increasing in several countries throughout Europe.

Table 3 - Volume of imports and exports (tonnes), volume value (USD 1000) and estimated value (USD) per kilogram of sea urchin (live, fresh, chilled, frozen, dried, salted or in brine; fermented, smoked), taking into account the most important European sea urchin markets in each category, in 2017 (FAO, 2019).

Imports				Exports			
		Volume value	Value/kg			Volume value	Value/kg
Live, fresh or chilled				Live, fresh or chilled			
France	192	2016	10.5	Spain	182	2026	11.1
United Kingdom	72	757	10.5	Iceland	132	1141	8.6
Italy	64	690	10.8	France	95	1198	12.6
Portugal	54	547	10.1	Portugal	43	501	11.7
Frozen				Frozen			
Italy	30	673	22.4	Netherlands	27	232	8.6
Spain	10	159	15.9	Spain	13	435	33.5
Netherlands	6	255	42.5	Belgium	2	47	23.5
Belgium	3	53	17.7	Iceland	2	123	61.5
Dried, salted or in brine; fermented, smoked				Dried, salted or in brine; fermented, smoked			
Italy	17	575	33.8	France	2	45	22.5
France	13	167	12.8	Netherlands	2	104	52.0
Spain	4	109	27.3	Spain	2	74	37.0
Netherlands	3	101	33.7	Germany	1	7	7.0
Prepared or preserved				Prepared or preserved			
Italy	32	1498	46.8	Iceland	27	116	4.3
Belgium	12	83	6.9	Spain	16	1426	89.1
France	10	452	45.2	Greece	15	76	5.1
Greece	4	89	22.3	Belgium	1	12	12.0

Sea urchin consumers expect year-round availability of the product, but the supply of sea urchin' gonads has been decreasing over the last years. Moreover, the average size of wild harvested sea urchins has steadily decreased in many regions compared to three or four decades ago (Sun *et al.*, 2015). Indeed, the increasing demand for sea urchins to meet international market demands from Japan, France, Italy, Turkey and Belgium, where sea urchin gonads are considered a culinary delicacy, is negatively affecting the natural stocks of sea urchins, mainly due to the overexploitation to which it is being subjected. Sea urchin captures are often quite limited and these countries are increasingly forced to import products from other countries such as Portugal, Greece, Ireland or Spain. Moreover, the increased demand coupled with the decreased levels of catch, resulted in an increased price of a kilogram of sea urchin gonads, depending on the season and species marketed (Sun *et al.*, 2015). There may also be future options to supply the emerging high-end restaurant niche market in several European countries or even opportunities to develop the processing methodologies of gonads from animals with low gonad yields or damaged urchins, for garnishes, sauces, and pates. This strategy might add economic value to this product, even with low-quality features; however, it is necessary to solve the issue related with the gap between the increased consumers demands and the decreased supply.



Figure 6 -*P. lividus* gonads.

Gastronomy and markets

Gonads are primarily marketable fresh, but can also be frozen, salted, baked, or steamed. Processing sea urchin gonads is extremely labour intensive, being a determinant factor for the final price of this product. The test needs to be cracked, manually opened using a special tool to expose the five gonads, which should be gently cleaned and removed. The gonads must be gently treated and their shelf-life, even when chilled, varies from 3 to 7 days, at most. Fresh, high quality grade gonads have the highest value on the market, presenting the best organoleptic features sought by the consumer. Freezing is an acceptable method for keeping processed low-quality gonads that will be further utilized in pâtés and sauces. The freezing process increases the gonads shelf-life and facilitates their transport and storage, but at the same time the ice crystals formed during the freezing process can negatively affect their quality. Furthermore, during defrosting, a loss of gonads firmness occurs, which can lead to the loss of about 25-50% of their value compared to fresh gonads (Stefánsson *et al.*, 2017). Processed gonads (dried, salted, and brined) are the least profitable category due to a lower demand. These processed sea urchin products are produced from low-quality sea urchin gonads or their by-products (Sun *et al.*, 2015).

In Japan, sea urchin gonads are included in several dishes (Lawrence, 2007), having a traditional role in the Japanese cuisine being served in sushi bars, restaurants and at wedding banquets with a small portion destined for household consumption (Sun *et al.*, 2015). Sea urchin gonads are hence considered a premium seafood being eaten raw as sashimi, served with rice as sushi or preserved in bottles mixed with brine or alcohol and salt (Andrew *et al.*, 2003). Raw sea urchins are normally arranged in traditional wooden trays and normally present the best organoleptic features (Figure 7). Sea urchin gonads can also be boiled in seawater, baked, and used as an ingredient in a variety of soups (Sun *et al.*, 2015). Product presentation mode is a critical aspect to safeguard the aesthetics of the gonads appearance (Stefánsson *et al.*, 2017), and an efficient and powerful way of attracting consumers and improve the product acceptance (Sun *et al.*, 2015).

Sea urchin gonads have also been popular in Mediterranean cuisine for centuries. In ancient Greece there was already a tradition of consuming this seafood, raw or well-roasted. During the Roman imperium, sea urchin gonads were consumed raw or preserved in salt (*“inpreparatos”*). Indeed, mosaics of that time have been found representing banquets where it is possible to see raw sea urchin gonads with vinegar, parsley or chopped mint (Soyer, 2013). Moreover, there is an antique receipt book called *“De re coquinaria libri decem”*, where Marcus Gavius Apicius, in 25 BC, uses sea urchin gonads with cooked must

and pepper (King, 2014; Lin, 2017). Nowadays, there is a well-established market for sea urchin gonads in the Mediterranean region. Due to historic and traditional reasons they are consumed in a variety of ways, depending on the regions: blended into sauces; with pasta, bread or simply used as an ingredient; or to embellish several dishes. Sea urchins have also found popularity outside traditional markets, with increasing ethnic populations and the growing popularity of sushi restaurants around Europe. Indeed, in 2016, sea urchins were one of the top ten food trends due to their unique flavour (Nestlé, 2016).



Figure 7 – Sea urchin gonads market presentation (photos from Shiki Singapore).

In Italy, sea urchin gonads are blended into sauces and pâté (Figure 8), served with pasta (Sun *et al.*, 2015) and incorporated into pizza, pastas, croutons, among others (Furesi *et al.*, 2014). It can also be served with bread, especially in Sicily, or with wine as an entrée (Wise, 2008). In Spain, sea urchin gonads are used as an addition to soups and stews, as a flavour intensifier, or as side dishes with truffle and cooked egg. They are also used as an ingredient in other dishes, for instance lamb jus, lasagne, gratin or scrambled sea urchin and, also, in the preparation of puddings or fish and seafood cakes. Sea urchin pate is the most appreciated conserve, most of it made in Asturias. In Greek and French cuisine, sea urchin gonads may also be served with bread or used as an ingredient in a variety of dishes. Greeks enjoy consuming sea urchins with liquor, sometimes pouring *ouzo* directly into the urchin test and then drinking the liquor-soaked gonads. French appreciate *Tarama*, which is basically an emulsion consisting of sugar-salted and smoked sea urchin gonads and vegetable oil, but with various other ingredients such as lemon juice, breadcrumbs and

colourants (Stefánsson *et al.*, 2017). Moreover, other traditional preparations of *P. lividus* are found in Provence, France: an omelette, an *oursinado* (prepared by mashing roe in a mortar and pestle and adding olive oil little by little to make a mayonnaise) with bread or potatoes and soup base or a *crème d'oursin* served with fish (Lawrence, 2007). In France, in retailers as Carrefour, Auchan and Leclerc, it is possible to find preserved or canned *P. lividus* gonads especially before Christmas (50 g glass jars costs about 100-120 €/kg) (Monfort, 2002). In the United Kingdom, sea urchin gonads are a complement to pasta or hollandaise sauce or served as a topping for baked potatoes and biscuits (Stefánsson *et al.*, 2017). In Portugal, besides the consumption of raw sea urchin in some traditional coastal villages, there is also a known traditional way of preparing sea urchin by placing the sea urchin gonads in-between needles. The pine needles are burned, and the sea urchin is cooked (Powell, 2013). Other countries that are not usual consumers of sea urchin also present different methods to cook sea urchin. For instance, Antilles people appreciate the *blaff d'oursins*, where sea urchin gonads are marinated in a mixture of salt, pepper, garlic, lemon juice and crushed allspice or a baked tarte with a mixture of browned onions, laurel, chives, logwood, garlic, lemon, salt and pepper, tomatoes, and roe (Lawrence, 2007). In the Philippines, sea urchin can be steamed, boiled, or grilled in an open fire before removing the roe, and then lemon juice is added. Another common dish in this region involves removing the Aristotle's lantern, gut, and coelomic fluid and leaving the gonads inside. Rice is placed inside the test and cooked before the test is removed, leaving the sea urchin shaped rice with the five gonads delightfully placed around it (Lawrence, 2007). In New Zealand sea urchin gonads are typically eaten raw with lemon or with toast after boiling them in saltwater during a few seconds. They also use a sauce made from the mixture of sea urchin gonads with olive oil and Hollandaise Sauce (Lawrence, 2007). Novel uses of sea urchins have also been reported, e.g. incorporations into a custard or ice-cream (Stefánsson *et al.*, 2017).



Figure 8 - Polpa di Riccio.

It is clear that the sea urchin European market is increasing and there could be opportunities for selling locally; however, marketing through locals, chefs, and friends will be required to grow this European market in a sustainable way (Grisolía *et al.*, 2012).

Indeed, Grisolia *et al.* (2012) showed that in the absence of previous experience with the product, a favourable opinion from a well-known chef or a friend works powerfully to increase the interest in this product. Also, it is well known that sea urchin presents a rather strong sea-flavour that may induce an initial rejection, but once the consumer gets used to it, it tends to become a strong acquired taste. Furthermore, the rising interest of consumers in adopting healthy eating habits has led to higher consumption of seafood products, based on their nutritional value and associated health benefits (ISSFAL, 2004). In fact, seafood, particularly sea urchin, are a good source of high-quality protein, low in saturated fat and rich in many micronutrients. Some studies explored the nutritional value of the *P. lividus* gonads (Arafa *et al.*, 2012; Montero-Torreiro *et al.*, 2003), however there is still scarce or even inexistent information regarding the nutritional value of specimens harvested in the Portuguese coast, as well as the variation between sex. Moreover, the market price and consumer acceptability of sea urchin gonads are influenced by their sensory traits, mainly the appearance, colour, texture, flavour, taste and size (Lourenço *et al.*, 2019). However, the sensory profile of this highly demanded gourmet product is not yet well described and may help enhance the gonads' marketability and acceptability.

Sea urchin sensory profile

Purchase and consumption drivers

The consumption of seafood products can be affected by: (1) supply-related barriers; (2) seasonal quality variation, geographic location and sex; (3) consumer aversion to consuming seafood because of a perceived difficulty in buying, preparing and cooking; (4) aspects related to intrinsic characteristics of the product such as the taste, texture, smell; (5) factors regarding the presentation mode, conservation method or the origin (fisheries or aquaculture); and (6) cultural and heritage factors, related to demographic and personal characteristics of the consumers (Almeida *et al.*, 2015; Birch *et al.*, 2012; Cardoso *et al.*, 2013; Gaviglio *et al.*, 2014; Hicks *et al.*, 2008; Musarskaya *et al.*, 2018; Myrland *et al.*, 2000). Ferreira (2006) demonstrated that seafood consumption is related to cultural values, while Murray *et al.* (2017) have stated that consumers elected price, intrinsic attributes and health benefits of the product as the most important key purchase determinants. It has become clear that the success and sustainability of commercial sea urchin, its economic viability and market acceptance strongly depend on a deeper knowledge of the preferences and patterns of sea urchin consumption by the Portuguese population.

In an attempt to improve knowledge and product success in the market, exploratory methodologies like qualitative research methods have been associated with the development of new concepts (Lawless *et al.*, 2013). This methodological approach requires a small number of participants and usually involves interviews or observations, which are less structured than controlled laboratory trials. These interviews with consumers made by trained investigators, allow to register the consumer's behaviour through notes, video or audio recordings or personal interactions (Jervis *et al.*, 2014). Flexibility is what differs from conventional descriptive sensory analysis, allowing for the information and flow of the study to adapt as new information appears (Lawless *et al.*, 2013). Indeed, the objective of qualitative research is to attain information about consumer perception and behaviour regarding a product with no previous assumptions and to analyse the data that emerges naturally (Nuttavuthisit, 2019).

Frequently, consumers struggle to express their true opinions regarding the choice or purchase drivers of the products, or they may even know the motives, but consider them too intimate or irrational that they rather not to share them with a strange interviewer (Donoghue, 2000; Mesías *et al.*, 2018). Moreover, they fear that their answers may be considered illogical or foolish and therefore, they prefer to mask their true intentions and

beliefs (Donoghue, 2000). In this context, other qualitative techniques as the *Word Association*, a projective technique that emerged from clinical psychology, is one of the most used techniques in consumer qualitative research because of its efficiency and easiness to use, to understand the different sides of human traits, underlying beliefs, thoughts and deep feelings, fears, or even unconscious perceptions that lead to an specific attitude or behaviour (Belk *et al.*, 2012). In this technique, participants are asked to write down the first words that come into their minds when thinking about a given stimulus (Roininen *et al.*, 2006). Indeed, this approach allow to explore the consumer insights about a given product or concept and to even access unrestricted mental associations to the stimulus. The first associations specified by the consumers are usually the most significant in their minds and normally related to their product-related behaviour and perception. In fact, these associations can characterize the consumer-driven choice and purchase (Guerrero *et al.*, 2000; Piqueras-Fiszman *et al.*, 2013; Roininen *et al.*, 2006). This methodology has been applied for different purposes by distinct authors. For example, Ares *et al.* (2008) explored the main associations between yoghurts and functional yoghurts using printed images. Ares *et al.* (2010b) used word association to point out important characteristics of milk dessert, using the original package as a visual stimulus. In another study, de Andrade *et al.* (2016) crossed the perception of Brazilian consumers regarding concepts of lamb meat with their frequency of consumption, not using any physical stimuli. Gastón Ares *et al.* (2015), in two-word association and cross-cultural studies, recognized the consumers' associations with wellbeing and food-related context. The concepts expressed in a word association task are considered spontaneous, contrary to the ones imposed in interviews or questionnaires. For this reason, this methodology is considered a quick, simple and useful approach in consumer qualitative research (Ares *et al.*, 2008; Roininen *et al.*, 2006).

Until now, only a few studies have assessed the consumer's motives and barriers to seafood consumption (Almeida *et al.*, 2015; Birch *et al.*, 2012; Cardoso *et al.*, 2013; Carlucci *et al.*, 2015; Hicks *et al.*, 2008; Musarskaya *et al.*, 2018; Myrland *et al.*, 2000; Pieniak *et al.*, 2010), however studies approaching the consumption of shellfish, namely sea urchin, have not yet been reported. The information about population preferences and consumption habits, as well as the knowledge that the consumer has on consumed species and the main factors influencing the consumer's choices are all key determinants for the valorisation of sea urchin products.

Quality and sensory attributes

Nowadays, wild-caught seafood is considered the quality reference for consumers (Claret *et al.*, 2014). Sensory properties such colour, freshness, and texture are crucial for the acceptance and choice of consumers concerning seafood (Calanche *et al.*, 2019), as well as for their purchase decision (Claret *et al.*, 2012; Verbeke *et al.*, 2007). Appearance features, that comprise different visual properties including colour, shape and texture (Spence, 2015), are the first sensory *stimuli* presented to a consumer, impacting the visual assessment and freshness perception of sea urchin gonads and consequently raising expectations about the product (Reisfelt *et al.*, 2009) (Figure 9). The perceived properties may induce positive sensations leading to product acceptance, or negative sensations leading to its rejection (Spence, 2015). Hence, visual features are essential regarding acceptance or rejection of a specific product (Cardello, 1996). Moreover, food choice largely reflects consumer preferences that are often associated with the taste, price, attitudes, beliefs and food knowledge, the search for convenience, health/wellbeing issues, and the need for humans to explore new food products. In other words, the determinants of everyone's food choices extend from their sensory and psychological preferences (mood, feeling or emotions) to livelihood issues (convenience, price/yield, variety, availability), attitudes and beliefs about food (Steptoe *et al.*, 1995). These aspects are increasingly gaining importance because of the consumer's perception about the sensory features of the products and their influence on their purchase criteria.



Figure 9 – Different ways of presenting *P. lividus* gonads to the consumer.

In this context, sensory analysis is considered crucial for food product development and success, assuming a relevant role in food evaluation and allowing for the assessment of its quality, acceptability and sensory profile (Giacalone *et al.*, 2013). Consumers can use intrinsic and extrinsic cues as quality indicators. Intrinsic cues are inherent to the product - appearance, taste, flavour and odour - while extrinsic cues include any external aspects, such the price or packaging (Gaviglio *et al.*, 2014). The appearance and taste of sea urchin gonads are the most important aspects of their quality, which vary depending on species (Phillips *et al.*, 2010a). The firmness, shape, granularity, and taste of the gonads have been assessed subjectively by eye, and colour has been visually assessed using colour cards or a grading system in *E. chloroticus* (Woods *et al.*, 2008), *S. droebachiensis* (Pearce *et al.*, 2003, 2004; Christopher M. Pearce *et al.*, 2002b), *H. erythrogramma* (Senaratna *et al.*, 2005), *S. intermedius* (Azad *et al.*, 2011) and *P. lividus* (Prato *et al.*, 2018b; Volpe *et al.*, 2018). Gonad colour has also been evaluated using a spectrophotometer in *E. chloroticus* (Phillips *et al.*, 2009; Phillips *et al.*, 2010b; Woods *et al.*, 2008), *S. droebachiensis* (Robinson *et al.*, 2002; Siikavuopio *et al.*, 2007), *S. franciscanus* (McBride *et al.*, 2004), *P. miliaris* (Suckling *et al.*, 2011; Symonds *et al.*, 2009), *S. intermedius* (Azad *et al.*, 2011), *T. gratilla* (Cyrus *et al.*, 2014), *M. nudus* (Takagi *et al.*, 2017) and *P. lividus* (Rocha *et al.*, 2019), or using the internationally recognized CIE L^* (lightness) a^* (redness) b^* (yellowness) colour space system. However, few studies have been carried out on descriptive assessment of the organoleptic features of sea urchin gonads, with most of the reported studies using a 4- or 5-point quantitative/intensity scale (Prato *et al.*, 2018b; Senaratna *et al.*, 2005; Takagi *et al.*, 2017; Volpe *et al.*, 2018; Woods *et al.*, 2008) or a 10-point intensity scale (Siikavuopio *et al.*, 2007). Nonetheless, when such grading systems and category scales are used to assess sea urchin quality, the ability to conduct statistical analysis to detect significant differences is limited (Moskowitz, 2004). Additionally, reliance on experienced assessors to evaluate quality based on 5-point quantitative/intensity scale is challenging, as experience tends to change personal preferences and introduce bias, consequently reducing genuine consumer perception. Descriptive analysis using unstructured line scales allows to accurately measure the intensity of sensory attributes (Barker, 2015) and a more comprehensive statistical analysis can be carried out. This descriptive approach has been previously used to assess the sensory quality (appearance, odour, taste, flavour, texture, and aftertaste) of gonads of *E. chloroticus*, in relation to sex and harvest location (Phillips *et al.*, 2009; 2010a; 2010b). Phillips *et al.* (2009) have shown that sex in *E. chloroticus* was the major factor influencing the sensory quality of gonads; the male gonad had a sweet taste and dairy flavour, while the female gonad had a bitter and sour taste, with an herbaceous and metallic flavour. The methodology and descriptive vocabulary of the 35 terms developed in this study to assess gonad sensory quality could be adapted to other species of sea

urchins, for instance to *P. lividus*. Indeed, as far as we know, there are no previous studies addressing the liking or acceptance of *P. lividus* gonads, neither studies tackling their sensory profile. There is still limited information about how consumer's perception is affected by the sensory properties of sea urchins, more specifically of *P. lividus*. Thus, it is essential to describe and quantify the exclusive sea urchin sensory attributes demanded by the consumer in order to select and deliver top quality gonads. This can also open opportunities for the enhancement of wild or farmed sea urchin gonads quality features through dietary modulation, allowing availability throughout the year. Furthermore, to completely use this valuable food resource, it is of extreme relevance to understand the factors that may influence its sensory properties, for instance the water temperature, seasonality, diet, sex, among others. Table 4 presents a general summary about methodological approaches used in sensory studies into sea urchins, published during the last 18 years (2002–2020), which considered the effect of several variables in the sensory properties of different sea urchin species.

To assess the sensory properties of a product, quantitative descriptive sensory analysis arises as one of the most satisfactory and effective methods to analyse quality characteristics of aquatic animals, as it generates objective descriptions of the perceived sensory attributes of products (Massa *et al.*, 2012; Olafsdottir *et al.*, 1997). Sensory evaluation often requires the use of a trained sensory panel to maintain proper control over the testing situation and to maintain a clear distinction between sensory and hedonic measurements. Even though using a trained panel for sensory analysis represents a sophisticated and valuable tool for food quality assessment, comparison of products, sensory mapping and product matching, the panel requires a deep knowledge, continuous training, and considerable financial investment, which could be a determinant limitation (Gacula, 1997). Moreover, trained panellists may evaluate attributes that are irrelevant to consumer choice (ten Kleij *et al.*, 2003). From a sensory perspective, it is crucial to explore the sensory space used by consumers to describe the products, granting a solid sensory evaluation, which is similar to the final perception of the consumer (Carr *et al.*, 2001). Moreover, the integration of the consumer's points of view in the initial stage of product development is essential to the product's success (Dijksterhuis, 2016; Kemp, 2013). In this perspective, the use of naïve consumers has been gaining increased prominence since they are a source of valuable data, mainly concerning the consumer preferences and perceptions regarding a product (Calanche *et al.*, 2019). During the last few years, there have been several fast and user-friendly technological developments that allowed the collection of sensory information from the consumers' perception of a specific product, with lower cost and shorter implementation time compared with the conventional profiling (Varela *et al.*, 2014). This is the case for Free-Choice Profiling, Projective Mapping, Flash Profiling, Sorting

and Check-All-That-Apply, which are profiling techniques frequently used and considered appropriate for working with untrained consumers (Gastón Ares *et al.*, 2015; Giacalone *et al.*, 2013; Jaeger *et al.*, 2014; Mamede *et al.*, 2016; Meyners *et al.*, 2013; Plaehn *et al.*, 2008; Reinbach *et al.*, 2014; Risvik *et al.*, 1994; Varela *et al.*, 2014; Williams *et al.*, 1984).

Free-choice profiling (FCP) is a sensory technique that can be used to describe a product in terms of their sensory characteristics: appearance, flavour, aroma, or texture. The basic principle of FCP is that each assessor uses his own list of sensory characteristics to evaluate the products. This technique revealed numerous advantages, such as the fact that extensive training is not needed and that the vocabulary development is time- and money-saving. However, FCP presents a drawback because the data analysis and interpretation are very complex and time-consuming due to the large number of individual attributes and their typology (Tárrega *et al.*, 2014). For that reason, other methods for evaluating differences perceived by consumers such as the Flash Profile, Ultra Flash Profile combined with Napping[®] and Check-All-That-Apply (CATA) are preferred compared to FCP, because these methods also produce a products' perceptual maps, but are quicker and more robust. Within the rapid descriptive methods, projective mapping (PM) is a simple sensory technique that allows panellists to express similarities and dissimilarities (Risvik *et al.*, 1994). Samples are projected onto a 2D surface (e.g. piece of paper), taking into account that samples perceived as similar should be placed closer to each other, while samples perceived less similar are placed farther apart. In this way, all samples are placed according to one another. Normally, assessors decide on their own discrimination criteria. PM is commonly paired with the Ultra-Flash Profiling (UFP) method (Pagès, 2003) to provide a set of sensory descriptors generated by the panel itself, aiming to assess global differences. The projections' Cartesian coordinates (x, y) and the frequencies of similar sample descriptors constitute the data. Another recent user-friendly methodology used to study the consumers' sensory response that has gained increased attention in recent years to obtain rapid product profiles from consumers, is the CATA ballot (Meyners *et al.*, 2014). This method allows a sensory evaluation by a panel constituted by naïve consumers, allowing to extrapolate the consumers perception and preference features regarding the product, to further help in its optimisation (Adams *et al.*, 2007; Ares *et al.*, 2010a). Consumers might be presented with a list of attributes (words or phrases), as well as hedonic responses, emotional responses, purchase intentions, potential applications, product positioning, or others, and asked to indicate which descriptors properly describe their interaction with the product (Adams *et al.*, 2007; Ares *et al.*, 2010a; Dooley *et al.*, 2010; Meyners *et al.*, 2014) and from which a sensory map of the products is constructed. Defined as a method which is quick and easy to implement, and based on the consumer vocabulary methodology, it has been widely used in different products from food to cosmetics (G. Ares *et al.*, 2015; Valentin *et al.*, 2012). The

CATA methodology summarizes the consumers' sensory evaluation, being the ideal number of participants between 50 and 100 tasters (Ares *et al.*, 2010a; Dooley *et al.*, 2010; Meyners *et al.*, 2014). However, in cases that CATA method is combined with a hedonic test, we should consider the number of consumers between 100 and 120 (Hough *et al.*, 2006). Hedonic or affective tests are often used to evaluate the acceptance or consumer preference of a product (Lim, 2011). The hedonic 9-point scale, that ranges from a negative polar extreme (1 - dislike extremely) to a positive extreme (9 - like extremely), is one of the more frequently used methods to measure liking (Meilgaard *et al.*, 2006; Peryam *et al.*, 1957). This effective and simple measure is used when the intention is to assess whether consumers like a food product and if they prefer one product instead of another. The type of answer retrieved permits the industry to take decisions regarding the improvement of their food products or development of new products and also to analyse the market potential for the product to be sold (Meilgaard *et al.*, 2006).

The CATA method is a great technique for discriminating samples and compared to the conventional descriptive analysis techniques, it is quicker to design experimentally and easier for the consumer to understand (Ares *et al.*, 2013; Ares *et al.*, 2011). However, the CATA format produces only qualitative data, indicating whether each term is suitable or not to describe the product, but does not allow a direct measurement of the intensity of the perceived sensory attributes, which could mask a potential discrimination between products that have similar sensory profiles, but vary in the intensity of those features (Ares *et al.*, 2014; Dooley *et al.*, 2010; Meyners *et al.*, 2016; Vidal *et al.*, 2018). To overcome this drawback, the use of attribute intensity measurements as a variant to CATA questions has been recommended by Ng *et al.* (2013) to enhance sample description and discrimination. In this context, Ares *et al.* (2014) introduced the Rate-All-That-Apply (RATA) questions. For this alternative approach, in order to obtain intensity-based responses using a CATA-style question, consumers are asked to indicate which terms are applicable to describe the sample, and if they do so, to rate their intensity using a 3 or 5-point scale. These quantitative techniques can be drawn based on previous assumptions or conclusions of the previous qualitative research (interviews, word associations, etc). Additionally, some quantitative findings can be explained through qualitative research from the individuals' experiences or reflections (Nuttavuthisit, 2019).

The identification of how the consumers perceive the product, as well as their consumption experience is the right path to further select and achieve a product with the sensory features sought by the consumer. Furthermore, understanding all the factors that influence gonad organoleptic properties, overall quality and aspects related to the marketability and consumers preferences, will allow for the production and regular supply of added value products and possibly widen exportations to the European and Asian market

and consequently improve the market price and consumer acceptability of this valuable resource.

Table 4 - General summary about sensory studies in sea urchin, published during the last 18 years (2002–2020).

Species	Sensory data collected from	Tools used for data collection	Sensory aspects evaluated	References
<i>S. droebachiensis</i>	2 assessors	Paint cards Intensity/quantitative scale	Colour, texture, firmness and taste	(Christopher M Pearce <i>et al.</i> , 2002; Pearce <i>et al.</i> , 2003, 2004; Christopher M. Pearce <i>et al.</i> , 2002a, 2002b)
	-	CIE Lab	Colour	(Robinson <i>et al.</i> , 2002)
	6 to 9 assessors	CIE Lab Continuous line scale	Smell, granularity, firmness, melting of gonads, taste and aftertaste	(Siikavuopio <i>et al.</i> , 2007)
<i>S. franciscanus</i>	-	CIE Lab Texture Analyzer	Colour, hardness and resilience	(McBride <i>et al.</i> , 2004)
<i>H. erythrogramma</i>	5 assessors	Demerit point grading system Hedonic scale	Colour, texture, firmness, shape, smell and taste	(Senaratna <i>et al.</i> , 2005)
	-	CIE Lab Colour grading system	Colour	(Brewster <i>et al.</i> , 2018)
<i>P. lividus</i>	3 assessors	Intensity/quantitative scale	Colour	(Shpigel <i>et al.</i> , 2005; Shpigel <i>et al.</i> , 2006)
	-	Paint cards Rating scale: excellent, acceptable or unacceptable	Colour was evaluated using	(Symonds <i>et al.</i> , 2007)
	-	Intensity scale Rating scale: excellent, acceptable or unacceptable	Colour	(Vizzini <i>et al.</i> , 2015)
	6 assessors	Intensity/quantitative scale Pantone colour standard chart	Colour, taste, texture and firmness	(Prato <i>et al.</i> , 2018b)

Species	Sensory data collected from	Tools used for data collection	Sensory aspects evaluated	References
	5 assessors	Electronic nose Intensity/quantitative scale	Taste, odour and colour	(Volpe <i>et al.</i> , 2018)
	-	Intensity scale Pantone colour standard chart Rating scale: excellent, acceptable or unacceptable	Colour	(Vizzini <i>et al.</i> , 2019; Vizzini <i>et al.</i> , 2018)
	-	CIE Lab Texture Analyzer	Colour, Firmness and resilience	(Rocha <i>et al.</i> , 2019)
	-	CIE Lab	Colour	(Santos <i>et al.</i> , 2019)
	-	Colour grading system	Colour	(Lourenço <i>et al.</i> , 2020)
	4 assessors	CIE Lab Intensity/quantitative scale	Colour, appearance and taste	(Woods <i>et al.</i> , 2008)
<i>E. chloroticus</i>	8 to 11 experienced assessors	CIE Lab Vocabulary development Unstructured line scales	Appearance, odour, taste, flavour, mouthfeel and aftertaste	(Phillips <i>et al.</i> , 2009; Phillips <i>et al.</i> , 2010c)
	7 to 9 experienced assessors	CIE Lab Unstructured line scales	Appearance, odour, taste, flavour, mouthfeel and aftertaste	(Phillips <i>et al.</i> , 2010a; Phillips <i>et al.</i> , 2010b)
	6 trained assessors	Olfactometer	Odours	(Niimi <i>et al.</i> , 2010)
	-	CIE Lab Colour grading system	Colour	(Brewster <i>et al.</i> , 2018)
	-	CIE Lab Pantone colour standards chart Rating scale: excellent, acceptable or unacceptable	Colour	(Suckling <i>et al.</i> , 2011; Symonds <i>et al.</i> , 2009)
<i>S. intermedium</i>	1 assessor	Intensity/quantitative scale CIE Lab	Colour, texture, firmness and taste	(Azad <i>et al.</i> , 2011)

Species	Sensory data collected from	Tools used for data collection	Sensory aspects evaluated	References
<i>M. nudus</i>	6 assessors	Intensity/quantitative scale CIE Lab Pantone colour standards chart	Colour and flavour	(Zhao <i>et al.</i> , 2015)
	12 assessors	CIE Lab Texture Analyzer Hedonic scale	Colour, flavour, sweetness, umami, richness, saltiness, texture, overall taste and hardness (instrumental)	(Takagi <i>et al.</i> , 2017)
	6 assessors	CIE Lab Texture Analyzer Desirability and intensity were evaluated using five grades	Colour, shape, smell and hardness (instrumental)	(Takagi <i>et al.</i> , 2019)
<i>T. gratilla</i>	1 assessor	Intensity/quantitative scale CIE Lab Rating scale: excellent, acceptable or unacceptable	Colour, texture and firmness	(Cyrus <i>et al.</i> , 2014)
	-	Intensity/quantitative scale	Colour, texture, taste and quality	(Tjendanawangi <i>et al.</i> , 2020)
<i>G. crenularis</i>	6 assessors	Pantone colour standards chart Intensity/quantitative scale	Colour and flavour	(Zhao <i>et al.</i> , 2014)

Echinoculture

Global production

The increasing worldwide demand for sea urchin gonads since the early 70s and its uncontrolled uptake has resulted in the overexploitation of natural stocks, yet still being unable to satisfy the expanding market (Ghisaura *et al.*, 2016). In the particular case of *P. lividus*, a species found in Portugal, Spain, France and Italy, the natural stocks are considered overexploited, where their fishery not sustainable and, consequently, their continuous exploitation will jeopardize natural populations (Sartori *et al.*, 2016b). Hence, both the dramatic depletion of the wild populations driven by the high market demand, and the need to produce gonads with consistent high-quality along the year, generated a worldwide interest in developing sea urchin aquaculture (designated by echinoculture) for the production of high-quality gonads to supply the expanding markets. In this context, echinoculture can be a potential solution to fill the gap between the growing demand and decreasing natural supply (Phillips *et al.*, 2010b). However, the preference for wild seafood over cultured seafood is a well-known premise that does not benefit aquaculture (Calanche *et al.*, 2019). For example, Claret *et al.* (2014) have revealed that consumers consider wild seafood fresher, healthier and more natural than aquaculture products. Overall, there is a need to improve consumer awareness for farmed seafood produced with distinct nutritional strategies, providing reliable information and building consumer trust, reducing perceived risk and improving product valorisation. It is crucial to help consumers perceive sustainability issues, as well as to educate and encourage them to follow a healthy and conscious consumption of natural resources, and consequently conserve our natural stocks.

Furthermore, according to the literature, the production of sea urchin species is still in its initial stages of development, but has gained an extreme interest in the last few years, for both commercial and scientific purposes, and is expected to grow in the next years (Sartori *et al.*, 2016b). The sea urchin life cycle is already controlled under captivity, but echinoculture is still largely dependent on wild-caught sea urchins, since it is faster and less expensive to produce from wild small-sized juveniles in aquaculture (Phillips *et al.*, 2010b). Sea urchins produced by aquaculture (from eggs to market) or simply by roe enhancement (capture of wild adults and increasing the size and quality of the roe) presently account for less than 1% of the sea urchins sold every year worldwide (Stefánsson *et al.*, 2017). Also, echinoculture can promote gonad growth, but cannot yet assure essential quality traits, such

as colour and flavour, compared to their wild counterparts (Lourenço *et al.*, 2019). Different factors can influence both the yield and quality of gonads, such as abiotic factors (e.g. temperature, salinity, photoperiod), nutritional factors (e.g. feed ingredients and chemical composition) and rearing conditions (e.g. stock density, water circulation) (Lourenço *et al.*, 2019).

With increased demand for farmed sea urchin at competitive selling prices, the improvement of aquaculture techniques, as well as the identification of the species nutritional requirements for optimal sea urchin somatic and gonadal growth are the main focus of the several published works (Agatsuma *et al.*, 2005; Agnello, 2017; Barker, 2015; Boudouresque *et al.*, 2020), always aiming to achieve an efficient and economically viable commercial scale production. To this end, several research groups around the world have focused their efforts to achieve a solution regarding the different bottlenecks limiting echinoculture development (Cuesta *et al.*, 2018; Pearce *et al.*, 2004; Phillips *et al.*, 2010b; Prato *et al.*, 2018a; Prato *et al.*, 2018b; Sartori *et al.*, 2016a; Shpigel *et al.*, 2005; Siikavuopio *et al.*, 2007; Vizzini *et al.*, 2015; Woods *et al.*, 2008; Zuo *et al.*, 2017; Zupo *et al.*, 2019).

Ultimately, the objective of sea urchin aquaculture is to increase gonad yields, improve gonad sensory attributes such as colour, taste, and texture and manipulate the reproductive cycle to allow for the marketing of sea urchins throughout the year rather than only during harvesting seasons. In general, intensive sea urchin aquaculture is a challenging endeavour, as the taste and appearance of urchin gonads from cultured urchins has so far lacked consistency in meeting the demands of this high-end market (Lourenço *et al.*, 2019).

Nutritional composition of the diets and roe quality

The quality and marketability of gonads is mainly determined by its size, taste, colour and consistency, which are influenced by the protein, lipid, carbohydrate and carotenoid content of diets (Cuesta-Gomez *et al.*, 2017; Taylor *et al.*, 2017). Natural diets in the form of macroalgae and kelps are a primary source of sea urchin food (Robinson, 2004). However, to maximize the economic viability of echinoculture production, sea urchins should be preferentially fed on formulated diets able to meet the species requirements (Suckling *et al.*, 2011). In this context, it has become clear that the success, sustainability and economic viability of commercial sea urchin aquaculture strongly depend on the development of suitable, cost effective and nutritionally balanced diets capable of producing high-quality gonads for the caviar-like industry (Eddy *et al.*, 2012; Christopher M Pearce *et al.*, 2002; Woods *et al.*, 2008).

Studies using semi-moist practical and semi-purified feeds demonstrated that sea urchin growth could be sustained by formulated feeds (Watts *et al.*, 2020). Other studies have compared commercially available and/or laboratory-prepared formulated feeds of natural macroalgae, showing that the use of macroalgae is neither commercially profitable nor sustainable (Prato *et al.*, 2018b; Schlosser *et al.*, 2005; Shpigel *et al.*, 2005). Nevertheless, the specific nutritional requirements of sea urchins for macronutrients, including protein, carbohydrates and lipids, and micronutrients, such as vitamins and minerals, and their impact in gonad growth and quality remain to be ascertained (Lourenço *et al.*, 2019). The primary focus of recent nutritional studies with sea urchins has been the evaluation of protein, lipid and carbohydrate requirements (Castell *et al.*, 2004; Gibbs *et al.*, 2013; González-Durán *et al.*, 2008; Hammer *et al.*, 2006a; Hammer *et al.*, 2006b; Hammer *et al.*, 2012; Kennedy *et al.*, 2007; Christopher M. Pearce *et al.*, 2002b; Senaratna *et al.*, 2005; Taylor *et al.*, 2017), nevertheless, the biological diversity of sea urchins suggests that nutrient requirements may potentially be species-specific. Thus, the nutritional requisites of the target species must be explored in order to achieve the greatest somatic and gonadal growth.

To date, only a few studies have evaluated the feasibility of increasing gonad size of *P. lividus* through the use of artificial diets with different protein and lipid levels (Carboni *et al.*, 2015; Prato *et al.*, 2018b; Zupo *et al.*, 2019), albeit without clear identification of this species nutritional requirement for maximal growth and gonad yield. In Carboni *et al.* (2015), a 22% dietary protein content improved somatic growth in relation to diets with 17% and 18% of protein and a 6.8% dietary lipid favoured gonadal size. Prato *et al.* (2018a) showed that a diet with 27% protein and 4.4% lipids promoted a higher gonad growth when compared with diets with 18/22% and 4.6/5.5% of protein and lipids, respectively. According to Zupo *et al.* (2019), the protein content of diets for *P. lividus* is strongly correlated with the GSI and should be incorporated up to 40-47%, keeping low lipid levels (<9%), to assure the highest GSI and theca diameter. Most studies into sea urchins showed that dry formulated diets with a protein content varying between 15 and 50% DM were able to simultaneously promote somatic and gonad growth (Lourenço *et al.*, 2018). Nonetheless, artificial diets that showed an approximate composition identical to fresh brown kelp, with protein level about 20-30%, carbohydrates level around 45-50% and with lipids level between 1 and 7%, improved growth performances for *S. droebachiensis* (Eddy *et al.*, 2012), *Pseudocentrotus depressus* (Akiyama *et al.*, 2001), and *L. variegatus* (Hammer *et al.*, 2004; Heflin *et al.*, 2012). Moreover, recently, Lourenço *et al.* (2020) evaluated six practical extruded diets with increasing protein levels (20-45% of dry matter, DM) in *P. lividus* adult specimens, observing that 30% DM dietary protein with 7% lipids concomitantly induced high nutrient utilisation and promoted highest GSI. Moreover, while dietary protein and lipid level can be

crucial for normal biological and metabolic processes and functioning, the quality and source of these macronutrients should also be considered (Hammer *et al.*, 2012). In fact, it is known that the growth rate is intimately related to the protein sources and the amino acid profile provided in the diet (Woods *et al.*, 2008). Phillips *et al.* (2009) and Woods *et al.* (2008) have shown that the amino acid profile in the diet has a high impact on growth, but also on the final product organoleptic characteristics. Indeed, high levels of leucine and valine in the diet were shown responsible for increased bitterness taste in gonads (Woods *et al.*, 2008), while sea urchin fed diets with high levels glutamate and glycine had sweet-tasting gonads and more intense umami taste (Phillips *et al.*, 2009; Woods *et al.*, 2008). Likewise, the quality of dietary lipids is crucial for the final sea urchin gonad quality (Givens, 2009). Essential fatty acids (EFA), for instance the linoleic (n-6) and linolenic (n-3) families of polyunsaturated fatty acids (PUFAs), must be supplied in the diet to improve sea urchin growth and quality; however, long chain-polyunsaturated fatty acids (LC-PUFA), such as 20:4n-6 (arachidonic acid, ARA) and 20:5n-3 (eicosapentaenoic acid, EPA) can be synthesized by sea urchins from their precursors, the linoleic (18:2n-6, LA) and linolenic (18:3n-3, ALA) FA, respectively (Carboni *et al.*, 2015; Castell *et al.*, 2004; González-Durán *et al.*, 2008). Tissue FA composition will reflect the dietary FA composition (Castell *et al.*, 2004; González-Durán *et al.*, 2008; Liyana-Pathirana *et al.*, 2002) and may hence compromise the nutritional value of sea urchin gonads.

Several studies had evidenced the impact of nutritional and feeding strategies on the final quality of farmed animal food products including those from aquaculture (Calanche *et al.*, 2019; Cuesta-Gomez *et al.*, 2017; Taylor *et al.*, 2017). In fact, manufactured diets have been shown to affect sensory quality of gonads (Phillips *et al.*, 2009; Phillips *et al.*, 2010b; Siikavuopio *et al.*, 2007; Volpe *et al.*, 2018; Zupo *et al.*, 2019). The texture and flavour were shown to be dependent on protein intake (Christopher M. Pearce *et al.*, 2002b), whilst colour and *umami* taste were related to the gonadal carotenoid profile (Shpigel *et al.*, 2005). An integrated approach is needed to close the gap between consumers' demand and aquaculture products, since the sensory features sought by the consumer can be met by the inclusion of specific ingredients or additives in aquafeeds (Calanche *et al.*, 2019). Future research efforts in aquaculture should take into account the importance of consumer behaviour and preferences regarding a determined product. In fact, to develop a successful industry for this gourmet product and target high market segments, it is important to meet the sensory quality standards, as well as to develop processing and preserving technologies to fulfil the consumer demands relatively to quality, storage time and preservation of their natural organoleptic features. The value of sea urchin gonads is associated with their excellence, which is related with their freshness. In turn, freshness is correlated with sensory traits such as flavour, odour and texture, but the appearance, mainly the colour,

stands out as the determinant factor that defines the marketability and price of sea urchin gonads (Robinson *et al.*, 2002).



Figure 10 – Recirculation aquaculture system (CIIMAR, Porto, Portugal).

Nowadays, it is clear that formulated feeds for sea urchin species are able to increase gonads size faster than when compared with natural feeds, in the same time period, but cannot still assure important quality traits, such as colour, texture and flavour, in relation to their wild counterparts (Lourenço *et al.*, 2019). For instance, gonads often present a pale white or yellow (cream) colour rather than the desired yellow/orange colour (Eddy *et al.*, 2012; Robinson *et al.*, 2002; Shpigel *et al.*, 2005). The future sustainable development of echinoculture depends on the ability of formulating diets able to produce sea urchin gonads with acceptable marketable colour (Robinson *et al.*, 2002). The bottleneck of improving colour can be thoroughly addressed by using carotenoids in formulated feeds. The main pigment accumulated in *P. lividus* gonads is echinenone (Rocha *et al.*, 2019), obtained from the metabolic conversion of dietary β -carotene via β -isocryptoxanthin (Kelly *et al.*, 2013; Symonds *et al.*, 2007). The concentration of echinenone in gonads was found to be dependent on the dietary β -carotene concentration, uptake and bioconversion (Shpigel *et al.*, 2006) and is strongly correlated with gonads colour intensity (Shpigel *et al.*, 2005). Studies on sea urchin fed diets supplemented with rich sources of β -carotene have succeeded in improving the desirability of gonad colouration (McBride *et al.*, 2004; Pearce *et al.*, 2004; Plank *et al.*, 2002; Robinson *et al.*, 2002; Suckling *et al.*, 2020b; Woods *et al.*, 2008), nevertheless species- and sex-specific pathways for carotenoid metabolism and gene expression have been suggested (Robinson *et al.*, 2002; Suckling *et al.*, 2020b).

In *P. lividus*, satisfactory improvement of gonad colour was obtained through dietary supplementation of pigments, using natural or synthetic sources of carotenoids, in particular β -carotene (Table 5). Several authors showed that the use of fresh macroalgae or vegetal leaves which are rich β -carotene sources are able to induce a marketable gonad colour in *P. lividus*; however, the use of fresh macroalgae to promote better gonad colour is not economically sustainable (Prato *et al.*, 2018b; Shpigel *et al.*, 2005; Vizzini *et al.*, 2015; 2019;

2018). Shpigel *et al.* (2005) showed that the most intense colouration of gonads was observed in sea urchins fed with *Ulva lactuca* and *Gracilaria conferta* for 12 weeks. Shpigel *et al.* (2006) demonstrated that natural β -carotene isomers (66 mg kg^{-1} of diet DM) induced better gonad colouration than synthetic forms (25 mg kg^{-1} DM), but dietary pigment concentrations were not comparable. Prato *et al.* (2018b) demonstrated that *P. lividus* developed a desirable and acceptable gonad colour when fed a mixed diet composed of 50% of a pelletized diet and 50% of fresh *Ulva sp.* (72% and 83% of the individuals in both trials showed a desirable colour based on market standards). Moreover, Vizzini and colleagues (2015; 2019; 2018) found that the use of unprocessed agricultural by-products (fresh vegetable leaves) to feed *P. lividus* feed was able to successfully improve sea urchin gonad colour. Contrarily, Shpigel *et al.* (2006) fed *P. lividus* with an agar-based diet prepared with *Dunaliella bardawil* powder, which is a natural source of β -carotene, and reported a mango-orange coloured gonad in over 90% of the treatment group. It was also stated that urchins fed with this diet showed greater concentrations of gonadal β -echinenone in comparison to sea urchins fed with other carotenoid pigments including all-trans β -carotene, zeaxanthin, lutein and cryptoxanthin.

It is clear that the recent published works aimed for the use of fresh or agar-based diets to improve *P. lividus* colour, which increases the transportation and storage costs, as well as the labour costs, both negatively impacting the efficiency and economic viability of echinoculture. Moreover, in most nutritional studies sea urchin gonadal colour and carotenoid profile was evaluated without sexual differentiation (Pearce *et al.*, 2004; Robinson *et al.*, 2002; Shpigel *et al.*, 2006; Symonds *et al.*, 2007). As aforementioned, it is evident that the development of suitable, cost effective and nutritionally balanced diets, using formulate extruded diets, is the right pathway to maximize the economic viability of echinoculture production and to produce high-quality gonads. Overall, carotenoid pigments added to formulated extruded feeds have so far failed to consistently produce *P. lividus* gonads of acceptable colour, suggesting that the concentration or source of pigments and/or the feed processing methods were still not optimized to produce wild comparable colour characteristics. However, colour is successfully manipulated by the use of dietary carotenoids, hence, in this scope, it is necessary to determine carotenoids requirements and identify the pathways leading to its absorption, metabolic conversion and deposition in sea urchin gonads.

Table 5 - General summary about *P. lividus* gonads colour enhancement studies.

Trial duration	Water Temp.	Diet type	β -carotene source	Diet β -carotene concentration (mg kg ⁻¹)	Effects	Reference
12 w	24 °C	Mix of the pellet diet + Macroalgae	Natural (<i>Ulva sp.</i> , <i>Gracilaria sp.</i>)	<i>Ulva sp.</i> : 3.95 <i>Gracilaria sp.</i> : 2.88	78% - good color, including 28% of the ideal mango orange color.	(Shpigel <i>et al.</i> , 2005)
4+8 w	20 °C	Agar based	Natural (<i>D. bardawil</i> powder) and synthetic	Natural: 66 Synthetic: 25	Natural diet - 90% mango-orange color	(Shpigel <i>et al.</i> , 2006)
3 m	16 °C	Fresh vegetables and macroalgae	Natural (Beet <i>Beta vulgaris</i> , cauliflower <i>Brassica oleracea</i> and the lettuce <i>Lactuca sativa</i> , and the marine macroalga <i>Ulva lactuca</i>)	-	<i>Lactuca sativa</i> - 80% mango-orange color	(Vizzini <i>et al.</i> , 2015)
1 m	28 °C	Mix of the pellet diet + Macroalgae	Natural (<i>Ulva sp.</i>)	-	61% red-orange to yellow-mango color	(Prato <i>et al.</i> , 2018b)
2 m	24 °C	Mix of the pellet diet + Macroalgae	Natural (<i>Ulva sp.</i>)	-	83% red-orange to mango color	
24 w	-	Fresh vegetables	Natural (lettuce <i>Lactuca sativa</i>)	-	90% - marketable gonad colour	(Vizzini <i>et al.</i> , 2018)
18 w	-	Mix of the pellet diet + Fresh vegetables	Natural (lettuce <i>Lactuca sativa</i>)	-	100% - sum of excellent and acceptable color	(Vizzini <i>et al.</i> , 2019)

Objectives of this thesis

P. lividus gonads are considered a prized and gourmet seafood delicacy, due to their unique flavour, making their commercialization highly profitable. This thesis aims to identify the organoleptic properties of sea urchin gonads that determine their marketability, consumer acceptance and purchase decisions, which will allow for the valorisation of this caviar-like product. Furthermore, this species has been overexploited and overfished, leading to the collapse of wild stocks; however, the demand for sea urchin products keeps increasing, boosting the search for alternative forms to supply markets. Aquaculture may accelerate the production of market-sized sea urchins, but market value and consumer acceptability of farmed urchins has to be assured by a high gonad quality. Therefore, a thorough characterization of the quality of wild harvest *P. lividus*, including its sensory profile, will firstly help define the consumers' quality demands aiming the selection of premium gonads. In a second phase, the ability of formulated diets to inducing high-quality gonads will be evaluated.

The main aim of this thesis will be achieved through the following specific objectives:

- 1) Characterization of the seasonal and sex variation of gonad quality (yield, nutritional value, colour and texture) and determination of the best harvest period (Chapter II);
- 2) Exploration of the consumers' and cuisine Chefs' perception, acceptance and consumption habits of this product, as well as the consumption related motives and barriers (Chapter III and IV);
- 3) Identification of the sensory profile, volatile profile and freshness criteria of sea urchin gonads during the best harvest period (Chapter V, VI and VII);
- 4) Modulation of sea urchin gonad growth and quality through dietary formulation (Chapter VIII and IX).

This work was constructed on a paper-based structure, six of which have been published in peer-reviewed indexed international journals (Chapter II, IV, V, VI, VIII, IX), and the remaining two have been submitted or are under submission (Chapter III, VII). Figure 11 summarises the methodological approach and identifies the major achievements of each chapter. In the second Chapter the harvest period for obtaining the best organoleptic gonadal features was identified. The following two chapters (III and IV) allowed for the understanding of the consumer's perception, acceptance and habits about this product category, in which a qualitative and quantitative methodology was used to study consumer behaviour. Chapters V to VII considered the sea urchin sensory properties preferred by the

consumers, leading to a final sensory ballot. Finally, the nutritional modulation of *P. lividus* growth and gonad quality in aquaculture systems was addressed in the last two Chapters (VIII and IX).

	Aim		Methodology	Scales	Panel	Data analysis	Originality
Chapter II	Characterization of the of the best quality harvest period for sensorial studies		Seasonal and sex variation of gonad quality	Nutritional value, colour (carotenoid deposition and colour measurements) and texture (instrumental firmness and resilience)	-	Descriptive analysis ANOVA	First analysis of seasonal and sex quality variation of <i>P. lividus</i> in Portugal
Chapter III	Product category and consumers relevant information		Web-based questionnaire Word Association	Attitudes, behaviours, product conceptualisation: rating scales, multiple-choice and free word association	309 + 228 participants in questionnaire	Descriptive analysis Correspondence analysis Content analysis Factorial analysis Chi-square	First consumer test on <i>P. lividus</i> and relevant information for sea urchins producers
Chapter IV	Chef's perception, acceptance and consumption habits of this product category		Interviews Napping®	Attitudes, behaviours, product conceptualisation with open-ended questions and ranking of similarities/dissimilarities	12 cuisine chefs	Descriptive analysis Inductive analysis with Nvivo Multiple Factor Analysis	Relevant information about sea urchin consumption habits and collection of the first sensorial attributes of <i>P. lividus</i>
Chapter V	Identification of the sensory features and odour active volatile compounds		Volatile analysis Napping®	Odour active volatile compounds quantification and ranking of similarities/dissimilarities	4 trained panellists	Descriptive analysis Non-parametric analysis Principal component analysis Multiple Factor Analysis	Jointly analysis of odour active volatile compounds and sensorial attributes (block design)
Chapter VI-VII	Identification of the sensory profile of <i>P. lividus</i>		Hedonic test CATA RATA	Liking and FACT (9-point hedonic scale) Sensory profile consumers (CATA and RATA Ballot)	60 consumers for CATA and 80 for RATA	Principal component analysis Correspondence Analysis Multiple Factor Analysis	Definition of Premium sea urchin gonads, based on the maximisation of liking and sensory profiles
Chapter VIII	Species nutritional requirements		Different dietary protein: lipid ratios	Growth and gonad quality, under a recirculating aquaculture system (RAS)	-	Descriptive analysis ANOVA	Best protein:lipid ratio to maximize <i>P. lividus</i> growth
Chapter IX	Gonads' colour and texture enhancement		≠ β -carotene sources and concentrations Hedonic test CATA	Growth and gonad quality Liking and FACT (9-point hedonic scale) Sensory profile consumers (CATA Ballot)	44 consumers for CATA	Descriptive analysis ANOVA Principal component analysis Correspondence Analysis Multiple Factor Analysis	Enhancement of <i>P. lividus</i> colour through diet incorporation of synthetic and natural β -carotene with different concentrations

Figure 11 – Overview of methodological approaches.

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Chapter II.

The effect of sex, season and gametogenic cycle on gonad yield, biochemical composition and quality traits of *Paracentrotus lividus* along the North Atlantic coast of Portugal

by

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The effect of sex, season and gametogenic cycle on gonad yield, biochemical composition and quality traits of *Paracentrotus lividus* along the North Atlantic coast of Portugal

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Sea urchin population harvest in the North Atlantic coast of Portugal was characterized in terms of gonad yield, nutritional composition and important market-related traits, over one reproductive cycle (March 2016 to March 2017). Most of the quality attributes showed a seasonal variation strongly dependent on sea urchin sex. Maximum gonad yield (18%) was observed in March 2017. A single spawning event occurred between May and July. Gonads are rich sources of protein (12–18% WW) with low fat content ($\leq 6\%$ WW), that increase during the gametogenic stages of recovery and growing (November–December). Polyunsaturated fatty acids were the dominant class in both sexes (4.2–14.7 mg.g⁻¹ WW), being preferentially accumulated in females. Total gonads carotenoid varied seasonally, with the highest level being observed in males during spawning season. Echinonone was the main pigment present in gonads, showing highest concentrations in males during spawning and gonad recovering. During the growing and early maturation period gonads were more reddish, yellowish and brighter, as well as more firm, irrespectively of the sex. Based on all seasonal changes affecting gonad yield and quality, the period between November and February seems the most suitable to harvest high quality gonads in the Atlantic coast of Portugal.

Sea urchin gonads (roe) are a prized and gourmet seafood delicacy, due to its unique flavour¹. The global volume of sea urchin captures in 2016 reached 28575 tonnes, whilst farming has further contributed with 10057 tonnes². Japan is an important driver of sea urchin market and represents 75% of total imports (11116 tonnes) of major sea urchin product forms (live, fresh or chilled, frozen and dried, salted, or brined) estimated in 14739 tonnes in 2016². Urchin roe can reach a high commercial value as demand often exceeds the market supply. Recently, excessive exploitation and destructive harvesting methods have caused the depletion of wild stocks³.

The sea urchin *Paracentrotus lividus* has being intensively harvested in most of its geographical range, throughout the Mediterranean Sea and the North eastern Atlantic, from Scotland (northern) to Canary Islands (southern)⁴. In Europe, the natural stocks found in France, Italy and Spain are considered overexploited, even so intensive harvesting is still occurring: in the North regions of Spain landings reached 520 tonnes in 2014⁵. In

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Portugal, *P. lividus* is the dominant echinoid species in the rocky shores⁶ and landings are mainly concentrated in the North Atlantic coast (Viana do Castelo) where harvests reached 28 tonnes in 2015⁷. Commercial harvesting has increased over the last decade, which might reflect the demand to supply markets of nearby regions, such as North Spain⁸.

The main market for sea urchin gonads is Japan where the price for fresh gonads is considerable higher than for frozen or dried/salted; fresh roe can reach 40–100€/kg⁹. A premium gonad is characterized by its size combined with specific sensory traits sought by the consumers (taste, colour, texture and freshness). Desirable gonads present a bright yellowish-orange colour^{1,10}, *umami* taste¹¹, firm texture and high freshness¹². However, gonads size and sensory traits largely depend on several factors, namely: (i) sea urchin species, (ii) gonads maturation stage (reproductive cycle), (iii) environmental cues (water temperature, photoperiod) and (iv) animal nutritional status (food availability and nutritional value).

P. lividus reproduction follows a predictable seasonal pattern characterized by six gametogenic stages¹³. The annual reproductive cycle of this species can present one or two spawning seasons, depending on the habitat, latitudinal location and environmental conditions to which populations are subjected to⁴. Some studies have investigated the role of exogenous factors, such as temperature, photoperiod and primary production in sea urchin gametogenesis and spawning. However, it has been suggested that the environmental cues that trigger spawning may differ from those that stimulate gonadal growth and development¹⁴. Byrne¹³ observed that temperatures lower than 13 °C may inhibit spawning of *P. lividus* populations in Ireland. Shpigel, *et al.*¹⁵ reported that short days and increasing temperatures (18–22 °C) enhanced gonad development and gametogenesis, while Spirlet, *et al.*¹⁶, Spirlet, *et al.*¹⁷ suggested that the spawning event may be triggered by shorter day-length. Latitudinal patterns can also be found in echinoids reproductive cycles, the gonad index of *P. lividus* showed a clear geographical trend with the Atlantic populations reaching higher GSI than the Mediterranean populations¹⁸.

During the reproductive cycle, gonads from both females and males increase in size (yield) over two distinct periods: before gametogenesis, when nutrient reserves are stored in the nutritive phagocytes, and during gametogenesis, when increased number and size of gametes is observed¹⁴. Gonads are preferentially consumed before the beginning of gametogenesis, when their nutritive phagocytes are in maximum volume and rich in nutrients, attributes that contribute to a sweeter flavour and firmer texture. Once gametogenesis begins, the taste and texture of gonads deteriorates, mainly due to the utilization of yolk protein into synthesis of new compounds¹⁹.

Gonad colour is a pivotal factor determining its quality and marketability. The desirable yellow-orange colour is determined by carotenoids deposited in the gonads, mainly the accumulation of echinenone that is its dominant pigment¹⁰. Gonad echinenone content is limited by the availability, uptake and bioconversion of β -carotene from dietary sources, in particular from algae^{20,21}. The consumption of different algae with different carotenoid profiles influences the concentration of pigments present in sea urchins²². Also, diet type (natural or formulated) can drastically influence not only the biochemical composition of gonads but also their organoleptic properties, such as flavour and texture^{11,23}.

To date, apart from a few studies focusing environmental²⁴, ecological^{6,25} and conservational⁸ issues of Portuguese populations of *P. lividus*, little is known about important market-related traits of this high valued species and its potential to commercialization. This study aims to characterize the seasonal variations of *P. lividus* gonads in terms of maximum yield, nutritional value, colour and texture over one reproductive cycle. The possible relationship between sex, season and gametogenic cycle and selected market-related traits was discussed, for the first time in a wild population, and the best period to harvest high quality gonads in the North Atlantic coast of Portugal was determined.

Results

Environmental condition. Detailed data on sea water temperature was obtained through daily records of an oceanic buoy located close to our study area. The annual variation of water temperature was minor and ranged between 11.8–16.4 °C (Fig. 1A). Temperature steadily rose from the lowest value recorded in March 2016 (11.8 °C) to its maximum (16.4 °C) observed during June–July 2016. Then, it slowly decreased until reaching 13 °C in February 2017. Records of day length indicated a seasonal variation, with a gradual increase of light hours from winter to summer season, followed by a decrease afterwards. Similar day lengths were registered in march-16 (12 h:12 m) and march-17 (11 h:47 m)²⁶.

Gonad index and gametogenic stages. Total body weight (105 ± 17.1 g) and test diameter (6.4 ± 0.4 cm) of all sampled animals were not significantly different over time ($P > 0.05$). This evidences a size-class consistency in the evaluated sea urchin groups, allowing a direct comparison of gonadal quality traits over the 13-month period. Gonad weight decreased to almost half of its value from March-16 to June (10.3 ± 2.7 g to 5.5 ± 2.3 g, respectively), then slowly increased until March-17 (15 ± 3.3 g), consequently affecting GSI values over the year. The annual reproductive cycle of *P. lividus* population from Praia Norte is presented in Fig. 1A. The GSI varied significantly over the 13-month period ($P < 0.05$), but was similar between sexes ($P \geq 0.05$, Supplementary Table S1). GSI decreased from March 2016 (9%) to a minimum level of 5% in May–June; then values steadily increase until March of the following year, when they peaked (18% for females and 15.6% for males). Changes on the GSI reflected gonads maturation stage (Fig. 1B). During March–April 2016, females and 50% of males presented gonads at stage III (pre-mature), indicating the beginning of gametogenesis. From June to July 2016 spawning occurred; most of the sea urchins presented empty gonads at the partly spawned (V) or spent (VI) stages. The recovery (I) and growing (II) stages of gonads were more noticeable from November to December 2016, in both sexes. During this period, it was possible to identify a large amount of nutritive phagocytes as well as the presence of primary gametes in the gonads. The onset of gonads growth (November) was closely followed by an increase of gonad size and volume (GSI), that peaked in March 2017, when maturation was undergoing and mature gametes were accumulated in gonads (stage III and IV).

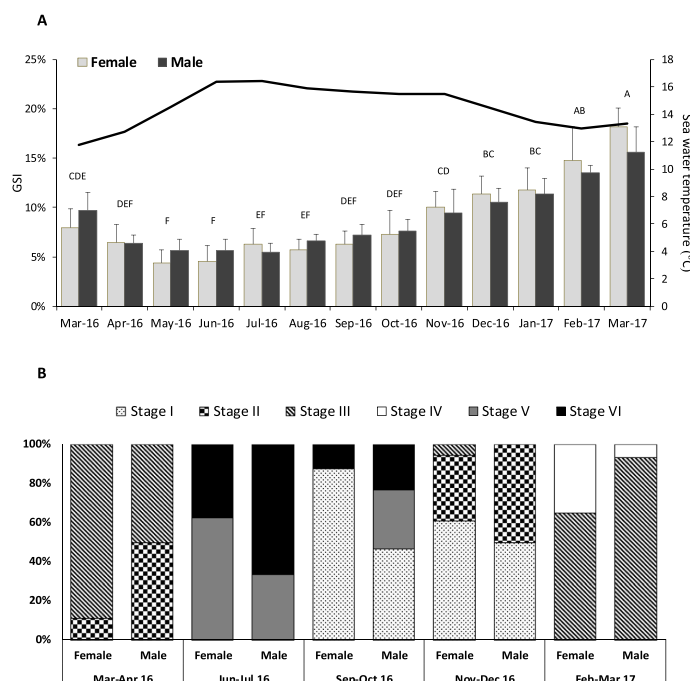


Figure 1. Annual reproductive cycle of females and males *P. lividus* in Praia Norte (Portugal); (A) variations in the gonad somatic index (GSI) over a 13-month period; (B) Gametogenic stages of ovary and testis through histological examination. Values are mean $\pm$ standard deviation; per month the 18 individuals were pooled by sex: N = 3 pools of female gonads and N = 3 pools of male gonads were established. Superscripts in capital letters (^{ABC}) indicate significant differences between months ($P < 0.05$).

Biochemical composition. The nutritional value of sea urchin gonads is presented in Fig. 2. All analysed parameters were significantly affected by sex and time ($P \geq 0.05$, Supplementary Table S1). Protein was the major component of gonads for both male and female *P. lividus* (Fig. 2C). Males had significantly higher protein content (14–18% WW) than females (12–15% WW). Both sexes showed highest protein content in November, during gonad growing (stage II) ($P < 0.05$). Lipid content of gonads (Fig. 2D) showed a significant interaction between sex and time ($P < 0.05$, Supplementary Table S1). Likewise proteins, the highest lipid content (6%) was observed in November, during gonad growing (stage II). Minimum levels (2–3% WW) were achieved at the beginning of the spawning season, in May–June, when gonads were mostly spent (stage VI). Energy content in gonads (Fig. 2E) followed a similar pattern of total lipids, with both main factors interacting significantly ($P \geq 0.05$). Again, gonad energy was at minimum levels during spawning (3–4 kJ/g WW), and achieved maximum content (7 kJ/g WW) in November, during growing. Both sexes showed similar moisture content, ranging from 66 to 81% over the year (Fig. 2A) however females had significantly higher moisture during October and November than males. Moisture was highly correlated with the lipid content ($r = 0.97$, Table 1). Ash content was higher in male (2.7–3.4% WW) than in female (1.6–2.4% WW) gonads ($P < 0.05$) and peaked in June during spawning (Fig. 2B).

The fatty acid (FA) composition of female and male gonads of *P. lividus* is shown in Table 2. In both sexes, the polyunsaturated fatty acids (PUFA) were the dominant FA class (4.2–14.7 mg.g⁻¹ WW), followed by saturated fatty acids (SFA) (2.3–11.0 mg.g⁻¹ WW) and monounsaturated fatty acids (MUFA) (2.2–7.7 mg.g⁻¹ WW). The most represented PUFA was 20:5n-3 (EPA), varying between 1.5–4.8 mg.g⁻¹ WW followed by 20:4n-6 (ARA; 1.2–3.7 mg.g⁻¹ WW), 18:4n-3 (0.1–2.5 mg.g⁻¹ WW) and 18:3n-3 (ALA, 0.08–0.85 mg.g⁻¹ WW). Reduced amounts of 22:6n-3 (DHA; 0.02–0.11 mg.g⁻¹ WW) were found in gonads of both sexes. Females had significantly higher levels of PUFA than males, particularly from the n-3 family ($P < 0.05$), however both DHA and ARA levels were similar between sexes ($P \geq 0.05$). As a reference for the consumption of seafood as part of a healthy diet, the levels of EPA + DHA in fresh gonads varied between 1.5–4.9 mg/g, being highest in November for both sexes. Most PUFA showed a seasonal variation in their content, being generally higher in November during gonad growth (stage II). Among SFA, the main compounds were 16:0 (1.3–6.2 mg.g⁻¹ WW), 14:0 (0.5–3.3 mg.g⁻¹ WW) and 18:0 (0.3–0.7 mg.g⁻¹ WW). Total SFA were significantly higher in females than males, and in November compared to the other months ($P < 0.05$). MUFA were mainly represented by 20:1n-9 (0.6–1.8 mg.g⁻¹ WW), 20:1n-11 (0.5–1.5 mg.g⁻¹ WW) and 18:1n-7 (0.3–1.2 mg.g⁻¹ WW), which were in higher amount in female gonads and preferentially concentrated in November (growing stage of gonads) ($P < 0.05$). A positive correlation was found between total lipids and EPA and DHA levels ($r = 0.67$ and 0.45 , respectively). EPA and ARA were negatively correlated to L* ($r = -0.58$ and -0.48) and positively correlated to β -carotene ($r = 0.40$, Table 1).

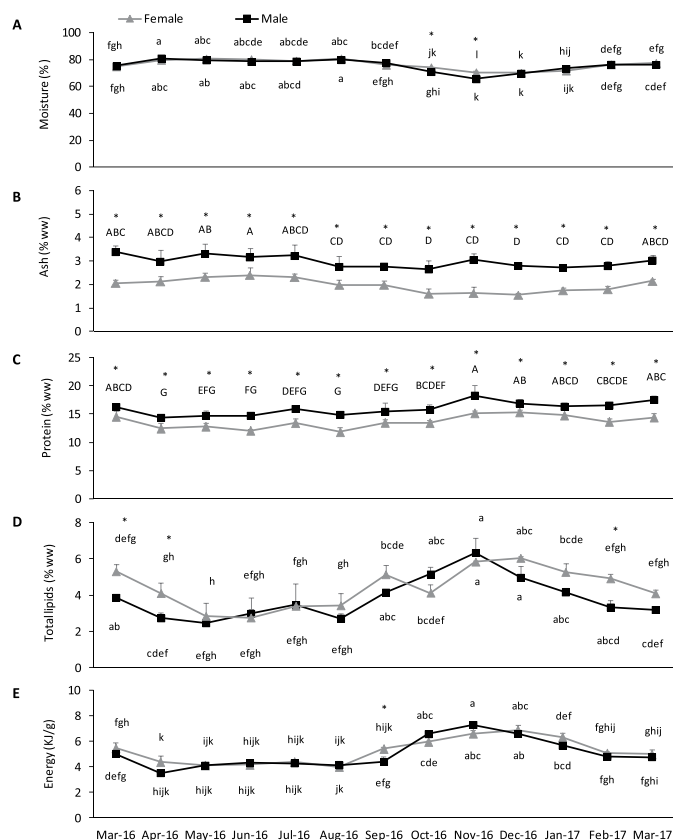


Figure 2. Changes in (A) moisture (%), (B) ash, (C) protein, (D) total lipids (% WW) and (E) energy (KJ/g WW) contents in gonads (ovary and testis) of *P. lividus* collected at Praia Norte (Portugal) over a 13-month period. Values are mean $\pm$ standard deviation; per month the 18 individuals were pooled by sex: N = 3 pools of female gonads and N = 3 pools of male gonads were established. Superscripts in capital letters (^{ABC}) indicate differences between months, asterisk (*) indicate differences between sexes and superscripts in lowercase letters indicate significant differences between sex X month (^{abc}) ($P < 0.05$).

Gonad colour. The seasonal variation of colour parameters lightness (L^*), redness (a^*) and yellowness (b^*) in *P. lividus* gonads is presented in Fig. 3. A significant interaction between sex and month factors was found for L^* and a^* parameters, while b^* showed a clear effect of both factors ($P < 0.05$, Supplementary Table S1). Overall, L^* values of gonads varied from 33 in September to 54 in February, and only in this month males showed brighter gonads than females ($P < 0.05$, Fig. 3A). Over the sampled period, females had more reddish and yellowish gonads than males, with a^* values ranging between 15–30 and b^* from 19–44. The highest value of a^* and b^* was achieved in January, yet levels were also high during gonad maturation period. Lower levels were found during spawning (June–July) for both parameters (Fig. 3B,C). A positive correlation was found for L^* and protein content ($r = 0.41$) as well as for lipid content of gonads and a^* and b^* ($r = 0.75$ and 0.72). Also, b^* and a^* showed to be correlated in gonad ($r = 0.77$; Table 1).

Total carotenoid content in gonads of females and males of *P. lividus* is presented in Fig. 4. There was a significant interaction between the effects of sex and month on carotenoid content yet, no differences were found for each factor separately (Supplementary Table S1). Males' carotenoid content was significantly higher in June–July (159 to $174 \mu\text{g.g}^{-1}$ WW) during the spawning period when compared to February–March 2017 (61 to $72 \mu\text{g.g}^{-1}$ WW), months prior to spawning characterized by high GSI values. By contrast, levels of total carotenoids did not vary significantly in females (61 to $103 \mu\text{g.g}^{-1}$ WW) over the 13-month period. A negative correlation ($r = -0.45$, Table 1) was found between total carotenoid content and GSI, meaning that higher levels of carotenoids occur during and after spawning.

Five carotenoid pigments were identified and quantified in the gonads of both sexes: α -, β -carotene, echinenone, lutein and β -cryptoxanthin. Levels of individual pigments are shown in Table 3. The gonads of both sexes broadly had similar carotenoid profiles (Supplementary Fig. S1), and echinenone was the dominant carotenoid in gonads, irrespectively of sex. Higher echinenone levels were observed in males during September and November

		1	2	3	4	5	6	7	8	9	10	11	12
Yield (%) and Biochemical composition (%WW)	(1)	GSI											
	(2)	Moisture											
	(3)	Protein		−0.69**									
	(4)	Lipids		−0.97**									
Fatty acids (mg.g ^{−1} DM)	(5)	ARA							−0.58**				
	(6)	EPA				0.67**			−0.48*	0.61*			
	(7)	DHA				0.45*							
Colour parameter (CIE)	(8)	L*	0.63**		0.41*								
	(9)	a*				0.75**							
	(10)	b*	0.66**	−0.54**		0.72**				0.77**			
Carotenoid analysis (μg.g ^{−1} WW)	(11)	Total carotenoid	−0.45*						−0.52*		−0.39*		
	(12)	β-carotene				0.40*	0.40**		−0.36*			0.71**	
	(13)	Echinenone			0.52**							0.77**	0.72**

Table 1. Values of Pearson's correlation coefficients between yield, biochemical composition and colour determination (carotenoids and CIE parameters) of *P. lividus* gonads determined for a 5-month period. N = 30, for the five months analysed: April, June, September and November 2016 and February 2017. ** $P < 0.01$ (two-tailed) and * $P < 0.05$ (two-tailed); bold values indicate negative correlations.

(approx. $171 \mu\text{g.g}^{-1}$ WW, $P < 0.05$) compared to females. β-cryptoxanthin was the second highest pigment in gonads; it had similar levels in both sexes ($P \geq 0.05$) and evidenced a seasonal variation ($P < 0.05$, Supplementary Table S1). A slight but significant decrease of β-cryptoxanthin levels occurred in February 2017, from 20.4 to $18.9 \mu\text{g.g}^{-1}$ WW. Levels of β-carotene were consistently higher than those of α-carotene and did not differ significantly between sex. Between February–April 2016 (before spawning) levels of β-carotene decreased to $10.88 \mu\text{g.g}^{-1}$ WW, while in September (after spawning) levels peaked up to $26.58 \mu\text{g.g}^{-1}$ WW. The less abundant pigment in gonads was α-carotene (0.3 – $2.2 \mu\text{g.g}^{-1}$ WW), levels were significantly higher in male than in female gonads and peaked in June (during spawning). Females showed significantly higher amount of lutein pigment (0.5 – $8.7 \mu\text{g.g}^{-1}$ WW) compared to males (0.9 – $4.0 \mu\text{g.g}^{-1}$ WW), and the interaction between sex and month was found significant ($P < 0.05$). Lutein levels in females were higher from April to September, before and after spawning occurred.

Echinenone pigment was strongly correlated with α- and β-carotene as well as with total carotenoid content ($r = 0.60$, 0.64 and 0.77 , respectively, Table 1). Also, a positive correlation was found between this pigment and protein content of gonads ($r = 0.72$). Although carotenoids are lipid soluble compounds there was no correlation between echinenone and the lipid content ($r = 0.11$, $P = 0.56$). β-carotene levels were positively correlated with both total carotenoid and α-carotene content in gonads ($r = 0.71$ and 0.44). Also, α-carotene was negatively correlated to GSI ($r = -0.49$, Table 1).

Gonad texture. Firmness of *P. lividus* gonads varied significantly within the three studied months, but remained similar between males and females (Supplementary Table S1). Gonads presented greater firmness in November (33.6 – 38.4 g) during growing stage (II) than in September (25.3 – 25.5 g, Table 4), the post-spawn period where most females were in recovery stage (stage I) and more than 50% of males still presented mature or spent gonads, as observed in Fig. 1B. Gonad resilience was not affected over time and was also similar between sexes, ranging from 77.8 to 80.3% (Table 4). Firmness was negatively correlated with moisture ($r = -0.63$, data not shown) whereas resilience was not correlated with any measured variable.

Discussion

Different aspects of the reproductive cycle of *P. lividus* were evaluated in this study to fully characterize gonads production, nutritional value, colour and texture in a wild population from the Atlantic coast of Portugal. Our results indicate the occurrence of a single annual spawning event during late Spring and Summer (June–July), coinciding with the rise of seawater temperatures up to its maximum value (16.4°C). Spirlet, *et al.*¹⁶ pointed that populations of echinoids from temperate waters exhibit similar reproductive patterns: nutrient storage during Fall and Winter followed by a long spawning season during late Spring and Summer. A single annual spawning season was also described in populations from the Atlantic coast of France, Ireland and Spain^{13,16,27} and in the Mediterranean Sea²⁸. However, the occurrence of two spawning events during an annual cycle has been described for *P. lividus* in the Mediterranean Sea²⁹ and in the central West coast of Portugal⁶, where seawater temperatures are usually higher than those reported in presently studied area.

In this study, the highest GSI value (18%) observed in March 2017 was almost 2.5 times greater than the highest value registered in a southern population (8%) of Portugal⁶. The differences found in the two Portuguese populations might be explained by local environmental conditions, habitat and seasonality factors. According to Ouréns, *et al.*¹⁸, the GSI values of *P. lividus* show a geographical trend, where the Atlantic populations reach higher values than the Mediterranean ones. Also, a latitudinal pattern in the Atlantic populations was described, with higher GSI values at higher latitudes. Environmental cues such as food availability and quality, sea water temperature or/and photoperiod have been suggested to modulate *P. lividus* reproductive potential¹⁴. Interestingly, in this study, the mean GSI value observed at March–16 (9%) varied considerably from that observed in march–17

Sex	Female					Male					Two-way ANOVA P-value		
Month	Apr-16	Jun-16	Sep-16	Nov-16	Feb-17	Apr-16	Jun-16	Sep-16	Nov-16	Feb-17	Sex	Month	X
14:0	2.4 ± 0.6 ^{B*}	3.0 ± 0.5 ^{B*}	2.7 ± 0.4 ^{B*}	3.3 ± 0.3 ^{A*}	2.2 ± 1.1 ^{B*}	0.5 ± 0.1	1.3 ± 0.4	1.0 ± 0.3	2.8 ± 0.7	0.6 ± 0.1	<0.01	<0.01	0.2
16:0	3.5 ± 1.0 ^{B*}	4.9 ± 0.5 ^{B*}	4.9 ± 1.0 ^{B*}	6.2 ± 0.3 ^{A*}	4.0 ± 2.0 ^{B*}	1.2 ± 0.2	2.8 ± 0.9	2.3 ± 0.4	5.4 ± 1.4	1.5 ± 0.3	<0.01	<0.01	0.6
18:0	0.4 ± 0.1 ^C	0.5 ± 0.04 ^{BC}	0.8 ± 0.3 ^{AB}	0.8 ± 0.1 ^A	0.5 ± 0.2 ^C	0.3 ± 0.05	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.3 ± 0.1	0.2	<0.01	0.6
20:0	0.2 ± 0.01 ^{C*}	0.2 ± 0.01 ^{BC*}	0.3 ± 0.1 ^{AB*}	0.3 ± 0.01 ^{A*}	0.2 ± 0.1 ^{BC*}	0.1 ± 0.01	0.1 ± 0.03	0.2 ± 0.01	0.3 ± 0.1	0.1 ± 0.01	<0.01	<0.01	0.4
Σ SFA	6.8 ± 1.8 ^{B*}	8.9 ± 1.1 ^{B*}	9.1 ± 1.8 ^{AB*}	11.0 ± 0.4 ^{A*}	7.2 ± 3.7 ^{B*}	2.3 ± 0.5	5.0 ± 1.6	4.3 ± 0.8	9.7 ± 2.3	2.6 ± 0.6	<0.01	<0.01	0.4
16:1n-7	0.6 ± 0.2 ^{B*}	1.0 ± 0.2 ^{AB*}	0.8 ± 0.1 ^{AB*}	1.1 ± 0.2 ^{A*}	0.8 ± 0.4 ^{B*}	0.1 ± 0.02	0.4 ± 0.2	0.3 ± 0.1	0.9 ± 0.3	0.2 ± 0.1	<0.01	<0.01	0.5
18:1n-9	0.4 ± 0.1 ^{B*}	0.6 ± 0.2 ^{AB*}	0.4 ± 0.1 ^{B*}	0.6 ± 0.1 ^{A*}	0.4 ± 0.2 ^{B*}	0.1 ± 0.05	0.3 ± 0.1	0.2 ± 0.04	0.6 ± 0.1	0.2 ± 0.1	<0.01	<0.01	0.3
18:1n-7	0.7 ± 0.2 ^{B*}	0.9 ± 0.1 ^{B*}	1 ± 0.2 ^{AB*}	1.2 ± 0.1 ^{A*}	0.7 ± 0.3 ^{B*}	0.3 ± 0.03	0.6 ± 0.2	0.5 ± 0.1	1.1 ± 0.3	0.3 ± 0.04	<0.01	<0.01	0.3
20:1n-11	1.1 ± 0.2 ^{BC*}	1.5 ± 0.1 ^{A*}	1.3 ± 0.2 ^{AB*}	1.5 ± 0.1 ^{A*}	0.9 ± 0.4 ^{C*}	0.6 ± 0.1	1.1 ± 0.3	0.8 ± 0.1	1.4 ± 0.3	0.5 ± 0.1	<0.01	<0.01	0.3
20:1n-9	1.2 ± 0.3 ^{BC*}	1.7 ± 0.3 ^{AB*}	1.3 ± 0.3 ^{BC*}	1.7 ± 0.2 ^{A*}	1.2 ± 0.6 ^{C*}	0.7 ± 0.05	1.4 ± 0.4	0.9 ± 0.1	1.8 ± 0.6	0.6 ± 0.1	<0.01	<0.01	0.6
22:1n-9	0.7 ± 0.2 ^{B*}	0.8 ± 0.1 ^{AB*}	0.7 ± 0.2 ^{B*}	0.8 ± 0.1 ^{A*}	0.5 ± 0.3 ^{B*}	0.2 ± 0.03	0.6 ± 0.1	0.4 ± 0.1	0.9 ± 0.2	0.2 ± 0.03	<0.01	<0.01	0.1
Σ MUFA	5.3 ± 1.3 ^{B*}	7.1 ± 0.9 ^{AB*}	6.2 ± 1.2 ^{B*}	7.7 ± 0.5 ^{A*}	5.1 ± 2.5 ^{B*}	2.2 ± 0.2	4.8 ± 1.4	3.7 ± 0.7	7.5 ± 1.9	2.2 ± 0.3	<0.01	<0.01	0.4
18:2n-6 (LA)	0.2 ± 0.1 ^{B*}	0.3 ± 0.1 ^{B*}	0.3 ± 0.1 ^{B*}	0.4 ± 0.01 ^{A*}	0.2 ± 0.1 ^{B*}	0.1 ± 0.01	0.1 ± 0.1	0.1 ± 0.02	0.4 ± 0.1	0.1 ± 0.03	<0.01	<0.01	0.2
18:3n-6	0.05 ± 0.01 ^{B*}	0.1 ± 0.01 ^{B*}	0.1 ± 0.01 ^{B*}	0.1 ± 0.01 ^{A*}	0.1 ± 0.03 ^{B*}	0.01 ± 0.01	0.04 ± 0.02	0.03 ± 0.01	0.1 ± 0.02	0.02 ± 0.01	<0.01	<0.01	0.3
18:3n-3 (ALA)	0.3 ± 0.1 ^{B*}	0.5 ± 0.1 ^{B*}	0.8 ± 0.2 ^{A*}	0.8 ± 0.01 ^{A*}	0.4 ± 0.3 ^{B*}	0.1 ± 0.02	0.2 ± 0.1	0.2 ± 0.01	0.7 ± 0.2	0.2 ± 0.04	<0.01	<0.01	0.2
18:4n-3	0.6 ± 0.1 ^{B*}	1.1 ± 0.1 ^{B*}	2.5 ± 0.6 ^{A*}	2.5 ± 0.2 ^{A*}	1.1 ± 0.8 ^{B*}	0.1 ± 0.04	0.4 ± 0.2	0.7 ± 0.3	2.0 ± 0.5	0.5 ± 0.1	<0.01	<0.01	0.1
20:2n-6	0.2 ± 0.1 ^{BC}	0.3 ± 0.1 ^{AB}	0.3 ± 0.1 ^{BC}	0.4 ± 0.01 ^A	0.2 ± 0.1 ^C	0.3 ± 0.03	0.4 ± 0.1	0.4 ± 0.04	0.6 ± 0.1	0.2 ± 0.01	0.05	<0.01	0.3
20:3n-6	0.1 ± 0.02 ^B	0.1 ± 0.02 ^{AB}	0.1 ± 0.01 ^B	0.1 ± 0.01 ^A	0.1 ± 0.04 ^B	0.1 ± 0.01	0.1 ± 0.03	0.1 ± 0.01	0.2 ± 0.04	0.1 ± 0.01	0.2	<0.01	0.6
20:4n-6 (ARA)	1.8 ± 0.4 ^C	2.2 ± 0.2 ^{BC}	3.0 ± 0.6 ^{AB}	3.2 ± 0.3 ^A	1.9 ± 1.2 ^C	1.5 ± 0.2	2.4 ± 0.6	2.2 ± 0.3	3.7 ± 0.6	1.2 ± 0.1	0.3	<0.01	0.4
20:3n-3	0.3 ± 0.1 ^C	0.5 ± 0.1 ^{BC}	0.8 ± 0.2 ^{AB}	0.9 ± 0.1 ^A	0.4 ± 0.2 ^C	0.2 ± 0.05	0.5 ± 0.1	0.6 ± 0.1	1 ± 0.2	0.3 ± 0.04	0.3	<0.01	0.6
20:4n-3	0.2 ± 0.03 ^{B*}	0.3 ± 0.01 ^{B*}	0.7 ± 0.2 ^{A*}	0.8 ± 0.1 ^{A*}	0.3 ± 0.2 ^{B*}	0.1 ± 0.02	0.3 ± 0.1	0.4 ± 0.1	0.7 ± 0.1	0.2 ± 0.05	0.02	<0.01	0.5
20:5n-3 (EPA)	2.2 ± 0.6 ^{C*}	2.6 ± 0.2 ^{BC*}	4.4 ± 0.8 ^{AB*}	4.8 ± 0.4 ^{A*}	2.9 ± 1.7 ^{C*}	1.5 ± 0.2	2.3 ± 0.6	2.6 ± 0.4	4.8 ± 0.5	1.7 ± 0.2	0.01	<0.01	0.4
22:4n-6	0.1 ± 0.01 ^{bc}	0.1 ± 0.01 ^{bc}	0.1 ± 0.01 ^{bc}	0.1 ± 0.01 ^{bc}	0.04 ± 0.02 ^c	0.05 ± 0.01 ^{bc}	0.1 ± 0.02 ^{ab}	0.1 ± 0.01 ^{ab}	0.1 ± 0.02 ^a	0.04 ± 0.01 ^c	<0.01	<0.01	0.04
22:5n-3	0.1 ± 0.02 ^c	0.1 ± 0.01 ^{bc}	0.1 ± 0.02 ^{bc}	0.2 ± 0.01 ^{bc}	0.1 ± 0.05 ^c	0.1 ± 0.03 ^{bc}	0.2 ± 0.04 ^{ab}	0.2 ± 0.03 ^{abc}	0.3 ± 0.04 ^a	0.1 ± 0.01 ^c	<0.01	<0.01	0.04
22:6n-3 (DHA)	0.05 ± 0.01 ^B	0.05 ± 0.01 ^B	0.05 ± 0.01 ^B	0.1 ± 0.03 ^A	0.04 ± 0.01 ^B	0.03 ± 0.01	0.1 ± 0.02	0.05 ± 0.01	0.1 ± 0.04	0.02 ± 0.01	0.9	<0.01	0.3
Σ PUFA	6.3 ± 1.5 ^{C*}	8.4 ± 0.9 ^{BC*}	13.3 ± 2.9 ^{AB*}	14.5 ± 1.2 ^{A*}	7.9 ± 4.9 ^{C*}	4.2 ± 0.7	7.2 ± 2.1	7.8 ± 1.2	14.7 ± 2.4	4.6 ± 0.6	0.02	<0.01	0.4
EPA + DHA	2.3 ± 0.6 ^{C*}	2.7 ± 0.2 ^{BC*}	4.5 ± 0.8 ^{AB*}	4.9 ± 0.4 ^{A*}	2.9 ± 1.7 ^{C*}	1.5 ± 0.2	2.4 ± 0.6	2.6 ± 0.4	4.9 ± 0.6	1.8 ± 0.2	0.01	<0.01	0.4
Σ n-6	2.4 ± 0.6 ^B	3.1 ± 0.5 ^B	3.7 ± 0.8 ^{AB}	4.3 ± 0.3 ^A	2.6 ± 1.5 ^B	1.9 ± 0.3	3.2 ± 0.9	2.9 ± 0.3	5.0 ± 0.9	1.6 ± 0.2	0.3	<0.01	0.4
Σ n-3	3.8 ± 0.9 ^{B*}	5.2 ± 0.5 ^{B*}	9.5 ± 2.1 ^{A*}	10.1 ± 0.9 ^{A*}	5.2 ± 3.2 ^{B*}	2.2 ± 0.4	4.0 ± 1.1	4.9 ± 0.9	9.6 ± 1.6	3.0 ± 0.5	<0.01	<0.01	0.30
n-6/n-3	0.6 ± 0.01 ^b	0.6 ± 0.03 ^b	0.4 ± 0.01 ^c	0.4 ± 0.01 ^{bc}	0.5 ± 0.03 ^{bc}	0.8 ± 0.1 ^a	0.8 ± 0.01 ^a	0.6 ± 0.04 ^b	0.5 ± 0.05 ^{bc}	0.5 ± 0.03 ^{bc}	<0.01	<0.01	<0.01

Table 2. Gonad fatty acid composition (mg.g⁻¹ WW) of *P. lividus* females and males collected at Praia Norte (Portugal) at five distinct periods. Values are mean ± standard deviation; per month the 18 individuals were pooled by sex: N = 3 pools of female gonads and N = 3 pools of male gonads were established. Within a row, superscripts in capital letters (^{ABC}) indicate significant differences between months, asterisk (*) indicate differences between sexes and in lowercase letters (^{abc}) indicate significant differences between sex X month ($P < 0.05$). Σ SFA includes: 12:0, 13:0, iso-14:0, 15:0, iso-15:0, anteiso-15:0, iso-16:0, 17:0, iso-17:0, anteiso-17:0, 22:0, 24:0. Σ MUFA includes: 14:1n-5, 16:1 n-9, 17:1n-7, 20:1 n-7, 22:1 n-11, 24:1 n-9. Σ PUFA includes: 16:2n-4, 16:3n-4, 16:4n-1, 18:2n-4, 18:4n-1, 21:5n-3.

(17%). Since sea water temperature (mean of 12.5°C) and day-length (mean of 12 h light) followed a quite similar variation pattern in these particular months, it is possible that changes in food availability or food type could have affected the development and maturation of gonads and thus partially explain the marked variation on GSI values.

GSI showed a seasonal variation, but did not differ between sexes. Similar results were observed in other *P. lividus* populations from Portugal⁶, Ireland¹³ and Mediterranean Sea³⁰. Sellem and Guillou³¹ highlighted the importance of using histological analysis to interpret GSI variation; these authors related punctual decreases of GSI values with changes in gonad reserves rather to gamete release. However, changes in the GSI observed over the study period, namely gonad size and weight, followed the gonad maturation pattern described by histological analysis: an increase of size was observed during the growing (II) stage due to nutrient storage in the non-germinal cells, continued until reaching the maximum yield at maturation (IV) stage then, with spawning, gonads became smaller and empty in content (stages V and VI).

The biochemical composition of *P. lividus* gonads showed a clear seasonal variation also varying with sex. Protein was the major component of gonads, ranging between 12–18% WW while total lipid content was considered low (<6% WW). All biochemical parameters were within the range of values previously reported for *P. lividus*^{27,28,32}. Moreover, the seasonal variation of nutrient content in gonads reflected the reproductive cycle.

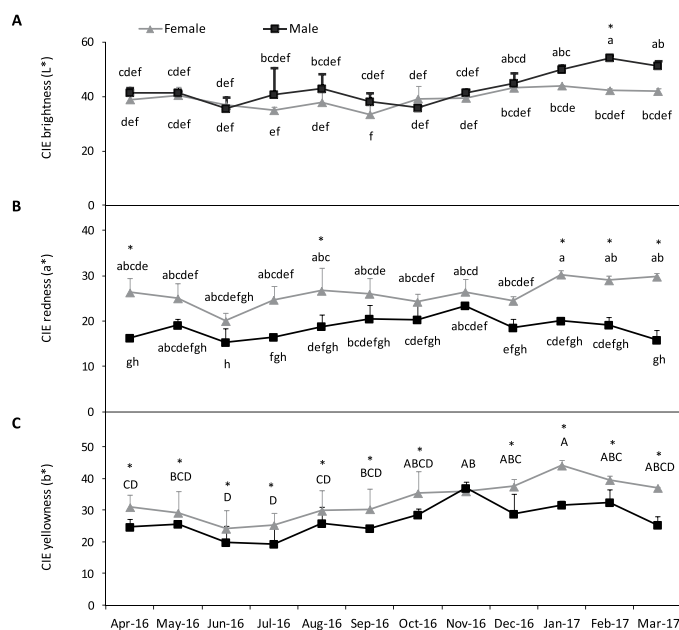


Figure 3. Colour parameters (A) lightness, L^* (B) redness, a^* and (C) yellowness, b^* measured instrumentally in gonads of *P. lividus* (ovary and testis) over a 13-month period. Values are mean $\pm$ standard deviation; per month the 18 individuals were pooled by sex: N = 3 pools of female gonads and N = 3 pools of male gonads were established. Superscripts in capital letters (A, B, C) indicate differences between months, asterisk (*) indicate differences between sexes and superscripts in lowercase letters indicate significant differences between sex X month (abc) ($P < 0.05$).

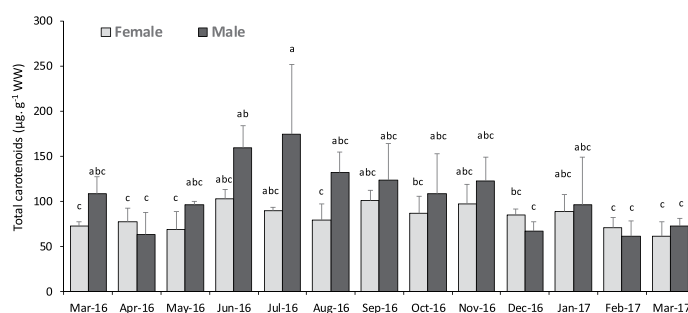


Figure 4. Total carotenoid content in gonads (ovary and testis) of *P. lividus* collected at Praia Norte (Portugal) over a 13-month period. Values are mean $\pm$ standard deviation; per month the 18 individuals were pooled by sex: N = 3 pools of female gonads and N = 3 pools of male gonads were established. Superscripts in lowercase letters indicate significant differences between sex X months (abc) ($P < 0.05$).

Previous reports showed that the best period to harvest gonads with high nutritional quality and commercial value is during the growing period, generally prior to the onset of gametogenesis, when the nutritive phagocytes are full³³ and accumulate higher levels of protein, lipid and carbohydrates³⁴. In the present study we observed that gonads reached their highest content of protein, lipid and energy in November (growing stage, II) and maintained this high nutritional value until January-February (pre-maturation stage, III), before full maturation of gametes, suggesting this period as desirable for harvesting nutrient-rich gonads. Afterwards, when gonads were mature and peaked their GSI (March-17), their nutritive content started decreasing, in terms of lipids and energy, reinforcing that gonad quality deteriorates with gametes development. Changes in the biochemical content of gonads can impair their flavour, leading to a loss of economic value. Murata, *et al.*³⁵ described that mature ovaries of *Hemicentrotus pulcherrimus* had a bitter taste, which was strongly correlated with the presence of a novel sulfur-containing amino acid, pulcherrimine. Furthermore, it was shown that the yolk protein stored in

Sex	Female					Male				
Month	Apr-16	Jun-16	Sep-16	Nov-16	Feb-17	Apr-16	Jun-16	Sep-16	Nov-16	Feb-17
Pigments										
α -carotene	1.0 $\pm$ 0.5 ^{AB*}	1.1 $\pm$ 0.7 ^{A*}	0.2 $\pm$ 0.02 ^{B*}	0.3 $\pm$ 0.2 ^{B*}	0.6 $\pm$ 0.2 ^{B*}	1.2 $\pm$ 0.7	2.9 $\pm$ 1.4	1.8 $\pm$ 1.2	0.8 $\pm$ 0.5	0.1 $\pm$ 0.1
β -carotene	12.3 $\pm$ 2.8 ^B	14.8 $\pm$ 6.2 ^{AB}	19.9 $\pm$ 4.6 ^A	22.5 $\pm$ 7.3 ^{AB}	14.3 $\pm$ 5.0 ^B	10.8 $\pm$ 5.7	19.2 $\pm$ 4.9	36.5 $\pm$ 19.9	23.1 $\pm$ 8.1	7.5 $\pm$ 3.3
Echinenone	33.6 $\pm$ 4.5 ^c	36 $\pm$ 17.0 ^c	64.1 $\pm$ 14.2 ^c	93.9 $\pm$ 16.8 ^{bc}	75.4 $\pm$ 28.7 ^c	83.2 $\pm$ 37.2 ^c	162.3 $\pm$ 16.9 ^{ab}	171.6 $\pm$ 57.3 ^a	170.7 $\pm$ 32.6 ^a	37.7 $\pm$ 7.5 ^c
Lutein	8.7 $\pm$ 3.9 ^a	6.7 $\pm$ 1.4 ^{ab}	4.7 $\pm$ 0.5 ^{abc}	4.1 $\pm$ 1.6 ^{bc}	0.5 $\pm$ 0.2 ^c	1.0 $\pm$ 0.4 ^c	1.7 $\pm$ 0.6 ^c	1.0 $\pm$ 0.5 ^c	1.0 $\pm$ 0.1 ^c	4.0 $\pm$ 0.7 ^{bc}
β -cryptoxanthin	20.1 $\pm$ 0.3 ^A	18.2 $\pm$ 0.04 ^{AB}	19.9 $\pm$ 0.5 ^A	20.2 $\pm$ 0.8 ^A	18.7 $\pm$ 0.8 ^B	20.1 $\pm$ 0.7	19.7 $\pm$ 0.3	20.4 $\pm$ 0.4	20 $\pm$ 0.8	19.2 $\pm$ 0.4

Table 3. Content of carotenoid pigments found in gonads (ovary and testis, $\mu\text{g}\cdot\text{g}^{-1}$ WW) of *P. lividus* collected at Praia Norte (Portugal) at five distinct periods. Values are mean $\pm$ standard deviation; per month the 18 individuals were pooled by sex: N = 3 pools of female gonads and N = 3 pools of male gonads were established. Within a row, superscripts in capital letters (^{ABC}) indicate significant differences between months, asterisk (*) indicate differences between sexes and in lowercase letters (^{abc}) indicate significant differences between sex X month ($P < 0.05$).

Sex	Female			Male		
Month	Sep-16	Nov-16	Feb-17	Sep-16	Nov-16	Feb-17
Firmness (g)	25.5 $\pm$ 3.7 ^B	33.6 $\pm$ 8.2 ^A	31.2 $\pm$ 8.7 ^{AB}	25.3 $\pm$ 6.7	38.4 $\pm$ 3.6	23.3 $\pm$ 5.3
Resilience (%)	80.3 $\pm$ 0.3	80.0 $\pm$ 0.9	79.8 $\pm$ 3.2	79.7 $\pm$ 1.8	80.0 $\pm$ 1.5	77.8 $\pm$ 1.4

Table 4. Firmness (g) and resilience (%) of *P. lividus* gonads (ovary and testis) collected at Praia Norte (Portugal) at three distinct periods. Values are mean $\pm$ standard deviation; per month the 18 individuals were pooled by sex: N = 3 pools of female gonads and N = 3 pools of male gonads were established. Within a row, superscripts in capital letters (^{ABC}) indicate significant differences between months ($P < 0.05$).

nutritive phagocytes is used during gametogenesis to synthesize new proteins and other nitrogen substances¹⁹, which might contribute to changes in gonad flavour. Despite these observations, no correlation could be found between the biochemical components of gonad and the GSI (which reflected gametogenic stages), confirming previous observations by Montero-Torreiro and Garcia-Martinez²⁷. Likewise in many other species, the nutritional composition of edible tissues (gonads) seems to be highly dependent on sea urchin diet³⁶. Changes on feed type and abundance throughout the year may affect the assimilation of nutrients and modify gonad composition. Moreover, recent studies have evaluated the effect of natural (algae) and formulated diets on *P. lividus* gonad biochemical composition^{32,37,38} in order to promote the production of high valued gonads.

The FA profile of *P. lividus* gonads was in general agreement with data reported in the literature^{28,38–40}. But only a few of these studies discern sex as factor, mostly focusing on the effects of season or diet. Martínez-Pita, *et al.*³⁹ found differences in the FA profile of ovary and testis of *P. lividus*: females showed higher levels of 14:0, LA and ALA whereas males exhibited higher levels of 18:0 and ARA; in terms of FA classes, only SFA differed between sexes, being higher in females. These authors also observed differences in the FA profile of two distinct populations, which suggested that environmental factors, such as food source, may influence the gonad FA profile of individuals of the same sex. The source and availability of food, as well as other site-associated factors, should be taken into consideration when results are being compared with the literature, since it may mask the effects of sex or season. In our work, females showed much higher concentrations of PUFA, MUFA and SFA compared to males, and although concentrations were within the expected values for sea urchins, the variation between sexes was more pronounced compared to other studies of *P. lividus*³⁹ and *Psammechinus miliaris*⁴¹.

To our knowledge, this is the first time that effects of sex/season/gametogenic cycle on FA composition of *P. lividus* male and female gonads, has been presented for a wild population. The seasonal effect on FA composition of gonads was clear; females and males had preferentially higher concentrations of the sum of PUFA, MUFA and SFA classes in November, which was coincident with the period of gonad growth (stage II), when the gonads were richer in nutrients. It was possible to relate the season factor with the gametogenic cycle, yet no correlation was found between GSI and any FA in gonads. Carboni, *et al.*³⁷ showed that the FA profile of *P. lividus* (without sex distinction) changed during gametogenesis, however differences were related to dietary lipid intake and not to seasonality. As previously discussed, diet has an important role in determining the nutritional composition of gonads³⁶. Sea urchins are generalist grazers, but they often prefer feeding on large kelp (*Laminaria* ps.) species. The selective ingestion of certain macro algae and their respective nutritional value, have certainly contributed to modulate the FA content of sea urchin gonads once macro algae are rich in PUFA, particularly in EPA⁴². A recent study conducted in the North coast of Portugal with *Laminaria ochroleuca*, an algae consumed by *P. lividus*⁴, showed a seasonal pattern of growth and reproduction for this algae⁴³. Interestingly, *L. ochroleuca* accumulated more nutrients during its growth period (winter)⁴³, which was coincident with the increase of nutrient content of gonads, namely FA.

Regarding the most relevant PUFA, EPA was preferentially accumulated in the ovary and levels decreased with increasing maturity stage, from September–November to February 2017. Previous studies have reported a trend of EPA accumulation in the gonads of sea urchins^{37,41}. In contrast, ARA and DHA levels only suffered a

seasonal variation, being more concentrated in November, in both males and females. The recommended daily consumption of EPA + DHA to decrease the risk of cardiovascular diseases in humans is between 0.25–0.5 g⁴⁴. If sea urchins are consumed during the period of highest concentration of PUFA in gonads (the growing stage in November) an intake of 50 g of *P. lividus* (around 5 adults) can provide near 0.245 g of EPA + DHA, which covers the daily recommended dose to promote human health, reinforcing the nutritional importance of sea urchin.

Few studies have evaluated the seasonal variation of gonad colour from wild populations of sea urchins, despite the relevance of this trait for market valorisation. In most cases gonad colour was evaluated visually or instrumentally and without sex differentiation^{36,45,46}. The results obtained by the CIE L*a*b* system were within the range of values reported for wild *P. lividus* fed on macroalgae. Seasonal changes in gonad colour were also reported for *Strongylocentrotus franciscanus*¹² and *S. droebachiensis*¹, which may be related to changes in gonad yield and possibly maturation stage (gametogenesis). In the present study, a positive correlation between L*, b* values and the GSI was observed, suggesting that the increase of gonad yield, from the growing to the full maturation stage, led to increased brightness and yellowish colour. Moreover, previous findings in *P. lividus* showed that gonads during the spawning season had an unacceptable coloration (dark red/brown) whilst during the growing period exhibited an excellent/good coloration (bright orange or yellow)⁴⁵. In terms of colour, Cook and Kelly⁴⁷ classified as acceptable to excellent coloration (pale yellow to bright orange) gonads presenting (mean) values of 42 for L*, 20 for a* and 35 for b*. Our results showed that L* a* and b* values observed, in both sexes, between November (growing stage) and February (pre-mature stage) were similar or higher than those proposed by Cook and Kelly⁴⁷, suggesting that gonads presented a desirable coloration for market. Additionally, it was also in this period that gonads become nutritionally richer and increased in size, as previously discussed. By combining all these positive features, one can propose the period between November and February as the most suitable to harvest premium gonads. Moreover, colour values decreased at spawning (June), showing some loss of colour quality, as previously observed with the nutrient content.

Total carotenoids in gonads did not differ between sexes but showed some seasonality. Total carotenoid content varied with gonad maturation stages and followed a similar pattern in several sea urchin species, increasing during spawning (June) probably due to gonad loss of biomass, and decreasing afterwards. This is consistent with previous observations in *P. lividus*⁴⁵, *P. miliaris*⁴⁸ and *S. droebachiensis*⁴⁹. Carotenoids are pigments deposited in the gonads however, and according to Symonds, *et al.*⁴⁵, acceptability of gonad colour is not directly linked neither with the levels of individual carotenoids nor their ratios found in the gonad. In fact total carotenoids picked after spawning, when gonads are known to be unacceptable for consumers⁴⁵.

Echinenone was the dominant carotenoid in the gonad of both males and females, confirming previous reports for this species^{20,45}. Moreover, echinenone levels were highest in males after spawning and onset of recovery period (September–November) whereas females showed more steady levels over time. Although no detailed evaluation of carotenoids deposition in gametes (eggs and semen) was performed, the white colour of semen contrasts with the orange coloration of eggs, suggesting that males might have kept most of the carotenoids in their gonads during spawning, that could partly explain their highest concentration after spawning, when gonad volume is strongly reduced. Moreover, males showed a considerable variation of echinenone concentration after spawning, which was also observed by Symonds, *et al.*⁴⁵. This is probably related with the high variability in gametogenic stages of males observed post-spawning, that were found at recovery, partly spawned and spent stages. Also, no correlation could be found between echinenone in gonads and L* a* b* parameters, confirming previous observations in *P. lividus*^{20,45} and *P. miliaris*⁴⁸. Gonads present a high content of pigments but its biochemical nature failed to be correlated with data obtained by instrumental or sensorial analysis, suggesting that the visual perception of colour may be influenced by other factors, besides carotenoid pigments. In *P. lividus*, unacceptable gonad coloration was observed with both very low and high levels of echinenone and total carotenoids in gonads⁴⁵. These results suggest that carotenoids clearly made a contribution towards the red-orange pigmentation of the sea urchin gonad, but other factors may induce the sensorial evaluation of colour. The second major group of pigments present in *P. lividus* gonads were β -carotene and β -cryptoxanthin, which only differ seasonally and not between sex. These findings may reinforce the hypothesis that, in *P. lividus*, echinenone is likely to be formed by bio-conversion of β -carotene via β -isocryptoxanthin¹⁰ and its metabolism may occur mainly in gonads²¹. Symonds, *et al.*⁴⁵ also identified β -carotene as the second major group of pigments in *P. lividus* gonads, but in a different study, Shpigel, *et al.*²⁰ reported the absence of β -carotene in gonads of urchins fed with a natural algal diet.

Firmer gonads were observed in November, during the growing period (stage II), whereas in both sexes, lower gonad firmness was observed in September (after spawning), when gonads appear as spent. Firmness was negatively correlated with gonad moisture content ($r = -0.63$), being in agreement with McBride, *et al.*¹². These authors demonstrated that *S. franciscanus* gonads had higher moisture content and softer texture during maturation whereas the opposite was observed during the recovering and growing period, suggesting that gonads firmness was increased by lower moisture content. Few studies determined gonad texture, and were mostly focused on subjective evaluations^{23,50} rather than in instrumental analysis using a texture analyser¹². The effect of sex in gonad texture was never reported before. Resilience is considered a useful texture measure for market valorisation, since a strong relation between high resilience values and high-quality rated gonads was reported by McBride, *et al.*¹². In the present study, gonads resilience was not affected over time nor differed between sexes, being always higher than 75%. This may suggest that resilience remains acceptable throughout the year cycle.

In conclusion, this study clearly demonstrated that most of the evaluated quality attributes were mainly dependent on sea urchin sex, but also showed a seasonal variation. According to the present observations, it is suggested that the best period for commercial harvesting sea urchins in the North Atlantic coast of Portugal is between November and February. During this period, gonads are mainly at the growing and premature stages of gametogenesis, holding a good nutrient storage in their nutritive phagocytes, as demonstrated by the highest levels of protein, lipid, n-3 and n-6 PUFA and energy content, in both males and females. In addition, gonads

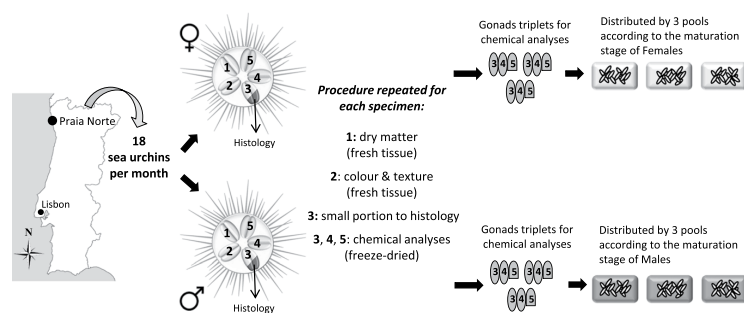


Figure 5. Summary of the monthly sampling protocol performed for *P. lividus* at Praia Norte (Portugal) over a 13-month period.

showed lower moisture content and simultaneously higher firmness, that is a valuable attribute. Also during this period, gonads were found more reddish, yellowish and brighter, irrespectively of the sex, being the values within those considered acceptable and desirable in the European market, were a bright and yellowish-orange gonad is rated premium. Moreover, as it was evidenced that most commercially important traits change seasonally compromising the gonad value, sea urchin production under controlled conditions (echinoculture) can be a sustainable alternative to assure a regular supply of high quality gonads. The present data can be further used to help tailoring new formulated feeds able to produce sea urchins with the desired gonad attributes for international markets.

Material and Methods

Ethics statement. All animal procedures respecting capture of wild specimens and tissue sampling were previously approved by the CIIMAR ethical committee, in compliance with the European Union Directive 2010/63/EU and the Portuguese law Decreto-Lei n.º 113/2013 on “protection of animals used for scientific purposes”. Wild sea urchins *P. lividus* were collected at Praia Norte (41°41'57"N, 8°51'13"W), Portugal, after authorization from the national maritime authority (Captaincy of the Port of Viana do Castelo) and the Portuguese Institute for Nature Conservation and Forests (ICNF). Moreover, the sampling site did not involve protected areas nor endangered/protected species in the Atlantic region, and followed the Portuguese law Portaria n.º 82/2011 on “minimum size for captures and harvesting”, implying that only commercial sized sea urchins (test diameter ≥ 5 cm) were collected.

Animals and sampling. Adult *P. lividus* were collected between March 2016 and March 2017, by snorkeling in subtidal rocky reefs of Praia Norte, Portugal. Sea temperature records from A Guarda (Spain) oceanic buoy (station nearby our sampling area; 41°54'16.8"N, 8°53'51.0"W) were obtained from CMEMS (http://marine.copernicus.eu-SST_GLO_SST_L4_NRT_OBSERVATIONS) through AquaSafe by Hidromod (<http://www.hidromod.com>). Day length data were obtained for Porto city (near our sampling site) and assessed from the Observatório Astronómico de Lisboa, University of Lisbon²⁶.

Eighteen ($N = 18$) sea urchins were collected monthly and transported to CIIMAR facilities in seawater. Individual records of test diameter and total wet weight were performed in laboratory; animals were then placed in an ice bath for 30 min before dissection (Fig. 5). The five gonads of each animal were carefully removed, damp-dried and weighted for individual gonad somatic index (GSI) determination, calculated as: [(g gonad wet weight/g total wet weight) $\times 100$]. The sex of the specimens was identified by direct observation of released gametes from the dissected gonads and later confirmed by histological examination. One gonad was used for instrumental assessment of colour and texture and another one for dry matter determination (Fig. 5). A small gonad sample was immediately fixed in 4% buffered formalin for 24 h and the remaining gonads were individually snap-frozen and kept at -80°C for subsequent biochemical analyses (Fig. 5). Fixed gonad samples were dehydrated, embedded in paraffin and transversely sectioned at $7\mu\text{m}$. Sections were stained with hematoxylin-eosin and observed under a binocular microscope (CX22LED, Olympus). The gametogenic stages of ovary and testes were identified according to Byrne¹³: recovery (stage I), with primary gametes and nutritive phagocytes; growing (stage II) with clusters of primary gametes and packed nutritive phagocytes, premature (stage III) with gametes at all stages of development and reduced amount of nutritive phagocytes; mature (stage IV) with mature gametes and few nutritive phagocytes; partly spawned (stage V) with loosely packed gametes and depletion of nutritive phagocytes and spent (stage VI) with gonads empty of gametes.

It is not possible to distinguish externally the sex of *P. lividus*, likewise other sea urchin species, yet both male and female individuals were collected monthly over the 13-month period, without needing any further sampling during the same month. Sex ratio in samplings varied over time: a minimum of seven and maximum of eleven individuals from the same sex were collected (sex ratio of 7:11, $N = 18$), but a ratio of 9:9 female:male ($N = 18$) was found in the majority of the sampled months (Fig. 5). Due to the small size of gonads, individual samples were pooled monthly by sex: three pools of female gonads ($N = 3$) and three pools of male gonads ($N = 3$) were established. Individuals from the same sex were pooled based on their maturation stage, after confirmation by histological analysis, as previous studies on *P. lividus* have related changes on nutritional composition and

carotenoid content of gonads with their maturation stage^{27,45,51}. The number of individuals in a pool was variable and dependent on the sex ratio and maturation stage of gonads, yet the three pools of male and three pools of female gonads were always ensured per month. Before analyses, gonad pools were quickly crushed in liquid nitrogen and reduced to a fine powder without defrosting. Half of this frozen sample was used for carotenoids analysis, whereas the rest was freeze-dried before all other biochemical analyses.

Biochemical composition. Chemical analyses were run in duplicate, following the AOAC⁵² procedures. Fresh gonads were analysed for dry matter after drying at 105 °C for 24 h, and samples of freeze-dried gonads were analysed for: ash content by incineration in a muffle furnace at 500 °C for 6 h (Nabertherm L9/11/B170, Germany); crude protein (N x 6.25) by a flash combustion technique followed by a gas chromatographic separation and thermal conductivity detection (LECO FP428, USA) and gross energy in an adiabatic bomb calorimeter (IKA C2000, Germany). Total lipids were determined following the method described by Folch, *et al.*⁵³ with dichloromethane-methanol (2:1) and gravimetric determination.

The fatty acid methyl esters (FAME) contained in total lipid extracts were transesterified by acidic methylation⁵⁴, as described by Campos, *et al.*⁵⁵. To each sample was added 1 mL of internal standard solution (1 mg C23:0/1 mL hexane; C23:0, Matreya LLC, USA). FAME were analysed in duplicate, using a Shimadzu GC-2010 Plus gas chromatograph (Shimadzu Europe GmbH, Germany), equipped with a flame-ionization detector and an Omegawax 250 capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness; Supelco, Bellefonte, USA). FAME were identified by comparing their retention times with known standards and quantified as mg.g⁻¹ of dry biomass, using the internal standard C23:0.

Colour determination. Gonad colour was evaluated instrumentally and by determination of the carotenoid content and profile. Colour was measured by the CIE 1976 (L*, lightness; a*, redness; b*, yellowness) method using a CR-400 colorimeter (Konica Minolta) at standard illuminant D₆₅. Readings were calibrated against a white tile and three replicate measurements were taken for each gonad and averaged to determine colour parameters.

Total carotenoid extraction was performed according to Symonds, *et al.*⁴⁵ with some modifications. Briefly, carotenoids from frozen gonad tissue (0.4–0.5 g) were extracted, in duplicate, in acetone (5:1, v:w). After centrifugation at 3500 × g for 10 min, at 8 °C, the supernatant was collected and the extraction procedure was repeated twice to obtain a colourless supernatant. Supernatants were evaporated to dryness under a constant stream of nitrogen, re-suspended in n-hexane and filtered with a 0.2 µL syringe filter for further analysis. Total carotenoid content was determined in a spectrophotometer (Shimadzu UV-1603, Japan), using the absorption maximum in hexane and the extinction coefficient (E^{1%_{1cm}) of 2500⁵⁶, based in the equation describe by Pocock, *et al.*⁵⁷:}

$$C = (E \times V \times 1000) / (100 \times G \times (E^{1\%}_{1cm}))$$

where, C = concentration of carotenoid (mg)/wet weight (g). E = maximal absorption of a known volume of carotenoid solution read at the wavelength of maximum absorption. V = sample final volume (mL). E^{1%_{1cm} = the extinction coefficient of a 1% (w/v) solution in a 1 cm cuvette at a defined wavelength. G = sample weight (g).}

The qualitative and quantitative profile of carotenoids was performed by high-performance liquid chromatography analysis (Waters Alliance Series 600, Milford, USA), and was restricted to five sampled months: April, June, September, November 2016 and February 2017, selected to cover the gametogenic stages identified in this study. Each carotenoid extract was suspended in n-hexane and 20 µL were injected in a reverse phase AcclaimTM C30 LC column (250 × 4.6 mm i.d., 5 µm particle size, Thermo Scientific) with a mixture of acetonitrile, methanol, dichloromethane, hexane and ammonium acetate (55:22:11.5:11.5:0.02, v-v:v:v:w) under isocratic conditions (1.0 mL/min, 25 min at 25 °C). Detection was achieved by a diode array detector (Waters 996 PDA, USA) at 454 nm. Retention times and spectra of compounds were analysed by comparison with pure standards and quantification performed by the calibration curves of α-carotene, β-carotene, echinenone, (Sigma, Portugal), lutein and β-cryptoxanthin (Extrasynthèse, France). Data were analysed using the Waters EmpowerTM 2 software and expressed as mg.g⁻¹ of gonad biomass.

Texture evaluation. An instrumental evaluation of firmness and resilience properties was performed in gonads using a TA.XT.plus analyser, fitted with a 5 kg load cell and controlled by Exponent v6 software (Stable Micro systems, UK). Firmness was defined as the force (g) required to compress a sample at 25% of its original height (at a 1.7 mm/s) using a 35 mm cylinder probe. Resilience was defined as the capacity of a sample to recover its original height after a compression with the same probe, being determined as: Resilience (%) = (post-test height/original height) × 100. Briefly, after firmness determination the gonad was compressed up to 60% of its original height and held that position for 2 s. The post-test height was recorded after 15 s. Tests were performed individually in fresh gonads samples in three months of the studied period – September, November and February – which represented the recovery (I), growing (II) and premature (III) stages of gametogenesis, respectively. These stages of gametogenesis were selected based on their relevance to gonad quality, since market acceptability is higher before gametes development and spawning.

Statistical analysis. Data are presented as means and standard deviation. Tests for normality and homogeneity of variances were performed by Kolmogorov-Smirnov and Levene's tests, respectively. A two-way ANOVA followed by HSD Tukey test, using sex (female and male) and month as independent variables, was performed with STATISTICS 6.4 package (StatSoft, Inc., Tulsa, OK, USA). Pearson's correlation was used to relate all parameters, independently of the sex factor. In all cases significant differences were considered when *P* < 0.05.

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Author Contributions

F.R. was involved in several analytic analyses (gonad biochemical, colour and texture determination and gametogenesis evaluation), analysed data, performed the statistical analysis and wrote the manuscript. L.B., S.M., B.R. were involved in biochemical analyses. A.O. was involved in the carotenoid pigments analysis. M.R.G.M. was involved in the fatty acid quantification. F.A. was responsible for the collection of wild sea urchins; A.J.M.F., M.P. and L.M.P.V. are research group supervisors. L.M.P.V. was responsible for the experimental design. All authors provided comments to the manuscript redaction.

Additional Information

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Chapter III.

Survey into the sea urchin consumption profile in the Portuguese population

by

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Format adapted for dissertation

Abstract

The consumption of shellfish is affected by many factors, namely those associated with culture, health and sensory properties. The main goal of this research was to explore and understand consumers' perceptions and behaviours regarding consumption of shellfish, namely sea urchin, applying a mixed methodology to 309 web-based questionnaires.

Consumers with a high sea urchin consumption profile stressed *Emotions* as the most important, while those with low consumption emphasised the *Unknown* as the main category. This could suggest that participants with high consumption patterns had a high level of familiarity with sea urchin, while low consumers had poor knowledge concerning this gourmet product. Moreover, participants with high gourmet shellfish consumption patterns were strongly associated with the category *Preparation methods*, suggesting a deep knowledge about the product, while the ones with low consumption were associated with *Exclusivity* of the product, mainly due to the high prices. The participants with low levels of food neophobia were significantly more willing to taste new shellfish products than participants with high levels of food neophobia to taste new shellfish products. This study allowed to characterize the main patterns of the shellfish and sea urchin consumption in Portugal.

Keywords: sea urchin; *Paracentrotus lividus*; free word association; shellfish; consumption frequency

1. Introduction

Seafood is a nutrient-rich food source, of which consumption is highly recommended due to its nutritional and health benefits (Institute of Medicine, 2007). Indeed, shellfish, particularly sea urchin, is a high-quality protein source, low in saturated fat and rich in several micronutrients. They are also a source of long-chain polyunsaturated omega-3, for instance eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids (Baião et al., 2019). These compounds are associated to the good development of foetuses and infants and also with the decreased risk of cardiovascular diseases and other chronic diseases in adults (Institute of Medicine, 2007).

Portugal is one of the European Union countries with higher average annual per capita consumption (55.9 kg), corresponding to a 160 g meal of seafood per day (FAO, 2018). Moreover, Portugal has an Exclusive Economic Zone of around 1,700,000 km² and a coastline of 942 km, which gives extremely prominence to fishery and fish products consumption. In Portugal, *Paracentrotus lividus* is the dominant echinoid species in the rocky shores (Baião et al., 2019) and landings reached 170 tonnes in 2018 (FAO, 2018),

being mostly exported to Spain, Italy and France. *P. lividus* gonads are considered a prized and gourmet seafood delicacy, due to their unique flavour, making their commercialization highly profitable. As a consequence, consumer demand and exploitation have been increasing over the last years, which resulted in the collapse of several natural stocks (Rocha et al., 2019). In Portugal, this product is under-consumed mainly because it is not part of the national gastronomy culture. However, the Portuguese population is mostly concentrated in coastal regions, so the potential to explore marine resources is vast, namely exotic and prized species like sea urchins. Furthermore, consumer shifts towards shellfish as key sources of healthy nutrients might present an opportunity for this high-valued species (Rocha et al., 2019).

It has become clear that the success and sustainability of commercial sea urchin, its economic viability and market acceptance strongly depend on the knowledge of the preferences and patterns of shellfish consumption by the Portuguese population. To date, only a few studies have assessed consumer's motives and barriers to seafood consumption, however, studies approaching the consumption of shellfish, namely sea urchin, have not yet been reported. Letarte, Dube, and Troche (1997) have been studying the relationship between seafood consumption and attitudes, Olsen (2003) has directed his efforts to health and diet beliefs, convenience and socio-demographic characteristics, while Myrland, Trondsen, Johnston, and Lund (2000) assessed the role of lifestyle.

The aim of this paper is to explore and understand Portuguese consumers' preferences and consumption habits regarding shellfish, and also consumer's motives and barriers to shellfish consumption, since understanding the factors influencing consumer choices is the key to the valorisation of sea urchin products.

2. Methods

2.1. Participants

The study was carried out in Portugal with 309 participants using a convenient, intentional and reasoned sampling. Respondents were selected through a convenience structured sample, with defined quotas for sex, age group and education level. Participants were recruited from Sense Test's consumer database (a sensory lab company).

2.2. Questionnaire

The questionnaire presented the following dimensions: (i) free-word association exercise, conceptualising shellfish and sea urchin; (ii) frequency and type of shellfish consumed; (iii) last time consumed. Specifically, participants were invited to write down the first four words that came to mind when they think about shellfish and sea urchin. This approach aimed to identify consumers' spontaneous associations with shellfish and

particularly with sea urchin. They described the verbal stimulus “shellfish” and “sea urchin”, using words, terms, sensations, associations or feelings that came to mind (Esmerino et al., 2017). Moreover, the consumers were also asked to answer the variety seeking (VARSEEK) and food neophobia (FNS) scales. Socio-demographic characteristics were also collected, namely, gender, age, education level, occupation type and residence municipality.

2.3. Statistical Analysis

Descriptive analysis was applied to characterise the participants, using frequency tables to systematise the results. For the word association task analysis, a first revision of each elicited word was performed to correct for typos and misspelt words. Data were analysed by grouping the responses into exclusive categories. Categories mentioned by at least 5% of the consumers were considered for further analysis. The answers were analysed according to the frequency of mention of the words in each category. A correspondence analysis (CA) was applied to obtain a two-dimensional representation of the frequency of consumption and the categories from the word association task. Regarding FNS, participants were divided into three groups using the 33rd and 66th percentile points as cut-off points: participants who scored lower than 18 were identified as “neophilics”; who scored higher than 34 as “neophobics”; and who scored between 18 and 34 as neither one nor other (“NOTO”) (Ristic, Johnson, Meiselman, Hoek, & Bastian, 2016). For the VARSEEK scores, the same methodology was used: participants who scored lower than 39 were classified as “low variety seekers (LVS)”; who scored more than 51 as “high variety seekers (HVS)”; and who scored between 39 and 51 as “medium VS (MVS)”. The existence of significant differences in the overall consumption frequency, FNS and VARSEEK of the consumers was analysed by chi-square test, using XL-STAT 2020® software.

3. Results and discussion

Factorial analysis was performed based on the frequency of consumption to define shellfish clusters (Table 1). Clam, shrimp, mussel and cockle were defined as common shellfish; goose barnacle, velvet crab, limpet and sea snail were defined as special shellfish; and oyster and sea urchin as gourmet shellfish. The majority of common shellfish, mainly shrimp and clam, are the ones with the highest consumption rates, while special and gourmet shellfish groups presented the lowest consumption rates. Most of the participants tended to have a low or moderate consumption of shellfish. Regarding the consumption of shellfish, 98.7% of the participants eat less than one serving of shellfish per week, falling below the recommendations of two to three servings of seafood per week (World Health Organization, 1990). Moreover, only 8.7% and 0.3% of the sample had a moderate and

high consumption of sea urchin, respectively, despite the campaign efforts to increase this product consumption and valorisation. Therefore, these results suggest that product valorisation campaigns might be necessary in Portugal to change dietary choices towards the consumption of gourmet products like sea urchin. In China and Japan sea urchin gonads have long been considered to be a nourishment for elder people and expectant mothers, because of their high content of vitamins, highly unsaturated fatty acids, and microelements (Liu & Chang, 2015). Hierarchical cluster analyses (HCA) was used on the attitudinal questionnaire to identify groups of consumers with similar food choice criteria, which resulted in three different clusters: low (LOW; 147 participants), high (HIGH; 73 participants) and high gourmet (HIGO; 89 participants) shellfish consumption (Table 2). The LOW group were composed mainly by middle age females with secondary or higher education level and had the lowest shellfish consumption level. HIGH is predominantly constituted by middle-age males and females with an intermediate education and had a high shellfish consumption. The HIGO group is also formed by middle-age males and females with secondary or higher education level and had a high consumption of gourmet shellfish products.

Table 1 - Food frequency consumption of different shellfish.

Shellfish (<i>Marisco</i>) (explained variance; alpha de Cronbach (α)) KMO = 0.893	Average consumption per month (Mean $\pm$ SEM)	Loadings
<i>Common</i> (<i>var</i> = 50.5%; α = 0.860)		
Mussel (<i>Mexilhão</i>)	1.38 $\pm$ (0.09)	0.761
Cockle (<i>Berbigão</i>)	1.28 $\pm$ (0.08)	0.746
Shrimp (<i>Camarão</i>)	2.42 $\pm$ (0.09)	0.797
Clam (<i>Amêijoia</i>)	1.99 $\pm$ (0.10)	0.874
<i>Special</i> (<i>var</i> = 13.9%; α = 0.849)		
Sea snail (<i>Búzio</i>)	0.31 $\pm$ (0.04)	0.640
Limpet (<i>Lapa</i>)	0.34 $\pm$ (0.04)	0.689
Velvet crab (<i>Navalheira</i>)	0.32 $\pm$ (0.04)	0.830
Goose barnacle (<i>Percebe</i>)	0.47 $\pm$ (0.06)	0.772
<i>Gourmet</i> (<i>var</i> = 7.3%; α = 0.640)		
Oyster (<i>Ostra</i>)	0.30 $\pm$ (0.04)	0.700
Sea urchin (<i>Ouriço-do-mar</i>)	0.15 $\pm$ (0.03)	0.853

A total of 179 and 260 different terms were mentioned when consumers thought about shellfish and sea urchin, respectively. Regarding the former, the most frequently mentioned words were *Shellfish* ($n = 109$), *Shrimp* ($n = 104$) and *Fresh* ($n = 72$), while for sea urchin the words *Spine* ($n = 161$), *Sea* ($n = 88$) and *Beach* ($n=45$) were the most cited words. As shown in Figure 1, *Shrimp* was clearly the most frequently mentioned word in the LOW and HIGO consumers' segments, while *Shellfish* was the most relevant word for consumers with high shellfish consumption (HIGH). Compared to the rest of the groups, the

LOW group gave more relevance to the words *Clam*, *Crab sticks*, *Fresh* and *Mussel*. Concerning sea urchin, *Spine* and *Sea* were the most significant words in consumers with low consumption profile, whereas *Tasty* was the most relevant word in the consumers with high gourmet shellfish consumption habits.

Table 2 - Sociodemographic characteristics of participants (n=309).

	Consumers' segmentation (%) ¹			
	Global	LOW	HIGH	HIGO
<i>Genre</i>				
Female	63.8	52.8	23.3	23.9
Male	36.2	38.4	24.1	37.5
<i>Age</i>				
18-29	20.4	60.3	20.6	19.1
30-54	61.2	48.1	20.7	31.2
55 and older	18.4	31.6	36.8	31.6
<i>Education level</i>				
Basic	20.1	37.1	33.9	29.0
Secondary	46.6	47.2	22.2	30.6
Higher (college)	33.3	26.2	19.4	54.4

¹ LOW – low shellfish consumers; HIGH – high shellfish consumers; and HIGO – high gourmet shellfish consumers.

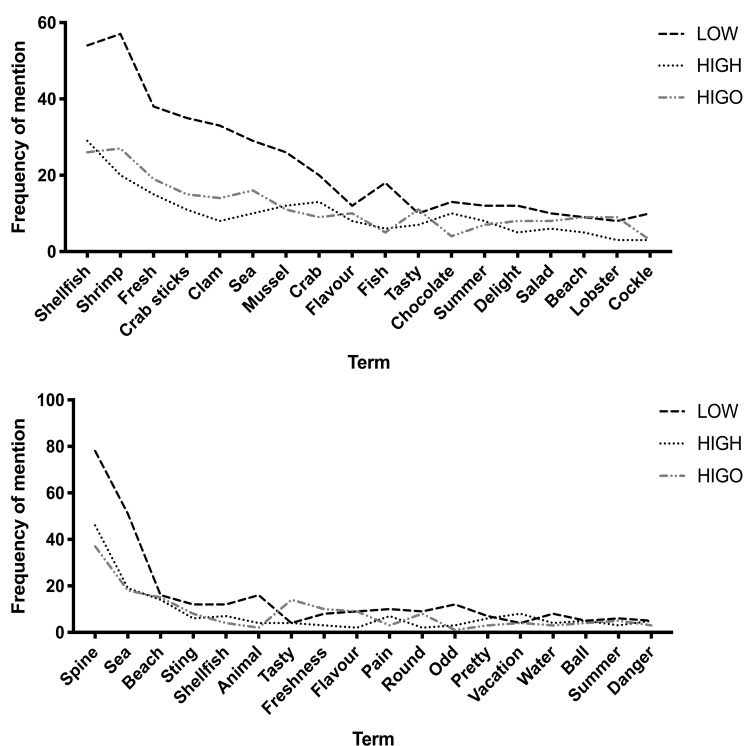


Figure 1 - Frequency of mention of the 18 most frequently words mentioned in the word association task relating to the consumers' segmentation: shellfish (left) and sea urchin (right). Abbreviations stand for: LOW – low shellfish consumers; HIGH – high shellfish consumers; and HIGO – high gourmet shellfish consumers.

Individual words concerning shellfish and sea urchin were grouped into 10 and 14 categories, respectively. As shown in Table 3, in all the consumer segments, the Shellfish

and Sensorial categories were the most salient when participants thought of shellfish. The most relevant words within the *Shellfish* category were *Shrimp*, *Shellfish*, *Clam* and *Mussel* whereas within the *Sensorial* category they were *Fresh*, *Flavour*, *Tasty* and *Delight*. All the words elicited within the *Sensorial* dimension had a positive connotation when thinking of shellfish. The category *Emotions* was more salient in HIGO compared to the rest of the segments, while the category *Other products* was less salient, when participants thought of shellfish. Moreover, for the LOW group the *Emotions* category was less salient, suggesting an extrinsic relationship towards these products. Indeed, in Figure 2, LOW males were distributed closer to the categories *Sea products* and *Shellfish* and LOW females with *Shellfish*, while HIGH females were associated with the categories *Health*, *Well-being*, *Preparation methods*, *Consumption moments* and *Other products*. HIGH males were correlated with *Sensorial*. Besides, HIGO females were associated with the categories *Sensorial*, *Emotions* and *Other features*, whilst males were linked with *Nature*, *Sea products* and *Shellfish*.

The *Sensorial* category was the most frequently mentioned among the three consumer segments when participants thought of sea urchin (Table 3). The most relevant words within the *Sensorial* category were *Tasty*, *Fresh*, *Round* and *Flavour*. The second most salient category in the three segments was *Appearance*, which was mainly related to *Spine*, *Sting* and *Shell*. *Spine* and *Sting* were elicited as being negative and could be related to the unknown or dangerous aspects when conceptualising sea urchin. The remaining categories were clearly less mentioned. It is interesting to highlight that some participants in all segments mentioned words related to *Health* and *Well-being* when thinking of shellfish or sea urchin. When participants thought of sea urchin, the frequency of mention of the categories *Other products* and *Preparation methods* were higher in HIGO than in the rest of segments, while for the category *Unknown* was lower (Table 3, Figure 2). Furthermore, for the HIGO female group, the categories *Other products* and *Preparation methods* were more important than the others, whereas HIGO males made associations with the categories *Sensorial* and *Appearance*. This could suggest that the HIGO group had a deep knowledge about the product, referring to terms such as sensory characteristics, quality, and also the cuisine techniques to prepare it. The HIGH female group mentioned positive *Emotions* connotations, which is in agreement with the fact that hedonic aspects of food consumption have been recognized as key determinants of consumer choice and perception of the product (Lim, 2011). On the other hand, participants with low consumption of shellfish and sea urchin emphasised negative *Emotions* and *Appearance* attributes respectively, explaining the reasons for the low consumption. Food-elicited emotions have also been reported to be highly relevant for shaping preference patterns (Canetti, Bachar, & Berry, 2002; Macht, 2008). High shellfish female and sea urchin male consumers elicited

relevant associations with *Health/Well-being* when thinking about these products. The concept of well-being is directly correlated with quality of life and has been used to demedicalise the concept of health (Statham & Chase, 2010). Furthermore, pleasant emotions, elicited by the HIGH group in sea urchin categories, have been reported to be key determinants of subjective well-being (Diener, Oishi, & Lucas, 2003). Indeed, HIGH stated *Emotions* as one of the most important categories, but LOW emphasised the *Unknown* as one of the main categories. This could suggest that participants with high consumption patterns had a high level of familiarity with this gourmet product, while the LOW group had poor knowledge concerning sea urchin products. However, inexplicably, the HIGH cluster was associated with *No-reference* category (NR). Moreover, LOW is associated with *Exclusivity* of the product, mainly due to the price of sea urchin that can easily reach the value of 60\$/kg depending on the market and species (Unuma, Sakai, Agatsuma, & Kayaba, 2015). Thus, differences in the associations mentioned by participants from different segments can be explained by differences in consumers' relevant values and socioeconomic contexts, as previously reported for perceived general wellbeing (Diener et al., 2003). Moreover, when consumers thought of shellfish or sea urchin, the most relevant associations were sensory aspects, namely those related to both intrinsic product characteristics (colour, taste, appearance) and extrinsic characteristics (spines, colour, shape). Further research is necessary to understand how the interaction between product characteristics, personal traits and context could determine consumers' perception of these shellfish products. It is also important to mention that small differences in the sociodemographic characteristics in the three consumer segments could have been partly responsible for some of the identified differences in the associations regarding shellfish and sea urchin. Due to cultural factors, gender's importance not only concern the preferences, but also the consumption frequencies (data not shown). Indeed, females had more associations with psychological and physical categories such as *Health* and *Well-being*, as well as *Emotions* and *Consumption moments* when compared to males. However, males had a higher shellfish consumption than females, as already previously described by Lopes et al. (2017).

Table 3 - Frequency of mention of the dimensions identified in the word association task relating to the consumers' segmentation: shellfish (above) and sea urchin (below). Results from chi-square per cell test.

Category	Word Examples	Global	Consumers´ segmentation ¹		
			LOW	HIGH	HIGO
Word association to shellfish					
Shellfish	Mussel, shrimp	431	226	94	111
Sensorial	Flavour, delight	269	117	65	87
Sea products	Crab sticks, algae	133	72	24	37
Health/Well-being	Rest, vacation	91	40	26	25
Other products	Guylian chocolate, rice	80	40	27	13 (-)**
Nature	Sea, sand	77	36	13	28
Preparation methods	Grilled, raw	75	33	22	20
Emotions	Pleasure, joy	39	10 (-)**	9	20 (+)**
Other features	Expensive, luxury	21	8	4	9
Consumption moments	Dinner, friends	14	5	8	6
Word association to sea urchin					
Sensorial	Tasty, fresh	321	148	71	102
Appearance	Spine, shell	216	103	61	52
Nature	Rocks, water	174	90	40	44
Shellfish	Shellfish, crab	103	54	14 (-)**	35
Emotions	Pain, curiosity	97	39	33 (+)*	25
Health/Well-being	Summer, beach	88	31 (-)*	28	29
Sea products	Coral, molluscs	50	29	7	14
Animals	Snails, starfish	42	26	8	8
Unknown	Unknown	33	27 (+)***	6	0 (-)***
Consumption moments	Family, friends	27	9	9	9
Preparation methods	Grilled, sauce	24	9	0 (-)***	15 (+)**
NR ²	-	22	8	10 (+)*	4
Other products	Tangerine, lemon	21	6 (-)*	1 (-)**	14 (+)***
Exclusivity	Rare, expensive	17	8	4	5

Effect of the chi square per cell. (+) or (-) indicate that the observed value is higher or lower than the expected theoretical value: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

¹LOW – low shellfish consumers; HIGH – high shellfish consumers; and HIGO – high gourmet shellfish consumers. ²No reference.

Regarding personal traits, HIGO had significantly higher neophilic values than LOW or HIGH, while neophobic values were highest in LOW (Table 4). There were also a higher number of participants with a low shellfish consumption profile being classified as LVS and a higher percentage of high gourmet consumption participants who were categorised as HVS. The participants with low levels of food neophobia were significantly more willing to taste shellfish products than participants with high levels of food neophobia. Indeed, the consumers' willingness degree increased with decreasing levels of neophobia. Thus, consumers with a high shellfish consumption profile are more likely to display adventurous behaviour in purchasing and consuming unfamiliar shellfish products compared to those with low exposure and involvement with shellfish products, as it was found in several food

studies (Hayes & Pickering, 2012; Ristic et al., 2016). This is in accordance with the lower FNS and higher variety-seeking values of the HIGO consumers in Table 4. There were no significant gender differences in the shellfish neophobic and variety-seeking segments (Table 4). Participants with ages between 30 and 54 returned a significantly lower FNS and higher VARSEEK values than their younger and older counterparts. However, shellfish-related neophobia did not follow a regular increase with age, as reported in other food studies (Ristic et al., 2016; Tuorila, Lähteenmäki, Pohjalainen, & Lotti, 2001). In this study, education was segmented into three groups, namely basic, secondary or higher education. There was a higher proportion of neophiliacs with secondary and higher education than neophobics, indicating that there was a negative correlation between neophobia status and education. Participants with basic education presented higher mean FNS score and lower variety-seeking values than those with tertiary education, though without statistically significant differences. The decreasing pattern of neophobia and increasing pattern of willingness to try new products has been associated with participants having greater access, knowledge, experience and exposure to novel foods and with increasing education level (Tuorila et al., 2001).

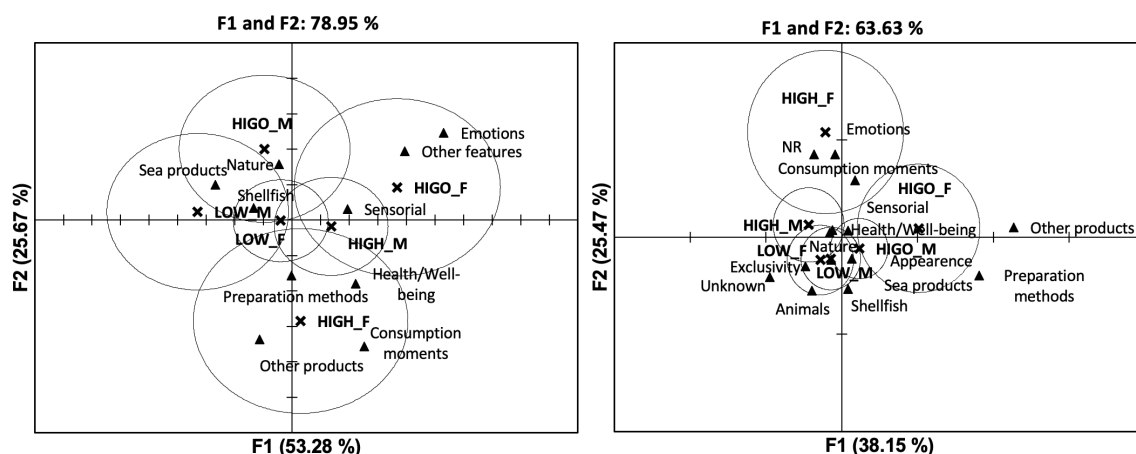


Figure 2 - Representation of the categories, consumers' segments and gender in the first and second dimensions of the correspondence analysis: shellfish (left) and sea urchin (right). Abbreviations stand for: LOW – low shellfish consumers; HIGH – high shellfish consumers; HIGO – high gourmet shellfish consumers; F – female; and M – male.

In conclusion, considering the status of psychological and physical *Health* and *Well-being*, as well as positive *Emotions* regarding shellfish, health-promoting strategies could help in the recognition of the effects of shellfish, on health and change food selection patterns towards healthier and gourmet dietary choices. This study allowed to characterize the main patterns of the seafood consumption in Portugal, while unveiling the conceptualizations associated with sea urchins. It will be useful for the aquaculture industry, as well as for the consumer science community as the information obtained can be used to improve the sea urchin marketing and commercialization.

Table 4 - Proportion of participants in neophobic (left) and VARSEEK (right) segment by consumers' segmentation and demographics (n = 309). * $p < 0.05$, ** $p < 0.01$.

	FNS ¹	Neophobic segment			χ^2	VARSEEK ³	VARSEEK segment ⁴			χ^2
	(mean $\pm$ SEM)	Neophilic [< 18]	NOTO ² [18 to 33.8]	Neophobic [> 33.8]		(mean $\pm$ SEM)	LVS [< 39]	MVS [39 to 51]	HVS [> 51]	
<i>Consumers' segmentation⁵</i>					15.379**					14.367**
Global	26.0 $\pm$ 0.6	73	158	78		44.0 $\pm$ 0.5	75	150	84	
LOW	28.1 $\pm$ 0.8 ^a	21	83	43		41.6 $\pm$ 0.8 ^b	44	76	27	
HIGH	24.9 $\pm$ 1.2 ^{ab}	21	33	19		45.4 $\pm$ 1.1 ^b	17	29	27	
HIGO	23.6 $\pm$ 0.9 ^b	31	42	16		46.7 $\pm$ 0.7 ^a	14	45	30	
<i>Genre</i>					0.988					0.994
Female	26.4 $\pm$ 0.8	47	97	53		43.9 $\pm$ 0.7	48	92	57	
Male	25.4 $\pm$ 0.9	26	61	25		44.0 $\pm$ 0.8	27	58	27	
<i>Age</i>					11.642*					3.923
18-29	27.2 $\pm$ 1.4 ^a	11	36	16		42.0 $\pm$ 1.2 ^b	17	33	13	
30-54	24.8 $\pm$ 0.7 ^b	48	102	39		45.1 $\pm$ 0.6 ^a	41	90	58	
55 and older	28.7 $\pm$ 1.5 ^a	14	20	23		42.2 $\pm$ 1.3 ^b	17	27	13	
<i>Education level</i>					14.441**					2.566
Basic	28.5 $\pm$ 1.3	13	22	27		42.0 $\pm$ 1.4	19	30	13	
Secondary	25.0 $\pm$ 0.8	34	81	29		44.4 $\pm$ 0.7	33	71	40	
Higher (college)	26.1 $\pm$ 1.0	26	55	22		44.5 $\pm$ 0.9	23	49	31	

¹FNS – Food Neophobia Scale; ²NOTO - neither one nor other; ³VARSEEK – variety seeking scale; ⁴LVS - low variety seekers; MVS – medium variety seekers; HVS – high variety seekers;

⁵LOW – low shellfish consumers; HIGH – high shellfish consumers; and HIGO – high gourmet shellfish consumers.

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Chapter IV.

Dimensions for the valorisation of sea urchin (*Paracentrotus lividus*) gonads production through the eyes of experienced chefs

by

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Abstract

The main goal of this research was to explore and understand Chefs' perceptions regarding sea urchin gonads sensorial features, production and use, with an emphasis on echinoculture. This study used a mixed methodology, where three groups of chefs were interviewed: Michelin star, from Ericeira and from Algarve, these being the two main regions of sea urchin consumption.

During the interviews, Chefs exposed that there is some apprehension regarding the consumption of sea urchin mainly due to their appearance. Moreover, this product is yet to be explored in Portugal where it still sits in a niche market, emphasizing the need to advertise benefits and sensory pleasure that this product could provide. Furthermore, the existent preconception and suspicion about aquaculture products and their acceptance should be countered, since the future availability of these gourmet products and other seafood commodities with high-quality will be dependent on their production in sustainable culture systems. The Chefs also performed sensory evaluation through the application of Projective Mapping with Ultra Flash Profiling, showing that sea urchin gonad's sensory properties were mainly influenced by sex.

These results emphasize the need for communication and marketing strategies through locals, chefs and gastronomic events, focused on the promotion of consumers awareness about the exclusivity of this product and the importance of aquaculture for the future of the seafood market.

Keywords: gonads; interviews; Michelin star Chefs; projective mapping; sensory features.

1. Introduction

In recent years, there is a growing consumers' interest in sea urchin gonads for their exclusive sensory traits (Lourenço et al., 2019) that influences their market price and consumer acceptability (Sun and Chiang, 2015). Sea urchin gonads are also a good source of high-quality protein, long chain polyunsaturated omega-3 and micronutrients, and are low in saturated fat (Baião et al., 2019, Rocha et al., 2019). But FAO (2020b) has recently reported that worldwide sea urchins capture decreased by almost 50% from the peak in 1995, reaching 58,528 tonnes (live weight) in 2018, as sea urchin stocks were overfished, leading to the collapse of native stocks. Live and fresh sea urchin dominate the global trades volume with an average price per kilo of €9, followed by the frozen sea urchin gonads that had an average price of €30 (FAO, 2020a). In Southern Europe, the most exploited and economically important species is *Paracentrotus lividus*. Its edible gonads are a prized and gourmet seafood delicacy, being one of the world's costliest seafood products (Boudouresque and Verlaque, 2020). Currently, the increasing demand for sea urchin

gonads has resulted in the intense harvest of *P. lividus* in Europe (Boudouresque and Verlaque, 2020). In Portugal, the commercial harvesting of this species is increasing as observed by the raise in landings from 60 to 170 tonnes, between 2016 and 2018, mainly for exportation to Spain, Italy and France (FAO, 2020a). Although Portugal is one of the European Union countries with higher average annual per capita consumption of seafood (55.9 kg), and a huge Exclusive Economic Zone (1,700,000 km²) and coastline (942 km), which gives prominence to fishery and fish products consumption (FAO, 2020a), this caviar-like product is yet under-consumed mainly because it is not part of the national gastronomy culture, except in some local villages, like Ericeira, in the Centre, and a few in Algarve. However, new emerging markets outside the traditional markets will appear in the next few years, because sea urchin is being seen as a novel, trendy and healthy product, whose interest has been increasing in several countries throughout Europe (Stefánsson et al. 2017). In this context, the Portuguese sea urchin market has a greater potential to develop and follow other Mediterranean countries trend (Stefánsson et al., 2017). Moreover, it is latent the increased importance and influence that Celebrity Chefs have on customers' behaviour, during the last few years. The role of Chefs is changing in our society, allowing for the valorisation of gourmet products (McBride and Flore, 2019, Manso and Moura, 2017, Chen et al., 2017). More recently, the European Commission has launched a campaign named 'Taste the Ocean', in which top Chefs promote the consumption of sustainably caught or produced fish and seafood (European Commission, 2021), thus emphasizing the interest of involving Chefs in the process of promoting sea urchin gonads.

The continued growth of sea urchin gonads consumption around the world boosted the interest in aquaculture to supply market demands (Sartori et al., 2016). Although aquaculture practices allow for the production of market-sized sea urchins throughout the year, their market value and consumer acceptability may be compromised. Therefore, an integrated approach is needed to fill the gap between consumers' demands and aquaculture products. For instance, the sensory features sought by the consumer can be met with the inclusion of new alternative additives in the aquafeed (Calanche et al., 2019) and the aquaculture acceptability could be improved through marketing and media communication (Olsen and Osmundsen, 2017).

Considering the role of Chefs in society, with an impact on the food debate in the media and society (Manso and Moura, 2017), one way to push sea urchin as being a novel and trendy product, obtained sustainably, is marketing it through local Chefs (Grisolía et al., 2012). Grisolía et al. (2012) showed that in the absence of previous experience with the product, a favourable opinion from a well-known Chef increases the interest in the product. It has become clear that the success of commercial sea urchin, its economic viability and market acceptance, strongly depend on the deeper knowledge of the Chefs' and

consumers' preferences and patterns of sea urchin consumption. In this scope, the main goal of this research is to evaluate the Chefs' perceptions regarding sea urchin gonads sensory features, production and use, with an emphasis on the future aquaculture production of the delicate *P. lividus*.

2. Material and methods

A mixed methodology (Guerrero and Xicola, 2018) was applied, combining a qualitative methodology (interviews) with the application of Projective Mapping paired with Ultra Flash Profiling (Pagès, 2003).

2.1. Interviews

Taking into account the exploratory nature of the subject, a face-to-face semi-structured interview was carried out with the Chefs to obtain in-depth descriptions of their opinions, perspectives and motivations towards sea urchin attributes and culinary applications. The criterion to select these participants was based on the idea that people in charge of the entire restaurant and kitchen and particularly accountable for all kitchen operations are the most important source of information on how to create, and design potential new dishes and menus made from sea urchin. In this context, Portuguese Michelin-star Chefs (MsC), Chefs from the Ericeira (EriC) and the Algarve region (AIC) were interviewed. The first were chosen for their exquisite trend-setting cuisine, while the Chefs from the Ericeira and Algarve region were interviewed considering their vast knowledge about the product and cultural uses for cooking it. Ericeira is a village located 35 km northwest of the capital of Portugal, Lisbon, presenting a vast wild sea urchin population, with a long-standing tradition of culinary exploitation of this delicacy, with the own name of the village deriving from the Portuguese name for sea urchins: *ouriço-do-mar* (Santos, 1998). In the South coast of Portugal, the Algarve region, it is common to harvest and consume seafood, including the sea urchin.

A semi-structured interview guide of open-ended questions was developed taking into account three dimensions, regarding sea urchin subjects: i) selection criteria and culinary applications ii) consumers' perception, and iii) Chefs' perception of aquaculture. Chefs were interviewed individually by the first author, at a time and place of their choice, usually at their restaurants, between March 30th and April 7th, 2018. Interviews lasting, on average 23.40 ($\pm$ 7.32) min, were voice and image recorded and then transcribed verbatim, following informed consent given by all participants.

The transcripts of the interviews were explored in a qualitative data analysis software, QSR NVivo12 (Copyright® QSR International Pty Ltd, Melbourne, Australia). Interviews were analysed using a thematic analysis procedure that involves a progression

from description to interpretation data (Braun and Clarke 2006). A comprehensive process of data coding and identification of dimensions, consistencies and discrepancies across dimensions was undertaken and explored to provide an in-depth understanding of the texts (Patton, 1990). To illustrate the analysis, direct quotes by the Chefs were transcribed, serving as a description of the topic explored. The quotes used in this text were translated into English.

2.2. Descriptive sensory analysis

2.2.1 Sea urchin samples

Two different officially reported harvest locations along the Portuguese coast were selected for this study: Viana do Castelo, North Region of Portugal [41°41'49.8"N 8°51'04.5"W] and Ericeira, Central Region of Portugal [38°57'40.3"N 9°25'13.1"W]. Both areas are semi-exposed to ocean swells and the annual seawater temperature ranges from 12 to 20 °C in Viana do Castelo and from 14 to 22 °C in Ericeira. To assure the required number of specimens for this study, sixty fresh and alive *P. lividus* (with around 5 cm diameter and 80 g) were simultaneously harvested on rocky intertidal shores in each of the selected places and kept at 6 °C until analysed.

The sea urchins were maintained at 6 °C during the transportation and processing, following most common procedures (Kelly et al., 2015) and were served whole to Chefs at 15 °C, to increase the aromatic intensity, in transparent PET containers, blind-labelled with random three-digit codes.

2.2.2 Projective mapping with Ultra Flash Profiling

Each Chef analysed a total of eight sea urchins, four from each harvesting location, evaluating their external appearance and odour. Further on, he/she opened the tests with a kitchen scissor and carefully removed the five gonads of each sea urchin using a spoon. These were washed in saline water (36 ‰) and placed in a white porcelain plate (12 cm) so that the Chef could further analyse the appearance, taste, odour and texture. The sea urchins' sex was evaluated through visual appearance by the main researcher: live males presented a pale-yellow colour and oozed a white liquid through the gonads, while the live females presented an orange colour and oozed an orange liquid through the gonads (Baião et al., 2021a, Baião et al., 2020).

Projective mapping was carried out by the Chefs in a piece of A1 paper (59.4 x 84.1 cm), according to the methodology described in Baião et al. (2020), except for the Ultra-Flash Profiling, where the Chefs were asked to generate sensory descriptors of the individual gonads, or groups, by directly dictating to the first author of this paper, also describing the differences between the samples. Multiple Factor Analysis (MFA) was

performed to compare the product position on the profiling maps produced by the projective mapping and the ultra-flash profile binary data, identifying if a particular attribute was associated with a given sample (Louw et al., 2013, Nestrud and Lawless, 2008), using XLSTAT 2020 (Addinsoft, USA).

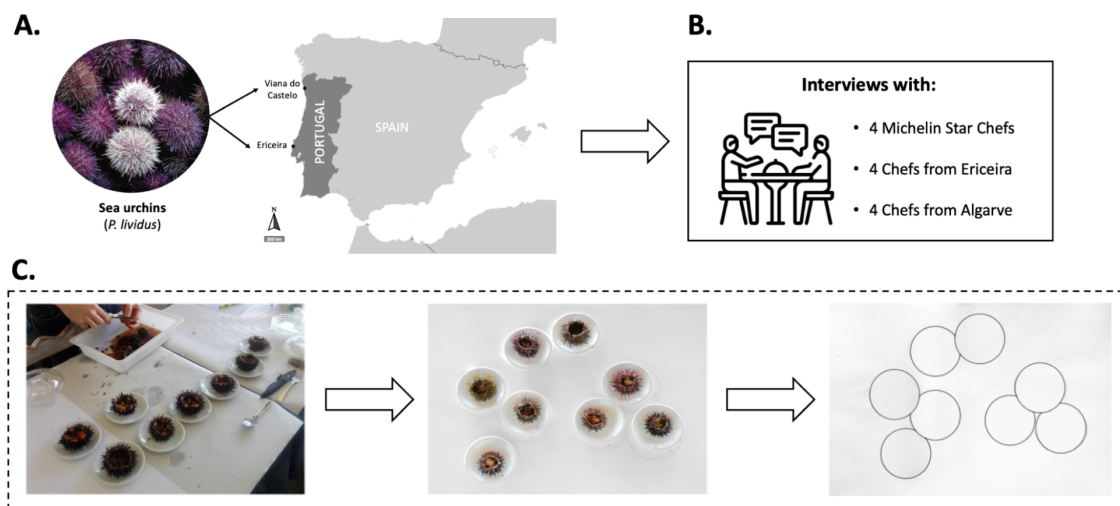


Figure 1 - Scheme of sampling procedures of sea urchins (A), chefs' interviews (B) and Projective mapping paired with Ultra-Flash Profile (C). For each sample, the Cartesian coordinates (x_i and y_i) were calculated by measuring the distance (cm) relative to the left and bottom edges of the sheet.

3. Results and discussion

All of the four MsC were Executive Chefs in restaurants with an author type cuisine. On the other hand, all four EriC were Chefs in restaurants with a traditional type of cuisine, three of them owning the restaurant. Of the four AIC, one was Executive Chef in a restaurant with an author type cuisine, and the other three were Chefs in restaurants with a traditional type of cuisine, with one of them being the owner of the restaurant (Table 1). To note that Executive Chef is the individual who prepares the dishes and organizes all the kitchen of the restaurant, besides supervising the services of the cooks and planning menus. It is relevant to note that despite Viana do Castelo being one of the harvest locations in Portugal, there is no tradition in the region's restaurants to serve this delicacy, with most of the harvest being exported to the North of Spain (FAO, 2020a) and therefore, Chefs from the region were not included.

Table 1 - Characterization of the restaurants and Chefs' work situation.

Participant	Work bound	Chefs	Type of establishment/Cuisine	Origin	Nº Staff/Nº Cooks
P1	E.C	MsC	Restaurant/Author	North of Portugal (Oporto)	6/2
P2	E.C.	MsC	Restaurant/Author	North of Portugal (Oporto)	20/3
P3	E.C.	MsC	Restaurant/Author	North of Portugal (Oporto)	6/2
P4	E.C.	MsC	Restaurant/Author	North of Portugal (Oporto)	20/3
P5	C / O	EriC	Restaurant/Traditional	Central of Portugal (Ericeira)	4/1
P6	C / O	EriC	Restaurant/Traditional	Central of Portugal (Ericeira)	12/2
P7	C	EriC	Restaurant/Traditional	Central of Portugal (Ericeira)	8/2
P8	C / O	EriC	Restaurant/Traditional	Central of Portugal (Ericeira)	12/2
P9	C	AIC	Restaurant/Traditional	South of Portugal (Algarve)	8/3
P10	E.C.	AIC	Restaurant/Author	South of Portugal (Algarve)	10/2
P11	C	AIC	Restaurant/Traditional	South of Portugal (Algarve)	8/3
P12	C / O	AIC	Restaurant/Traditional	South of Portugal (Algarve)	8/3

Abbreviations are: Algarve Chef (AIC), Chef (C), Executive Chef (E.C.), Ericeira Chef (EriC), Michelin star Chef (MsC) and Owner (O).

Table 2 - Main categories that emerged from Chefs interviews transcripts analysis, regarding d culinary applications of sea urchins and sea urchin and aquaculture perceptions.

Category	Examples of sample quotes		
	MsC (n=4)	EriC (n=4)	AIC (n=4)
Culinary practices (230 references)			
Sensory attributes (42 references)	<i>"it is important to taste and smell the sea and iodine"</i> [P3] <i>"they have a very strong taste, very intense"</i> [P2] <i>"the inner aspect is not inviting and then the taste is to strong"</i> [P1] <i>"feel the essence of the sea"</i> [P4] <i>"the bright orange colour with a firm texture are the better ones"</i> [P3] <i>"some people like it a lot and some people do not like it, because of the intense flavour"</i> [P2] <i>"This animal entails different negative myths, for instance that is not good or that is too strong, and this idea went through generations. Indeed, I remember that this animal was used to play because of the spines, but was not seen as edible animal"</i> [P1]	<i>"I rather a bitter, salty and iodine flavour and odour"</i> [P5] <i>"intense and bittersweet flavour"</i> [P6] <i>"explosion of flavour"</i> [P7] <i>"it is known to have a characteristic flavour and a bright orange colour"</i> [P7] <i>"there is a preconception relatively to this product and a lot of my clients have afraid to try it"</i> [P6] <i>"It is not a product that is widely advertised in Portugal, people are a little afraid of consuming it. We have to evangelize the people for the consumption of sea urchin"</i> [P8] <i>"Spanish and French markets essentially of Europe are very demand of this type of product. But in Portugal (...), although this situation is being overcome with the Ericeira sea urchin festival"</i> [P5] <i>"the public is not yet educated for this. This turns out to be a very exotic product yet (...) not the microbiological risk, but the total ignorance"</i> [P8]	<i>"dive in the sea"</i> [P10] <i>"texture similar to caviar"</i> [P12] <i>"creamy and firm texture"</i> [P9] <i>"colour determines acceptance because is correlated with flavour intensity"</i> [P11] <i>"... a spectacular element that immediately gives us the sea in each dish and well accepted by avant-garde customers ... it has a very strong flavour and some more conservative customers do not accept or dislike it"</i> [P12]
Culinary applications (92 references)	<i>"I use this caviar to give irreverence to my dish"</i> [P3] <i>"secondary element that ends up having a big statement in the mouth and plate (...) a simple sauce is sufficient to demonstrate a great magnificence in the plate"</i> [P1]	<i>"Ericeira comes from the Ouriceira word (sea urchin – ouriço in Portuguese) and it is part of our tradition to use sea urchin in the preparation of our recipes"</i> [P8] <i>"The sea urchin has a leading role in my dishes. The main ones are the ones I've told you about, the sea urchin risotto"</i> [P5] <i>"au natural gonads for entree recipes"</i> [P5 and P6]	<i>"I use sea urchin in my plates to enhance the flavour and also to give the caviar texture experience to my customer"</i> [P12] <i>"transfer the sea flavour to the dish"</i> [P10] <i>"we use sea urchin predominantly au natural and to enhance the flavour of the sauce"</i> [P11]

Category	Examples of sample quotes		
	MsC (n=4)	EriC (n=4)	AIC (n=4)
	<i>"I do not use sea urchin as the main element of the dish, but as a supplement to transfer the sea flavour to the dish" [P2]</i> <i>"welcome appetizer to the customer" [P4]</i> <i>"it's a secondary ingredient, but it stands up with splendour in the dish" [P1]</i> <i>"it is normally used in crème brûlée, cocktail and appetizers" [P3]</i>	<i>"we use this product to desserts, risotto, sushi steamed ... and we also apply it in rice and açorda, a typical Portuguese dish" [P5]</i> <i>"... only one minute on the steam to increase the firmness" [P6]</i>	
Risk perception (23 references)	<i>"it is a sensitive product that needs to be handle with extra care" [P3]</i> <i>"as other shellfish, sea urchin has a very short shelf-life" [P4]</i> <i>"I only work with reputable and serious suppliers because I am aware of the risks to my customers" [P2]</i> <i>"it is necessary to keep them fresh along the process of harvesting, transport and storage in the restaurant" [P2]</i>	<i>"I work with sea urchin for about 20 years and It was never reported any incidents with this product in Ericeira" [P5]</i> <i>"... as long as they are properly captured and preserved" [P8]</i>	<i>"I do not use it when toxins are reported in Sagres or other nearby beaches" [P12]</i> <i>"in my restaurant sea urchins with different appearance, colour or smell are eliminated" [P9]</i> <i>"it is important to have controlled and low temperature to keep the sea urchin fresh and alive during transport and storage" [P10]</i> <i>"cooking it certifies the safety of the product, however this type of product requires to be au natural because of its taste and texture" [P11]</i>
Purchase criteria (32 references)	<i>"freshness and buy the product from trusted suppliers" [P1 and P2]</i> <i>"we take into account the brightness and the state of the spines and we use it for three days when correctly acclimatized" [P2]</i>	<i>"the flavour and size of the gonads" [P7]</i> <i>"I harvest the sea urchins myself and I normally use them for a week" [P6]</i> <i>"firstly, I open one to see the size and appearance of the gonads and, then if they seem good enough, I start to harvest the quantity necessary for the week" [P6]</i>	<i>"fresh and alive" [P8]</i> <i>"the exterior appearance (spines and colour) and odour (sea and iodine smell)" [P12]</i> <i>"during the beginning of the year when they present the best organoleptic features" [P9]</i>

Category	Examples of sample quotes		
	MsC (n=4)	EriC (n=4)	AIC (n=4)
Availability (18 references)	<i>"the sea urchin availability is seasonal, and we can have it between February and April" [P3]</i> <i>"sea urchin does not appear in our menu ... the supply is not constant ... we use it as a welcome appetizer"</i> <i>"sometimes we need to use canned sea urchin" [P1]</i>	<i>"there is limitation in the supply of sea urchins" [P7]</i> <i>"i use it in every menu" [P5]</i> <i>"the best organoleptic features are present between November and February" [P6]</i> <i>"sea urchin is a product that only exists a few months of the year, it does not exist all year round ... during the winter months sometimes is difficult to harvest it ... so we make sea urchin dishes only by order" [P8]</i>	<i>"its availability is seasonal, so we try to use it about twice a year" [P10]</i> <i>"even in these months as it is winter the supply is not constant" [P9]</i> <i>"the lack of quality of the product that reaches at the restaurant" [P10]</i>
Aquaculture (94 references)			
Perception of aquaculture (10 references)	<i>"the aquaculture has a bad reputation because of its production methods, nutrition and use of antibiotics" [P2]</i> <i>"the fish are produced like the chicken and that is not good marketing" [P3]</i> <i>"in my opinion fish produced in aquaculture presents lower quality standards in terms of flavour and texture, compared with the wild ones" [P1]</i>	<i>"aquaculture have a bad image ... hormones and antibiotics" [P7]</i> <i>"aquaculture could be positive due to their water quality and nutrients intake control ... to be developed in perfect zootechnic conditions for human consumption" [P8]</i>	<i>"...affect the products texture, flavour and consumers' health" [P10]</i> <i>"fish do not have space to swim what affects negatively its texture ... has more abdominal fat than wild fish" [P9]</i> <i>"... offers quality certification and guarantees the freshness of the product" [P11]</i>
Farmed sea urchin (84 references)			
Acceptance (34 references)	<i>"my consumers and I have a preference for wild seafood" [P2]</i> <i>"fish are produced like chicken and that is not good marketing" [P1]</i>	<i>"I am aware that aquaculture would allow availability throughout the year" [P6]</i> <i>"aquaculture products will always be of inferior quality" [P5]</i>	<i>"customers associate wild fish with better firmness and flavour" [P10]</i> <i>"wild fish are associated with greater health benefits" [P9]</i>

Category	Examples of sample quotes		
	MsC (n=4)	EriC (n=4)	AIC (n=4)
Nutrition (10 references)	<i>"I am aware of the advantage of aquaculture controlling food and growth, however for now wild animal have better organoleptic features" [P3]</i> <i>"I am aware that aquaculture would allow availability throughout the year ... if the quality were similar to wild sea urchins, I would probably start using it" [P4]</i>	<i>"there is a preconception regarding the use of antibiotics and hormones ... my customers do not want aquaculture products because of this preconception" [P6]</i> <i>"aquaculture can offer more food security as they use product tracking methodologies" [P7]</i>	<i>"aquaculture do not know what it is in the water and food that they give to the fish, however an offshore aquaculture is different ... probably they have similar water and food supplies as the wild fish" [P11]</i> <i>"I look for the best product regardless of the source – aquaculture or wild" [P12]</i>
	<i>"aquaculture have a more controlled feeding process, designed to promote the growth of the animal and address the nutritional needs of the animal and consequently the human" [P1]</i> <i>"although aquaculture presents nutritional and control benefits, its products have inferior flavour and texture" [P2]</i>	<i>"aquaculture do not use adequate food for fish ... they use substances as corn, while the wild ones have a more balanced diet" [P6]</i> <i>"aquaculture is positive in the sense that it will control the quality of the water where the sea urchin will be produced as well as the quality of nutrients for the sea urchin to develop in perfect conditions for consumption. Also, could offer quality certification and guarantee of freshness status of the product" [P8]</i>	<i>"aquaculture is improving the fish nutrition however wild seafood will always be better" [P12]</i> <i>"it is the consumer demands that defines the use of wild or aquaculture products" [P10]</i>
	<i>"I look for the best taste, regardless of the value" [P2]</i> <i>"customers can perceive differences between an aquaculture and wild product, mainly in terms of flavour and texture" [P2]</i>	<i>"sea urchin aquaculture probably would negatively affect attributes such as iodine and colour" [P7]</i> <i>"aquaculture can hardly produce something similar and with the same quality as the sea urchins from Ericeira" [P5]</i> <i>"for me the difference between aquaculture and wild is not noticeable" [P7]</i>	<i>"I believe that the wild seafood has more sensory advantages, because the food is different, it is natural and, therefore, the flavour and texture must be better, more natural." [P10]</i>
Sustainability (22 references)	<i>"the development of aquaculture ... would decrease their overexploitation in the wild" [P4]</i>	<i>"it will certainly be more sustainable than over-exploiting wild stocks" [P8]</i>	<i>"we could also preserve the wild state of what exists ... it allows to preserve the environment and species" [P12]</i>

3.1. Interviews

Two broad levels of analysis were identified which combined many dimensions, cutting across the different topics of discussion: i) culinary practice and ii) aquaculture (Table 2). All of the twelve participants reported these two broad levels in their interviews. Each of these themes is described below.

3.1.1. Culinary practices

The culinary practices category gives an overview of how selected Chefs perceive the main factors that drive their costumers to accept or reject sea urchin in their dishes. This may allow us to better understand the criteria used by the Chefs to select, purchase and prepare sea urchin gonads to meet the demand. About 230 references (out of 324) were produced by the twelve participants during the interviews, highlighting the relevance of this subject.

3.1.1.1. Sensory attributes

According to the Chefs' speeches, the consumers' acceptance of sea urchin is not unanimous, because, as referred by one MsC: "some people like it a lot and some people do not like it" [P2]. For our Chefs, consumers have a general feeling of dislike about sea urchin products, particularly due to their "exotic appearance". Appearance features, that comprise different visual properties including colour, shape and texture (Spence 2015b), are the first sensory stimuli presented to consumers that impact the visual assessment and freshness perception of sea urchins' gonads and, consequently, raising expectations about the product (Reisfelt et al. 2009). Or, as referred by one Chef, this is particularly remarkable for customers: "who are going to taste sea urchin for the first time, [often consider] sea urchin as having a repugnant appearance, which is not whatsoever attractive to try it, so they ask to have it cooked" [P6]. On the contrary, consumers who are willing to experiment with new shellfish products are more likely to exhibit exploratory behaviour in purchasing and consuming unfamiliar shellfish products (Hayes and Pickering, 2012, Ristic et al., 2016). Indeed, higher levels of consumers' willingness to try are associated with lower levels of food neophobia, which has been associated with greater access, experience and exposure to new foods (Tuorila et al., 2001).

The EriC considered that the sea urchin market in Portugal is actually in an immature phase and, in general, consumers outside the harvest region (like the case of the Ericeira region) do not know this product. This product is not widely announced in Portugal and people from other places are afraid of consuming it, a fact that was pointed out by EriC: "we have to evangelize the people for the consumption of sea urchin" [P8]. This EriC also mentioned that the annual sea urchin festival in the Ericeira region (occurring between

March and April) promotes sea urchin awareness and consumption. Additionally, the MsC mentioned that the sea urchin product: “entails different negative myths, for instance, that is not good or is too strong” [P1], thus increasing the aversion for many consumers. These myths and beliefs are particularly powerful once consumers have some prejudice, considering that this product has a bad taste or a slippery texture, decreasing its acceptance and consequently the chance of consumption.

The AIC also stated that for them, the sea urchin colour and overall appearance are the most important factors regarding consumer acceptance. The perception of positive product features may lead to the acceptance of the product, whilst negative features might lead to its rejection (Cardello, 1996, Spence, 2015a). It was generally agreed among all the Chefs interviewed, that some intrinsic attributes influence negatively the consumer acceptance of this product, namely their strong flavour, their repugnant appearance, and the pale and not appealing colour, that tend to present a white liquid and a bitter and acid flavour, as described by AIC and MsC. MsC also mentioned that canned sea urchin gonads present a product with lower quality regarding the odour, flavour and texture and some of the EriC said that cooked sea urchin tended to lose its characteristic flavour and texture, as also referred by Stefánsson et al. (2017) and Sun and Chiang (2015). Nevertheless, the Chefs also mentioned other intrinsic attributes that are better accepted among the consumers, namely their reddish-orange gonad and their sea smell. Indeed, most of the Chefs of our sample preferred a "reddish-orange gonad" with "intense and bittersweet flavour" and half of the sample also highlighted that this product is preferable for its "freshness", "iodine flavour and odour" and "sea smell", while one Chef of each group referred that natural roe with a "creamy and firm texture" is preferable. In this context, AIC defended in their speech that this product is an outstanding element that can give the sea flavours in each dish and it is well accepted among vanguard customers, while conservative customers generally dislike it due to intense flavour and odour.

3.1.1.2. Culinary applications

Sea urchin gonads have been popular in Mediterranean cuisine for centuries. In Italy, sea urchin gonads are blended into sauces and *paté* served with pasta (Sun and Chiang, 2015) and incorporated in pizza, pasta, croutons, among others (Furesi et al., 2014). It can also be served with bread, especially in Sicily, or with wine as an entrance (Wise, 2008). In Spain, sea urchin gonads are used as an addition to soups and stews as a flavour intensifier or as side dishes with truffle and cooked egg. They are also used as an ingredient in other dishes, for instance, lamb juice, lasagne, gratin or scrambled sea urchin, among others and, also, in the preparation of puddings or fish and seafood cakes (Stefánsson et al., 2017). In Greek and French cuisine, sea urchin gonads may also be

served with bread or used as an ingredient in a variety of dishes. Greeks, for example, enjoy consuming sea urchins with liquor, sometimes pouring ouzo directly into the test of the urchin and then eating the liquor-soaked gonads (Stefánsson et al., 2017). In Portugal, the culinary applications vary according to purposes regarding the use of sea urchin by Chefs. The MsC prize the use of sea urchin as a delicacy, as also reported by Proville (2009), regarding the perception of several different sea urchin species by celebrity Chefs. They reported using the gonads to embellish and to transfer the sea flavour and odour and to give a caviar-like texture, thus enhancing the plate's sophistication. They also use this product in *crème brûlée*, appetizers and cocktails.

The EriC used sea urchin products as the main ingredient in various dishes, further reporting that the sea urchin has a "leading role in their dishes" [P5]. This may be justified by the fact that Ericeira comes from the word "*Ouriceira*" (deriving from "*ouriço*" - sea urchin in Portuguese), emphasizing that it is a long-established tradition to use sea urchin in the region's cuisine. The EriC normally uses this product au natural or as the main element in their dishes, for instance in risotto, and in the typical malandro rice: a saucy quite runny rice, in the sense that it has a lot of sauce so the proportion of broth to the rice, is higher than for dry rice); and *açorda*: a bread stew often described as a dry bread soup, combining generously seasoned bread mash, usually infused with olive oil, sautéed onions, and cilantro, with various shellfish. Eggs are added last and are vigorously mixed with the bread base, allowing them to cook and provide the desired thickness. They also steam or grill the sea urchin for some customers, although they rather not, because cooking the sea urchin attenuates the flavour and smell and increases the firmness. These later aspects have also been identified for other species of sea urchin (Stefánsson et al., 2017, Sun and Chiang, 2015), with the advantage of diminishing the contamination risk inherent to raw sea urchin (Santos-Ferreira et al., 2020). The AIC use sea urchin predominantly au natural in sauces to improve its flavour. They also defend that the product processing reduces the quality, for instance, the flavour and texture.

In general, all the Chefs prefer sea urchin au natural, due to their fresh and sea odour, sweet flavour and a firm and creamy texture, that allow customers to enjoy this "dive in the sea" [P10] experience, a real "explosion of flavours" [P7].

3.1.1.3. Risk perception

Considering that sea urchin is a very high perishable product and is preferentially prepared au natural by the Chefs, the sea urchin risk perception is a cause of concern among the interviewed. The MsC stated in their narratives that this product "is a sensitive product that needs to be handle with extra care" [P3]. In their opinion, sea urchin presents a short shelf-life and for that reason, they rather work with fresh sea urchins, making an

effort to have the best conditions to maintain the product fresh during harvesting, transport and finally during the storage in the restaurant. They keep the sea urchin at 6° C with high levels of humidity expecting to maintain them alive and fresh for about three days. On the contrary, the other two groups use the sea urchin captured for about a week. Indeed, EriC stated that they work with this product for a long time, and was never reported any incidents regarding food safety incidents or other related issues. All of them said to be careful during the storage and processing of sea urchin, however, they did not reveal the storage conditions that they use for sea urchin. Nonetheless, most common procedures maintain the live sea urchin at 6 °C during transportation and processing (Kelly et al., 2015, Baião et al., 2020). The AIC only use the sea urchin during the harvesting season and if the Portuguese Institute for Sea and Atmosphere, I. P. (IPMA, IP) does not report toxins in water or shellfish, prohibiting fishing and sea harvest. They rather eliminate sea urchin with different appearance, colour or smell, not taking any risks. They also keep the sea urchin in a controlled and low-temperature environment to maintain them alive and fresh.

3.1.1.4. Purchase criteria

Taking into account these intrinsic quality attributes, the freshness of the product was indeed the most important sea urchin criteria referred to by all of the Chefs interviewed. They rather buy alive sea urchin to guarantee the “maximum level of freshness and quality” [P11]. To guarantee this freshness, the MsC referred that they use only “fresh, bright and alive” [P1] wild sea urchin. The EriC and AIC give more attention to the size of the sea urchin because they associate the dimension with better flavour and bigger gonads. Moreover, AIC purchase criteria are also based on the external colour, spines appearance and iodine odour. The perceived properties related to the freshness of the product may induce positive sensations, influencing positively the consumer purchase decision (Claret et al., 2012, Verbeke et al., 2007). As referred previously, both, MsC and AIC only use sea urchins delivered by trusted and certified suppliers, but the EriC rather prefer to harvest themselves, picking sea urchins at their choice, instead of buying from other suppliers. This way, besides avoiding the middlemen commissions, they guarantee the freshest conditions and are also able to assess the sea urchin’s quality through their gonads size and appearance in situ, deciding to either continue harvesting or not.

3.1.1.5. Availability

The supply constraint hampers the sea urchin product availability and accessibility in the restaurants, due to their seasonality: in Portugal, *P. lividus* presents a seasonal quality variation, being only available for consumption with the highest-quality sensory features, from November to March (Rocha et al., 2019). All the Chefs reported that they usually use

sea urchin only for about one period per year (December to April), because: "There is a limitation of the supply of sea urchins" [P7]. As a result, according to the Chefs, the supply constraint is the main factor that limits the use of this product in their restaurants and their menus: "In Portugal, it is difficult to have fresh sea urchin, this is the difficulty we face daily. Sometimes we use canned sea urchin gonads, allowing us to use this product all year round, but the quality is lower" [P1]. This constrain is reinforced by the fact that during these winter months it is also difficult to harvest sea urchin, mainly due to harsh weather conditions. To answer to the shortage in the supply of sea urchin and the contamination risk inherent to the consumption of raw gonads, echinoculture emerges as a possible solution for stable and secure production of high-quality gonads, able to supply the expanding markets, filling the gap between the growing demand and decreased natural supply (Sartori and Gaion, 2016, Phillips et al., 2010b).

3.1.2. Aquaculture

The aquaculture category (94 references out of 324) produced by participants during the interviews) provided details about the Chefs opinions regarding their views towards aquaculture production and aquaculture product acceptability and marketability. As the Chefs did not know a lot about the aquaculture of sea urchin, the debate of aquaculture of sea urchin was not always straightforward, but they have built some parallelism from their knowledge regarding seafood aquaculture in general.

3.1.2.1. Perception of aquaculture

In general, the Chefs were very suspicious about the use of farmed sea urchin or other cultivated seafood products in their dishes. They all have a generally negative opinion regarding aquaculture and said that they avoid aquaculture seafood products, because in aquaculture production: "fish are produced like chicken and that is not good marketing" [P3]. This is an image transferred from intensive terrestrial livestock production, as supported by other studies (Claret et al., 2014, Vanhonacker et al., 2011, Verbeke et al., 2007), strengthening that perceptions of aquaculture are based more on emotions than awareness and knowledge (Vanhonacker et al., 2011). Indeed, aquaculture has a bad reputation because of negative beliefs regarding its production methods, nutrition and use of antibiotics, among media and general public opinion (Olsen and Osmundsen, 2017, Amberg and Hall, 2008, Schlag, 2010); it is also an activity that raises concerns about health, environmental, economic and socio-cultural issues (Olsen and Osmundsen, 2017). Our Chefs follow this negative evaluation in a culinary setting, as according to their opinion, aquaculture can negatively "affect the products texture, flavour and consumers' health" [P10]. Moreover, they perceive this as being the evaluation of their costumers, which is

following Claret et al. (2014). In their opinion, flavour and texture are affected by poor-quality nutrition and because fish in aquaculture could not swim freely and develop muscle fibres. Nevertheless, for some of the Chefs from each group, aquaculture seems to have some advantages regarding the water and nutrition quality control, the quality certification that guarantees the freshness of the product and the all-year-round availability, only found in aquaculture systems. These risks and benefits balance has also been reported by other authors, regarding the evaluation of aquaculture systems (Amberg and Hall, 2008, Mazur and Curtis, 2006, Rickard et al., 2018).

3.1.2.2. Farmed sea urchin

For restaurants, customers are the pillars of their business, so they try to offer a good service and make the customer satisfied by catering to their desires and wants. This may explain why the Chefs reported that in general they rather use wild seafood than an aquaculture product: consumers are very cautious about accepting products from aquaculture production. For example, most of the “customers associate a 300 grams’ fish with aquaculture, even if it is from the wild” [P3] because seabream or seabass from aquaculture are normally commercialized with around this standard weigh (FAO, 2020b).

The MsC reported in their interview’s that they look for the best flavour and quality in wild specimens regardless of the price, with this being valid for sea urchin, as for any other product. They believe that their customers are aware of this topic and that they can perceive differences between aquaculture and wild products, mainly in terms of flavour and texture. The EriC believe that aquaculture production of sea urchin will reduce its iodine and sea flavour and colour, which are the features that they want to distinguish in their sea urchin dishes. In their opinion, aquaculture will hardly produce sea urchin with the same quality and sensory features as the wild ones. One of the EriC stated that aquaculture could fill the gap concerning the seasonality of the product, however, he considers that it will be difficult to achieve the same natural flavours of sea urchin gonads [P5]. In the same way, the AIC believe that wild seafood has better flavour, mainly due to natural food quality. In this sense, they considered that the use of farmed products, namely sea urchins, may not be the best way to advertise their dishes, receipts and restaurants. This concern is not surprising, since Portuguese consumers have a better acceptance of wild compared with farmed seafood products, as reported by other Portuguese studies (Pinto de Moura et al., 2012, Moura et al., 2011). Only one of the Chefs referred to choosing the better-quality products regardless of the origin – wild or aquaculture.

Moreover, although aquaculture is seen by the Chefs as able to present “nutritional and control benefits” [P2] and also “guarantee the freshness status of the product” [P8], in the same way as the generality of consumers, they express a pronounced fear of the use

of antibiotics and hormones in aquaculture, which diminish the product quality. One of the twelve Chefs stated that “fish in aquaculture is fed with corn, not meeting the fish nutritional requirements, while wild fish have a balanced diet” [P6]. It is also known that nutrition in aquaculture is strongly correlated with the quality of the product and, nowadays, the nutrition could be adapted to meet the consumers demands (Calanche et al., 2019). The opinion concerning the nutrition of seafood is divided between the advantage of a more controlled diet designed to promote animal growth addressing its nutritional requirements, and the advantage of a more natural diet capable of inducing more natural sensory features (Calanche et al., 2019, Claret et al., 2016).

Taking into account these negative perceptions about aquaculture and the opportunities given by this food production system, namely in terms of sustainability and nutrition, there is a need to improve consumers’ and Chefs’ understanding of aquaculture production. To be promoted through increased trust in production systems and enhanced positive image of farmed seafood (Kaiser and Stead, 2002). Claret et al. (2014) have revealed that consumers considered wild seafood fresher, healthier and more natural than aquaculture products, so consumers need to be aware of the sustainability issues as well as to be educated and encouraged to follow a healthy and conscious consumption of natural resources, and to consequently preserve natural stocks. Moreover, consumers’ shift towards seafood as key sources of protein, minerals and PUFA and increased concern regarding seafood quality and security (Henchion et al., 2017) may represent an opportunity to explore the benefits of aquaculture. Indeed, most of the Chefs believe that aquaculture and fish nutrition is improving, and, in the future, the nutritional and quality profile will be similar to wild seafood. However, as explained by one of the Chefs, consumers’ demand is what defines the use of wild or aquaculture products in their restaurants.

Aquaculture may be a solution, but consumers’ perception of farmed products needs to be improved by providing reliable information focused on sustainable farming practices and safety issues.

3.3. Descriptive sensory analysis (perceptual mapping with comments)

After the interviews, the main sea urchin sensory features were assessed by each chef to better explore and achieve a deep knowledge regarding the perception of this gourmet product, following a projective mapping technique (Risvik et al., 1994) complemented with Ultra Flash Profiling (Pagès, 2003) to describe the sensory features of sea urchin. From the analysis of the Chefs’ comments, a list of 32 terms (Table 3) was generated and these were grouped within main categories: appearance, odour, taste, texture and aftertaste. The first model produced by MFA (Figure 2) explained 80.7% of the variation in the data over the first two dimensions. The separation of the samples along

dimension 1 was attributed to differences between the sea urchins' sex. Sex has also influenced sensory properties in *E. chloroticus* gonads, as described by Phillips et al. (2010c) and Phillips et al. (2009), *S. droebachiensis* and *H. pulcherrimus* (Lee and Haard, 1982, Murata et al., 2002) and *P. lividus* (Baião et al., 2020, Baião et al., 2021a), but intrinsic cues inherent to the sea urchin - appearance, taste, flavour and odour- have been suggested to potentially be species-specific (Phillips et al., 2010a).

Table 3 - List of 32 sensory attributes divided into four dimensions (original in Portuguese).

Sensory dimension (in Portuguese)	Descriptor (in Portuguese)
Appearance (Aparência)	appealing (<i>apetivo</i>); not appealing (<i>não apetivo</i>); fresh (<i>fresco</i>); not fresh (<i>não fresco</i>); pale yellow (<i>amarelo pálido</i>); vivid orange (<i>laranja vivo</i>); pale orange (<i>laranja pálido</i>); clay-like colour (<i>cor de tijolo</i>).
Odour (Odor)	sweet (<i>doce</i>); fresh/sea (<i>fresco/mar</i>); inodorous (<i>inodoro</i>); iodine (<i>iodine</i>); underdeveloped (<i>não desenvolvido</i>); rusty (<i>ferrugem</i>); mud (<i>lodo</i>).
Texture/Mouthfeel (Textura)	soft (<i>suave</i>); creamy (<i>cremoso</i>); firm (<i>firme</i>); slimy (<i>viscoso</i>).
Taste (Sabor)	slightly sweet (<i>ligeiramente doce</i>); sweet (<i>doce</i>); sea taste (<i>mar</i>), iodine (<i>iodo</i>); salty (<i>salgado</i>); very intense (<i>muito intenso</i>); bitter (<i>amargo</i>); soft (<i>suave</i>); pleasant (<i>agradável</i>); tasteless (<i>sem sabor</i>).
After taste (Sabor residual)	iodine (<i>iodo</i>); bitter (<i>amargo</i>); persistent (<i>persistente</i>).

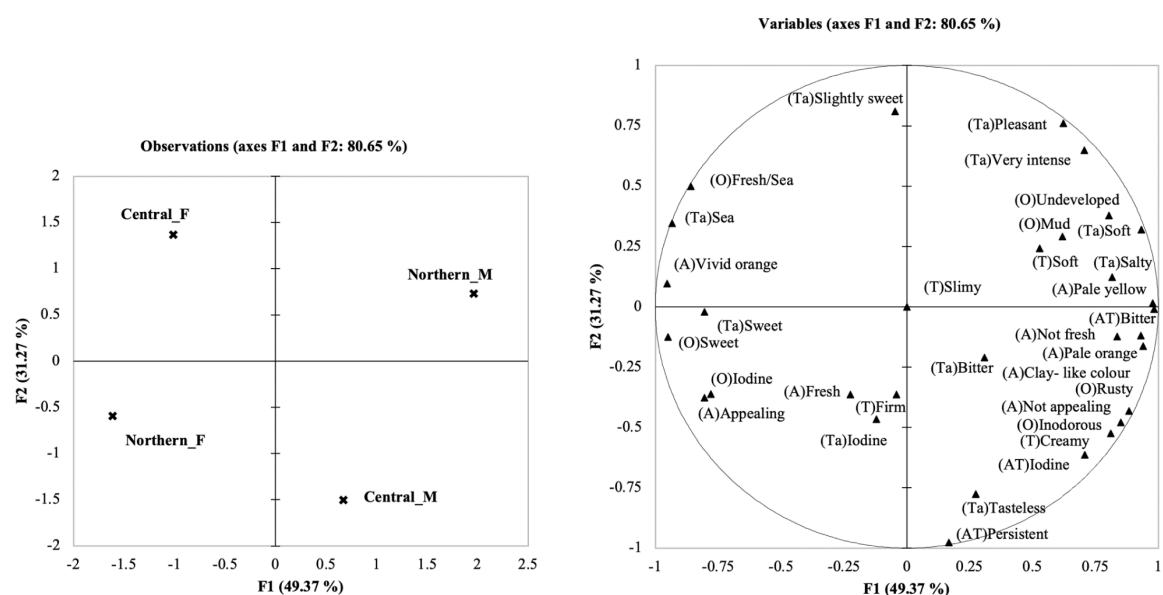


Figure 2 - MFA of projective mapping data. Individual factor map plot (Left) and correlation loading plot of individual variables (semantics) from the ultra-flash profile (Right), in accordance with the sex and harvest location (Viana do Castelo - Northern and Ericeira - Southern) of the different sea urchins. Abbreviations are M = male, F = female, (A) = appearance, (O) = odour, (T) = texture, (Ta) = taste and (AT) = after taste.

For our data, males from the North were represented by a very intense, salty and pleasant taste, but an underdeveloped and muddy odour and a soft texture, whereas the Central males were characterized for being tasteless and creamy, and for having iodine and persistent aftertaste. Notwithstanding, males, at both locations, tended to have a slightly bitter taste, a less appealing and less fresh appearance, a pale colour, a rusty odour, a soft texture and a bitter aftertaste, while females yielded a sweet and fresh sea/iodine flavour, an appealing overall appearance and a vivid orange colour (Figure 2). The location had no clear influence on the different sensory features, which is following Baião et al. (2020) and Baião et al. (2021a), which have demonstrated that location did not have a large influence on the taste, flavour and textural qualities of *P. lividus*.

The second model produced by MFA (Figure 3) explained 62.7% of the variation in the data over the first two dimensions. The separation of the samples along the diagonal that crosses quadrants 2 and 4 was also attributed to differences between the sea urchin sex. All the Chefs, during their interviews, associated the intense flavour, their repugnant appearance and the pale colour with undesirable intrinsic attributes that influences negatively the consumer acceptance of this product, linked mainly with male sea urchins. Moreover, the pale-yellow gonads are associated with the presence of a white exudate (sperm) and a bitter and acid flavour by the AIC and MsC, features also associated with male sea urchins. It is important to note that none of the Chefs was aware of the extrinsic gonad colour distinction between sea urchin sex. Nevertheless, the very intense salty taste and the firm texture was associated with a pleasant taste by the EriC, but could be a personal preference due to the high frequency of consumption of this product by this regional Chefs. Contrarily, the valorised and preferable attributes, such as the sweet taste, fresh and sea flavour, appealing appearance and bright-orange colour were associated with sea urchins' females. It was also relevant to state that the AIC, which referred to working more with sea urchins incorporated into sauces, to improve their flavour (see section 3.1.1.2), tended to be further apart from the evaluations of the other Chefs, as they would be less used to taste the sea urchin on their own, with no tasting enhancement or masking.

Colour and flavour are the most important product-intrinsic sensory features when it comes to setting the consumers' expectations regarding specific food (Spence, 2015b, a). The perception of positive properties, as the bright-orange colour, sweet taste and a fresh and sea flavour, may lead to acceptance of the product, whilst negative features, as a pale colour, not appealing appearance and intense flavour might lead to its rejection (Spence, 2015b, Cardello, 1996). Moreover, Chefs prefer a bittersweet flavour, firm texture and orange colour, which represents the majority of the Mediterranean countries' preferences (Sartori and Gaion, 2016). These sensory characteristics reflect the characteristics associated with the female. However, it is not whatsoever sustainable to consume only

females. The EriC Chefs, despite preferring sea urchin gonads with female traits, also note that it was possible to achieve an improved sensory balance on the plate by mixing the gonads from both sexes, thus combining their distinct sensory features.

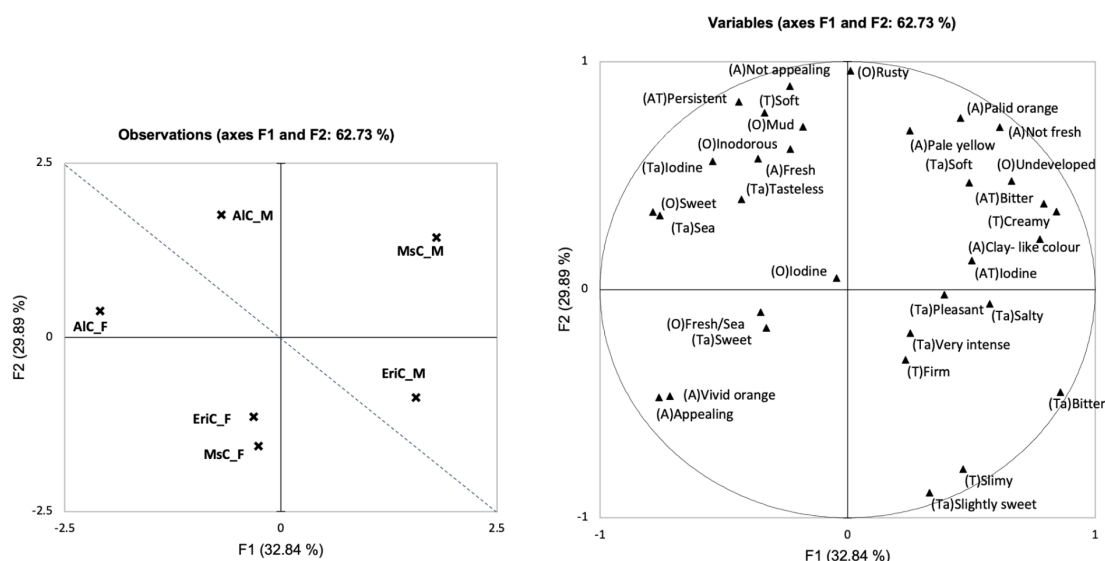


Figure 3 - MFA of projective mapping data. Individual factor map plot (Left) and correlation loading plot of individual variables (semantics) from the ultra-flash profile (Right), in accordance with the group of Chefs (MsC – Michelin star Chefs; EriC – Ericeira Chefs and AIC – Algarve Chefs) and sea urchin sex. Abbreviations are M = male, F = female, (A) = appearance, (O) = odour, (T) = texture, (Ta) = taste and (AT) = after taste.

One of the main barriers to the creation of a reliable export market for sea urchin gonads has been the seasonal and sex variability in product quality. Therefore, aquaculture could be explored as a means of contributing to the enhancement of gonad properties, such as colour, taste and texture, once these properties impact the visual assessment and freshness perception of sea urchin gonads, which are key purchase determinants. This could be achieved through the modulation of temperature and photoperiod, as well as dietary factors. Furthermore, aquaculture could allow the delaying of the gametogenesis of the gonads, keeping them in the pre-matured stage, and increasing the size (Baião et al., 2019) and quality (Baião et al., 2021b) of the gonads, adding economic value to this product.

Conclusions

The Chefs acknowledge the advantage of using sea urchin products, once this product allows the transference of the sea flavour and odour and other caviar-like features to their dishes. However, the lack of stability in the supply of a high-quality sea urchin product is a strong barrier to include it in the main dishes of the menu, unless a seasonal menu is in place. Although aquaculture products are not yet well accepted among Chefs and consumers, Chefs have the awareness that having availability throughout the year with high-quality can only be found in aquaculture systems. In this context, aquaculture success, sustainability and market acceptability depend on the development of suitable and nutritionally adequate diets to produce high-quality gonads for the caviar-like industry.

These results emphasize the need for communication and marketing strategies through locals, Chefs and gastronomic events, focused on the promotion of consumer awareness about the exclusivity of this product and the importance of aquaculture for the future of the seafood market.

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Chapter V.

**Analysis of volatile compounds in *Paracentrotus lividus* by HS-SPME/GS-MS
related to its sensorial properties**

by

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Analysis of volatile compounds in *Paracentrotus lividus* by HS-SPME/GS-MS and relation to its sensorial properties

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ABSTRACT

This study characterizes the sensory features and odour active volatile organic compounds (VOC) profile of *P. lividus*, taking into consideration the sex and harvest location. Sensory evaluation was performed by an experienced panel through the application of Projective Mapping paired with Ultra Flash Profile. VOC were determined by headspace solid-phase microextraction coupled to gas chromatography mass spectrometry (HS-SPME/GC-MS). Data showed that sea urchin gonad's sensory properties were mainly influenced by sex, while their volatile profile was affected both by sex and harvest location. Males tended to be sweeter, softer and with a pale-yellow colour, while females revealed an intense sweet tropical taste and a bright-orange colour. PCA unveiled variations in the levels of p-xylene, 1,2,4-trimethylbenzene and m-xylene between locations, whereas levels of β-pinene, 2,4-dimethyl-1-heptene, D-limonene and heptane discriminate sex. This study contributes to a better characterization of the sensory profile of sea urchin gonads aiming the selection of premium gonads.

1. Introduction

In the Mediterranean, sea urchin (*Paracentrotus lividus*) gonads have been considered a prized seafood product and a delicacy due to its flavour and texture. The increasing market demand for sea urchin gonads has resulted in the depletion of wild stocks (Baião et al., 2019). For this reason, sea urchins emerge as a novel species candidate for aquaculture diversification with high commercial value (Baião et al., 2019). Recently, several studies were carried out to improve sea urchin culture and research efforts have mainly focused in two major areas: the sea urchin life cycle and the gonad yield of wild and cultured sea urchin (Baião et al., 2019; Robinson, Castell, & Kennedy, 2002). The market price and consumer acceptability of cultured sea urchin gonads are influenced by their sensory traits, mainly the appearance, colour, texture, flavour, taste and size and therefore it is important to assess these quality attributes. In order to fully utilize this valuable natural resource,

it is crucial to understand the factors that influence the sensory properties and overall quality of *P. lividus* gonads.

To assess the sensory properties of a product, descriptive sensory analysis arises as one of the most satisfactory and effective methods to analyse quality characteristics of aquatic animals, as it generates objective descriptions of the perceived sensory attributes of products (Varela & Ares, 2014). Descriptive sensory analysis has been previously used to assess odour and flavour attributes in another sea urchin species (*E. chloroticus*), in relation to sex and harvest location (Phillips et al., 2009; Phillips, Hamid, et al., 2010). Phillips et al. (2009) have shown that the sex of *E. chloroticus* strongly influences sea urchin gonad taste; the male gonad has a sweet taste and dairy flavour, while the female gonad yields a bitter and sour taste and metallic flavour. In the last years, several rapid descriptive methods have gained popularity due to their lower cost and shorter implementation time than conventional profiling and may be performed by semi-trained or even naïve tasters

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(Varela & Ares, 2014). Within the rapid descriptive methods, projective mapping (PM) is a simple sensory technique that allows panellists to express similarities and dissimilarities (Risvik, McEwan, Colwill, Rogers, & Lyon, 1994). PM paired with Ultra-Flash Profiling (UFP) method (Pagès, 2003), provides a set of sensory descriptors generated by the panel itself.

The volatile composition of gonads can also be assessed to determine whether sea urchins can be distinguished based on location or sex, as shown by Niimi, Leus, Silcock, Hamid, and Bremer (2010). Gas chromatography coupled to mass spectrometry (GC-MS) has been applied to analyse various volatile components and contaminants in food samples (Kataoka, Lord, & Pawliszyn, 2000). Niimi et al. (2010) have used this technique in combination with olfactometry detector to investigate and identify the odour active volatile compounds in sea urchin gonads. Headspace solid-phase microextraction (HS-SPME) has been a valuable technique to understand the importance of volatiles and their contribution to odour and flavour attributes in other products. Although this method has been used in different environmental and food matrices (Mansur et al., 2019; Nathalie; Moreira et al., 2019; N. Moreira, Lopes, Cabral, & Guedes de Pinho, 2016; Sánchez-Palomo, Díaz-Maroto, & Pérez-Coello, 2005), it has not been previously applied in *P. lividus* samples.

The purpose of the current study was to identify the sensory features and odour active volatile compounds responsible for the differences within the sensory properties of *P. lividus* due to sex and harvest location, using very recent methodologies as PM paired with UFP and HS-SPME/GC-MS. This could improve the knowledge regarding the *P. lividus* gonads quality.

2. Material and methods

2.1. Sea urchin samples

Two different officially reported harvest locations along the Portuguese coast were selected (Viana do Castelo, Northern of Portugal [41°41'49.8"N 8°51'04.5"W] and Ericeira, Centre of Portugal [38°57'40.3"N 9°25'13.1"W]) for this study. Both areas are semi-exposed to ocean swells and the annual seawater temperature ranges from 12 to 20 °C in Viana do Castelo and from 14 to 22 °C in Ericeira. To assure the required number of specimens for this study, twenty fresh and alive *P. lividus* (with around 5 cm diameter and 80 g) were simultaneously harvested on rocky intertidal shores in each of the selected places, in February 2017, and kept alive at 6 °C until analysed. Sea urchins were dissected until two males and two females, from each local, were sampled based on visual appearance. The sex of the sea urchins was later confirmed by histological examination of a small gonad slice fixed in 10% formalin in seawater according to Baião et al. (2019). Then the five gonads of each sea urchin were carefully removed using a spoon maintaining the temperature at 6 °C to avoid temperature fluctuations, washed in saline water (36‰) and placed in a petri dish, so that each gonad could be distributed to each panellist. One of the gonads of each individual was placed inside chromatographic vials, sealed and stored at −20 °C for volatile compound analysis. This way controlling variability through a block design approach based on individual sea urchins.

2.2. Descriptive sensory analysis

The samples were maintained at 6 °C during transportation and processing, following most common procedures (Kelly, Carboni, Cook, & Hughes, 2015) and were served at 15 °C, to increase the aromatic intensity, in transparent PET containers, blind-labelled with random three-digit codes. Each panellist analysed two gonads from different males and two gonads from different females, for each harvesting location (total of eight samples per panellist) (Fig. 1A). The application of the block design, where every panellist evaluates gonads from the exact

same animals, with the block also extending to the volatile determination, guarantees a higher control of the biological variability.

Projective mapping was carried out by four expert panellists at Instituto Português do Mar e da Atmosfera (IPMA). These panellists were not specifically trained for the evaluation of sea urchin gonads, yet they had extensive experience in descriptive sensory analysis of several fish species and seafood products. Sensory evaluation was carried out in a test room equipped with individual booths in accordance with ISO standard 8589:2007 - Sensory analysis - General guidance for the design of test rooms (ISO, 2007).

The descriptive sensory analysis was carried out according to Phillips et al. (2009) in a sensory testing laboratory in IPMA. Filtered water and green apple quarters were provided for palate cleansing between samples. The eight gonads were simultaneously presented to each panellist, who were requested to lay out the products on a piece of A3 paper (29.7 × 42 cm) in such a way that the distance between each sample in the bi-dimensional space would reflect their similarity: if close together they were similar, if far apart samples were deemed as different. The panellists were asked to layout the sea urchin gonads in the paper taking into account the attributes from the different sensory dimensions. While the panellists gradually tasted the samples, they took notes of their sensory impressions.

The projective mapping was combined with an ultra-flash profile, as described by Pagès (2003). The panellists after placing the samples on the sheet were asked to generate sensory descriptors of the individual gonads, or groups, by directly writing on the sheet, in order to describe the differences between the samples. Moreover, they were asked to describe each sample using objective attributes, avoiding hedonic descriptors. For each sea urchin gonad, both X and Y coordinates were collected and compiled in a table together with the generated descriptors. Coordinates were calculated by measuring the distance (cm) relative to the left (x-coordinate) and the bottom (y-coordinate) edges of the sheet. For each panellist and for each sample, the experimenters assigned a "1" if a descriptor was cited for each particular gonad and a "0" if not. The data table obtained was constituted by four sub-tables corresponding to the four panellists and by eight rows corresponding to the eight tested gonads.

2.3. Volatile analysis

About 1 g of each sample from the sensorial analysis, stored at −20 °C, was placed in 20 mL vials and analysed by headspace solid-phase microextraction coupled to gas chromatography mass spectrometry (HS-SPME/GC-MS), method adapted from Barros et al. (2012). The volatile compounds were extracted by a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco Inc., Bellefonte, Pennsylvania), using a Combi-PAL autosampler (Varian Pal Autosampler, Switzerland). The extraction was carried out during 30 min at 45 °C with a stirring speed of 250 rpm after a 5 min incubation at the same temperature. Finally, 6 min of fibre desorption was placed into GC injector at 250 °C.

The GC-MS analysis was performed on a 436-GC model (Bruker Daltonics) coupled to a SCIION single quadrupole mass spectrometer (Bruker Daltonics), using a fused silica (Rxi-5Sil MS) capillary column (30 m × 0.25 mm internal diameter × 0.25 mm; Restek Corporation, U.S., Bellefonte, Pennsylvania) for chromatographic separation. The carrier gas was high purity helium (Gasin, Portugal) at a constant flow of 1 mL/min. The initial column temperature at 40 °C was kept for 1 min, followed by an increase of 5.0 °C/min from 40 to 250 °C (5 min) and from 250 to 300 °C (0 min). Single quadrupole mass spectrometry was conducted in the electron impact (EI) mode at 70 eV. The analysis was performed in full scan mode from 40 to 250 *m/z* with a scan time of 500 ms.

The identification of volatile compounds was accomplished by comparing the MS fragmentation with the mass spectra available in the National Institute of Standards and Technology (NIST) MS spectral

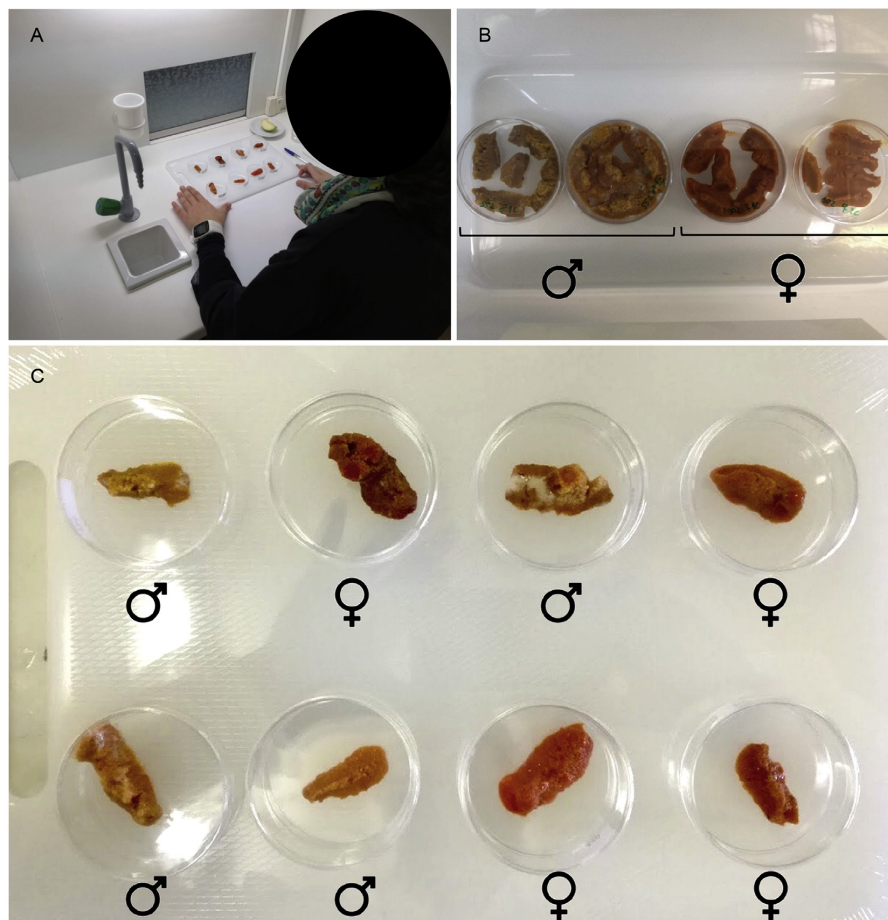


Fig. 1. Figure A shows an example of the samples' presentation to the panellist. Figure B shows an example of male (two pictures on the left) and female sea urchin gonads (two pictures on the right). Figure C shows an example of eight samples given to one of the trained panellists, signalling gonads from male and female individuals, the latter with a more vivid orange colour.

database (version 2.2, 2014) and by comparing the Kovats retention indices (RI) calculated for each compound with the RI reported in the literature. The identification was based on matching the retention time and mass spectra obtained from the sample with the authenticated standards injected at the same conditions.

2.4. Data analysis

The projective maps from each panellist and volatile compounds data were formatted according to Pagès (2003). Multiple Factor Analysis (MFA) was performed to compare the product position on the profiling maps produced by the projective mapping and the ultra-flash profile.

Peak areas corresponding to the identified volatile compounds were manually integrated. A data matrix was constructed with the areas of 45 compounds (Table 1) and normalized by each sample weight. Data were analysed using Principal Component Analysis (PCA). PCA and MFA were applied to the data matrix using the XLSTAT 2018 system software (Addinsoft, USA). The nonparametric Mann-Whitney test was performed to assess the individual statistical significance of normalized peak areas, considering statistical relevance for $p < 0.05$ (GraphPad Prism 6, USA). The bar plots represent the differences in compound

levels (expressed as the mean and standard deviation of the normalized peak area, $n = 4$) for each harvest location or sea urchin sex.

3. Results and discussion

3.1. Descriptive sensory analysis

A list of 28 terms was generated by the panel during the session to describe the sensory features of sea urchin and these were grouped within the main categories of sensory attributes: appearance, odour, taste and texture.

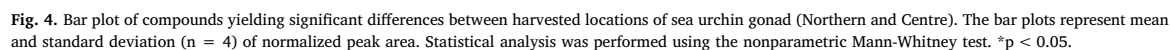
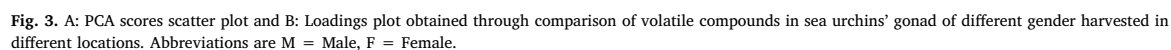
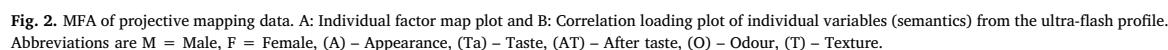
The model produced by MFA (Fig. 2) explained 59.9% of the variation in the data over two dimensions. The separation of the samples along dimension 1 was attributed to differences between the sea urchin sex (Fig. 2A). Sex also influenced sensory properties in *E. chloroticus* gonads, as described by Phillips et al. (2009) and Phillips, Niimi et al. (2010), *S. droebachiensis* and *H. pulcherrimus* (Lee & Haard, 1982; Murata et al., 2002). For our data, at both locations, males tended to be sweeter, softer and with a pale yellow colour when compared with females, while females yielded an intense sweet tropical taste and an orange colour (Figs. 1B and 2B). Phillips et al. (2009) have demonstrated that *E. chloroticus* male gonads had a sweet taste and dairy

Table 1

Volatile compounds identified in sea urchin roe by HS-SPME/GC-MS analysis and their odour descriptor associated.

Compounds	Specific group	CAS	RT (min)	RI	Most abundant ions (m/z)	Odour descriptor ^a	References ^b
Alcohols							
1-Penten-3-ol	C	616-25-1	2.90	–	41, 57	Tropical, fruity, bitter	Jonsdottir, Olafsdottir, Chanie, and Haugen (2008)
3-Methyl-1-butanol	N	123-51-3	3.62	–	42, 55, 70	Alcoholic, fruity, oil	Phillips, Hamid, et al. (2010)
1-Octen-3-ol	B	3391-86-4	9.65	980	43, 57, 72	Fishy, earthy, mushroom	Niimi et al., (2010), Phillips, Hamid, et al. (2010)
2,6-Dimethylcyclohexanol	N	5337-72-4	13.50	1108	43, 58, 71, 82, 95, 110, 128	NR	NR
4,8-Dimethyl-1-nonanol	N	33933-80-1	16.93	1226	43, 57, 69	NR	NR
Aldehydes							
3-Methylbutanal	B	590-86-3	2.64	–	44, 58, 71	Malt, aldehydic, sour	Quirós, López-Hernández, González-Castro, Cruz-García, and Simal-Lozano (2001)
Hexanal	B	66-25-1	4.89	806	44, 56, 72	Fruity, fresh, sweet	Phillips, Hamid, et al. (2010)
2-Ethyl-2-hexenal	C	645-62-5	9.21	965	41, 55, 69, 97, 126	NR	NR
Phenylacetaldehyde	B	122-78-1	11.51	1042	65, 91, 120	Honey, floral, bitter	Niimi et al. (2010)
Nonanal	N	124-19-6	13.35	1103	41, 57, 70, 82, 98, 114	Citrus, fresh, grapefruit	Quirós et al. (2001)
Benzene and derivatives							
Toluene	B	108-88-3	4.16	–	65, 91	Sweet, paint	Phillips, Hamid, et al. (2010)
<i>m</i> -Xylene	B	108-38-3	6.28	860	91, 106	NR	Phillips, Hamid, et al. (2010), Quirós et al. (2001)
<i>p</i> -Xylene	B	106-42-3	6.53	870	91, 106	NR	Phillips, Hamid, et al. (2010)
Styrene	N	100-42-5	7.07	892	51, 78, 104	Floral, sweet, balsam	Niimi et al. (2010)
Propylbenzene	N	103-65-1	8.83	952	65, 91, 120	NR	Quirós et al. (2001)
<i>m</i> -Ethyltoluene	N	620-14-4	9.04	960	105, 120	NR	Matiella and Hsieh (1990)
<i>p</i> -Ethyltoluene	N	622-96-8	9.14	963	105, 120	NR	Matiella et al. (1990)
Mesitylene	N	108-67-8	9.27	967	105, 120	NR	NR
1,2,4-Trimethylbenzene	B	95-63-6	10.02	993	105, 120	Plastic	Matiella et al. (1990)
Hemimellitene	N	526-73-8	10.83	1020	105, 120	NR	NR
<i>p</i> -Cymene	N	99-87-6	10.97	1022	91, 119, 134	NR	Matiella et al. (1990)
Hydrocarbons							
Heptane	B	142-82-5	3.11	–	57, 43, 71	Alkane, ethereal, bitter	Giogios, Kalogeropoulos, and Grigorakis (2013)
2,4-Dimethyl-1-heptene	B	19549-87-2	5.79	–	43, 55, 70, 83	NR	Giogios et al. (2013)
(<i>E,Z</i>)-1,3,5-Octatriene	C	40087-61-4	6.77	880	79, 91, 108	NR	NR
Monoterpenes							
α -Pinene	C	80-56-8	8.26	933	41, 77, 93	Fresh, earthy, sweet	NR
β -Pinene	B	127-91-3	9.54	977	41, 69, 93	Resinous, woody, pine	NR
α -Phellandrene	C	99-83-2	10.40	1006	77, 93, 136	NR	NR
Monoterpene_1	C	–	10.49	1009	67, 95, 138	NR	NR
(3 <i>E</i>)-3-Ethyl-2,5-dimethyl-1,3-hexadiene	C	62338-07-2	10.62	1013	67, 81, 109, 123, 138	NR	NR
(2 <i>E</i> ,4 <i>E</i>)-3,7-Dimethylocta-2,4-diene	C	6874-39-1	10.96	1024	67, 95, 138	NR	NR
<i>D</i> -Limonene	B	5989-27-5	11.10	1029	68, 93	Lemon, orange, fresh, sweet citrusy aroma	Niimi et al. (2010), Phillips, Hamid, et al. (2010), Quirós et al. (2001)
Eucalyptol	N	470-82-6	11.18	1031	43, 81, 108, 154	Fresh mint-like	Niimi et al. (2010)
β -Ocimene	C	13877-91-3	11.68	1048	41, 79, 93, 121, 136	Citrus, tropical, terpene	NR
γ -Terpinene	B	99-85-4	11.91	1055	77, 93, 121, 136	Bitter, herbal, lemon, tropical	NR
Norisoprenoids							
2,2,6-Trimethylcyclohexenone	N	2408-37-9	11.25	1034	56, 69, 82, 140	Citrus, honey, pungent	NR
β -Cyclocitral	N	432-25-7	16.70	1218	67, 81, 123, 137, 152	Fruity, herbal, sweet	NR
Sesquiterpenes							
Sesquiterpene_1	C	–	22.69	1442	91, 105, 133, 161, 204	NR	NR
Sesquiterpene_2	C	–	23.15	1460	91, 105, 133, 161, 204	NR	NR
Sesquiterpene_3	C	–	24.58	1519	91, 105, 131, 145, 159, 202	NR	NR
Trihalomethanes							
Bromoform	C	75-25-2	7.04	890	79, 91, 173, 252	NR	NR
Unknowns							
Unknown_1	N	–	5.42	826	79, 66, 91	NR	NR
Unknown_2	N	–	7.97	923	77, 91, 105	NR	NR
Unknown_3	N	–	9.46	974	41, 57, 70	NR	NR
Unknown_4	N	–	18.51	1283	55, 69, 83, 97	NR	NR
Unknown_5	C	–	23.26	–	81, 96	NR	NR

RT - Retention time. RI – Kovats retention indices determined using a commercial hydrocarbon mixture (C6–C20). NR – Not reported. ^a From Wishart (2011). ^b Compounds presents in sea urchin roe or other aquatic species in the literature. B - Compound present in both locations; N - Compound present in north population; C - Compound present in centre population.



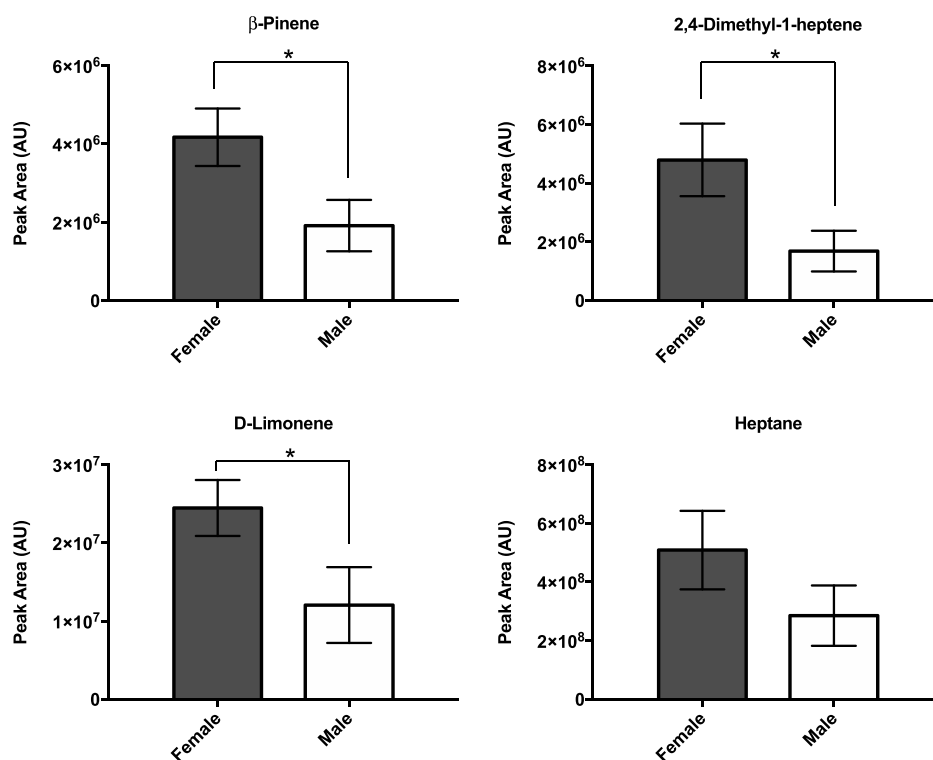


Fig. 5. Bar plot of compounds yielding significant differences between sea urchin gonad gender. The bar plots represent mean and standard deviation ($n = 4$) of normalized peak area. Statistical analysis was performed using the nonparametric Mann-Whitney test. * $p < 0.05$.

flavour, while female gonads had a bitter and sour taste, with an herbaceous and metallic flavour and longer duration of aftertaste. Moreover, for the samples of *P. lividus*, males tended to have an inodorous, discreet or sweet aroma, while females presented a fresh and tropical aroma (Fig. 2B). Niimi et al. (2010) have shown that females present an herbaceous, metallic and sulphur flavours and a marine and fresh odour probably due to the volatile compound α -limonene (Table 1). On the other hand, males have presented a dairy odour and flavour. A large number of sensory attributes were significantly different between sex, which was consistent with previous results for other sea urchin species (Niimi et al., 2010; Phillips et al., 2009; Phillips, Hamid, et al., 2010).

Location add no significant influence on the different sensory features, which is not in accordance with Phillips, Niimi, et al. (2010) and Niimi et al. (2010) that have demonstrated that location had a large influence on taste, flavour and texture qualities of *E. chloroticus*. Nevertheless, differences on geographical characteristics and distances between both locations were much more pronounced in their works, ranging from a delta (Doubtful Sound, southern east coast of New Zealand) to open ocean (Matheson Bay, northern east coast), distancing over 1200 km, while in our study both locations were at the west coast and distancing about 300 km.

3.2. Volatile analysis

A total of 45 volatile compounds were detected in *P. lividus* gonads, but 5 of them could not be identified (Table 1). Alcohols, aldehydes, benzene and derivatives, hydrocarbons, norisoprenoids and terpenes (mono- and sesquiterpenes) were the main classes of the identified compounds. Furthermore, one trihalomethane was detected in samples

from the Centre. Some of these compounds have been described in *P. lividus* gonads and other sea urchin species, as shown in Table 1. Some volatiles compounds, as 1-penten-3-ol, 2,6-dimethylcyclohexanol, 4,8-dimethyl-1-nonanol, 2-ethyl-2-hexenal, β -cyclocitral, *m*-ethyltoluene, *p*-ethyltoluene, mesitylene, 1,2,4-trimethylbenzene, hemimellitene, *p*-cymene, (*E,Z*)-1,3,5-octatriene, 2,2,6-trimethylcyclohexanone, α - and β -pinene, α -phellandrene, (3*E*)-3-Ethyl-2,5-dimethyl-1,3-hexadiene, (2*E*,4*E*)-3,7-dimethylocta-2,4-diene, β -ocimene and γ -terpinene, were tentatively identified by NIST14 and Kovats indices for the first time in gonads of this sea urchin species.

Differences in volatile profile of sea urchin gonads were observed between sex and harvest location in terms of number and class of compounds identified. A considerable number of monoterpenes, as α -pinene, α -phellandrene, (3*E*)-3-ethyl-2,5-dimethyl-1,3-hexadiene, (2*E*,4*E*)-3,7-dimethylocta-2,4-diene, β -ocimene and one unknown monoterpene at RT 10.49 min, as well two compounds from other classes (2-ethyl-2-hexenal and bromoform) were only identified in sea urchins from the Centre. Terpenes are mentioned to be naturally produced and contained in essential seaweed oils. In fact, the simplest terpenes, named monoterpenes, are abundant in seaweed oils (Naylor, Hanke, Manes, & Crews, 1983; Nollet et al., 2012) and have been recently identified in seafood and fish (Matiella & Hsieh, 1990; Niimi et al., 2010; Phillips, Niimi, et al., 2010; Quirós, J. M., Cruz-García, & J., 2001). Herbal, fresh, minty, sweet and woody are the typical odour descriptors for this compound class (Wishart, 2011). Bromoform, an insoluble and volatile gas present in marine air and seawater at trace concentrations, has been described as produced by phytoplankton and algae (Palmer & Reason, 2009). Such characteristic compounds found in the sea urchin gonads harvested in the Centre of Portugal have never

been previously reported in other species, and their presence can be related to the intake of different seaweeds by the sea urchin. The flora in the North of Portugal is similar to that found in Central Europe, however it differs from that in the Centre or South of Portugal that is mainly influenced by the Mediterranean and North zone of the West African coast (Soares et al., 2017). Also, Ardré (1971) has shown that while the number of red seaweed increase, the number of brown seaweed decrease along the Portugal coastline, from North to South. This may at least partially justify the observed differences in the presence and concentration of volatile compounds between harvested locations. Some benzene and derivatives compounds were only detected in Northern female samples, such as styrene, propylbenzene, *m*-ethyltoluene, *p*-ethyltoluene, mesitylene, hemimellitene and *p*-cymene. Unknown compound 1, 2 and 3, and 2,6-dimethylcyclohexanol were also found in this group (Table 1). Benzene and derivatives have been described in the literature as a result of sea pollution, however, some of those compounds seem to contribute to the sensorial properties of seafood products (Bencsath et al., 2015). Propylbenzene and *m*-xylene have been identified in *P. lividus*, as well as ethyl benzene (Quirós et al., 2001) which is used to produce styrene by xenobiotic-metabolising enzymes in cytochrome P450 1 (CYP1) of *Strongylocentrotus purpuratus* (Goldstone et al., 2006). Styrene has also been identified in *E. chloroticus* (Niimi et al., 2010). Floral and sweet are the common odour descriptor of this aromatic compound, being one of the potential aroma contributors. Additionally, *m*-Ethyltoluene, *p*-ethyltoluene and 1,2,4-trimethylbenzene have also been discovered in blue crab (*Callinectes sapidus*) (Matiella & Hsieh, 1990). Alcohols, as 2,6-dimethylcyclohexanol, appear to have a small impact in odour due to their high threshold values (Quirós et al., 2001). Although, 1-Octen-3-ol was not attributed to a specific location in this study, it has been previously identified by Niimi et al. (2010).

For animals from both sexes, the observed differences in volatile compounds were only observed in male gonads from the Northern region. Six more compounds were detected in these males than in the female group, namely 3-methyl-1-butanol, eucalyptol, 2,2,6-trimethylcyclohexenone, nonanal, β -cyclocitral and one unknown compound at RT 18.51 min. 3-Methyl-1-butanol, also known as isoamyl alcohol. This compound has been found in *E. chloroticus*, contributing to its alcoholic, fruity and oily flavours (Phillips, Niimi, et al., 2010). Quirós et al. (2001) proposed that the presence of high chain alcohols could be related with oxidoreductase activity in tissues. In fact, Nolle et al. (2012) have identified 3-methyl-1-butanol in cod, haddock and salmon samples, suggesting that their formation is due to amino acid and lipid degradation. Eucalyptol (1,8-cineol), as part of monoterpenes family and mainly present in aromatic plants and their essential oil fraction, has been reported in algae (Naylor et al., 1983). This monoterpene has also been identified in *E. chloroticus* (Niimi et al., 2010), contributing with a fresh and minty flavour. Carbonyl compounds, such as aldehydes and ketones, have been reported as having an important role in food flavour because of their low flavour threshold in comparison with other volatile compounds. The carbonyl flavour compounds are mainly resultant from lipid oxidation during storage and cooking (Nolle et al., 2012). Nonanal has previously been identified in various fish species (Yasuhara & Shibamoto, 1995) and also in *P. lividus* (Quirós et al., 2001). This aldehyde results from the oxidative degradation of oleic acid, presenting a characteristic fatty-oil aroma (Quirós et al., 2001). Other aldehydes such as hexanal, decanal, phenylacetaldehyde and 3-methylbutanal have been reported in *P. lividus* (Quirós et al., 2001), as well as in *E. chloroticus* (Niimi et al., 2010), *S. purpuratus* (Kanehisa et al., 2008) and *E. chloroticus* (Phillips, Niimi, et al., 2010). Norisoprenoids are products of carotenoid degradation which have an extremely low aroma threshold (Oliveira, Barbosa, Ferreira, Guerra, & Guedes De Pinho, 2006), having a significant impact in the aroma of several matrices, for instance in natural sea salts and in oysters' (Zhang, Li, Wang, Zhang, & Chen, 2009). β -Cyclocitral is a product of β -carotene thermal degradation and has been found in different microalgae

species (Van Durme, Goiris, De Winne, De Cooman, & Muylaert, 2013), being its odour described as grassy, woody, hay- or tobacco-like, according to their concentration. The present work was the first study to identify β -cyclocitral and 2,2,6-trimethylcyclohexenone in sea urchin gonads.

For assessment of other compounds responsible for the differences between sea urchin sex and their harvest location, PCA was applied to the spectra peak area of all volatile compounds identified. Fig. 3 shows the PCA scores (A) and loadings (B) plot of the volatile profile of sea urchins gonads analysed. In PCA scores plot (Fig. 3A), it is clearly observed two main clusters of Northern (left side) and Centre (right side) samples separated along PC1 with 44.4% of the variance. The volatile compounds responsible for this discrimination were interpreted in the PCA loadings plot (Fig. 3B) unveiling variations in levels of benzenes and derivative compounds (*p*-xylene, 1,2,4-trimethylbenzene and *m*-xylene) between Northern and Centre samples, as illustrated in the bar plots in Fig. 4. These compounds have been reported in some aquatic species (Matiella & Hsieh, 1990; Quirós et al., 2001), but no author has described the relationship between these benzene derivatives and their harvest location. Niimi et al. (2010) have also shown differences in the concentrations of the volatile compounds between harvest locations and suggested that these differences could be due to food availability and/or the reproductive stage. This could also apply to the present study where distinct availabilities of algae were described in the two selected harvest locations (Ardré, 1971).

A poor discrimination by sex was observed in PCA scores plot along PC2 with 21.4% of the variance (Fig. 3A). Differences in levels of β -pinene, 2,4-dimethyl-1-heptene, *D*-limonene and heptane are contributing for the discrimination of sex, as can be seen in PCA loadings (Fig. 3B) and bar (Fig. 5) plots. Hydrocarbons constitute one of the major groups of volatile compounds described in sea urchin by Quirós et al. (2001). In the present study only three compounds of this class were identified, being 2,4-dimethyl-1-heptene and heptane characteristic of the female group. However, these volatiles might not contribute to the flavour due to their high thresholds' values (Cha, Cadwallader, & Baek, 1993). Additionally, β -Pinene and *D*-limonene, as part of monoterpenes class, and due to their pleasant aromas, could have a positive impact on female sea urchin compared to male samples. *D*-Limonene has been identified in fish (Grigorakis, Taylor, & Alexis, 2003), crab (Matiella & Hsieh, 1990) and shrimp (Kubota, Shijimaya, & Kobayashi, 1986), as well as in sea urchin (Quirós et al., 2001). This terpene has been reported to be responsible for fresh, light, sweet and citrus aroma (Quirós et al., 2001), potentially contributing for the female group flavour. Robinson et al. (2002) have suggested sex-specific pathways of carotenoids metabolism and expression in *S. droebachiensis*, whilst Baião et al. (2019) have also reported sex-specific metabolism and deposition of nutrients (protein and lipids) in sea urchin gonads. Therefore, it is reasonable to suggest that males and females have different metabolism and deposition pathways of the compounds present in the algae-based diet.

It is also important to refer that *P. lividus* has a single annual spawning event during late Spring and Summer (May–July), coinciding with the rise of seawater temperatures up to its maximum value (16.4 °C) (Rocha et al., 2019). Spirlet, Grosjean, and Jangoux (1998) pointed that populations of echinoids from temperate waters exhibit similar reproductive patterns: nutrient storage during Fall and Winter followed by a long spawning season during late Spring and Summer. Rocha et al. (2019) also demonstrated that the biochemical composition of *P. lividus* gonads showed a clear seasonal variation also varying with sex. Previous reports showed that the best period to harvest gonads with high nutritional quality and commercial value is during the growing period, generally prior to the onset of gametogenesis, when the nutritive phagocytes are full (Walker et al., 2015, pp. 263–288) and accumulate higher levels of protein, lipid and carbohydrates (Marsh, Powell, & Watts, 2013). In the present study, the sea urchins were harvested in February, within the expected period for harvesting high

quality gonads, as suggested by Rocha et al. (2019). Afterwards, the changes in the biochemical content of gonads due to its maturation may impair their sensory profile, leading to a loss of economic value.

4. Conclusions

The combination of HS-SPME/GC-MS, projective mapping and ultra-flash profile provided information on the several volatile compounds and sensory descriptors, respectively, that discriminated sea urchin gonads samples in terms of their sex and harvest location. These differences between sea urchin gonads may explain the somehow inconsistent sensory properties observed within the sea urchin population previously reported by Rocha et al. (2019). Several volatile compounds have never been previously identified either in seafood or sea urchin, so it is important to understand their origin and the biochemical pathways by which they are formed. This study is of extreme importance for the aquaculture industry, as well as for consumer science community as the information obtained can be used to improve the growth and quality features of sea urchin gonads through dietary modulation, taking into account the sea urchin sensory profile.

CRedit authorship contribution statement

Luís F. Baião: Investigation, Resources, Writing - original draft. **Ana Sofia Oliveira:** Formal analysis. **Amparo Gonçalves:** Investigation, Resources. **P. Guedes de Pinho:** Writing - review & editing. **Luisa M.P. Valente:** Methodology, Writing - review & editing, Conceptualization, Funding acquisition. **Luís M. Cunha:** Methodology, Writing - review & editing, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Chapter VI.

Sensory profiling, liking and acceptance of sea urchin gonads from the North Atlantic coast of Portugal, aiming future aquaculture applications

by

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ABSTRACT

Sea urchin gonads are receiving increasing attention for their exclusive sensorial attributes. However, the sensory profile of this highly demanded gourmet product has not yet been well described, and it may potentially help enhancing the gonads' marketability and acceptability. The main goal of this study was to build a sensory profile of sea urchin (*Paracentrotus lividus*) gonads, to evaluate differences between sex, harvest location, as well as the impact of presentation on consumer acceptance. Sixty untrained panellists (regular consumers of seafood) were asked to evaluate eight samples of raw sea urchin gonads, divided by sex, harvest location and presentation (in a clear translucent glass bowl or inside a sea urchin test). The panellists evaluated overall liking and acceptance (Food Action Scale), followed by a Check-All-That-Apply (CATA) ballot with a list of 38 sensory attributes divided into four dimensions: appearance (9), odour (8), texture (7) and taste (14), launched in Sense Gest. From a three-way ANOVA, no significant effect of sex, harvest location and presentation were identified for both liking and acceptance. However, a significant interaction between presentation and sex was identified: females with gourmet presentation were rated higher than females presented in a bowl; and males presented in a bowl were favoured in relation to gourmet presentation. Sensory profiling clearly separates gonads according to sex, with females being more appealing than males that presented a milky white fluid. Gonads were also separated according to the presentation method: the white background of the bowl seemed to highlight the orange colour of females; the gourmet presentation favoured the females in relation to the males, mainly because the male gonad colour and white-colour exudate were emphasized by the background of the sea urchin test. Moreover, the gourmet presentation in both sexes was correlated with a fresh, tropical and pleasant odour. This study concluded that sea urchin with an orange gonad and a sweet, fresh and tropical flavour is preferable, which will allow future nutritional research efforts to be focused on the enhancement of these gonad attributes.

1. Introduction

Sea urchin gonads has proven to be a culinary delicacy prized in many European countries, as well as in Chile, North America, Asia, and especially Japan, which accounts for around 80% of worldwide demand of sea urchin products (FAO, 2020). Currently, sea urchin gonads are one of the most valuable seafood products, mainly in Japan where market demands for this delicacy has been growing for decades (Stefánsson, Kristinsson, Ziemer, Hannon, & James, 2017). In Europe, in

most of its geographical range, there are several species of urchins, but the most economically important and appreciated species is *Paracentrotus lividus*, intensely harvested in France, Ireland, Spain, Portugal, Italy, Croatia and Greece (Boudouresque & Verlaque, 2020; Guidetti, Terlizzi, & Boero, 2004). *P. lividus* gonads are considered a prized and gourmet seafood delicacy, due to their unique flavour, making their commercialization highly profitable, mainly in Mediterranean countries. Nowadays, the consumption of *P. lividus* is mainly limited to France, Italy and Spain, and to a lesser extent to Greece, being France the

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main consumer country of sea urchin in Europe, being the second world consumer, after Japan (Stefánsson et al., 2017; Sun & Chiang, 2015). From a commercial perspective, high-quality sea urchin gonads are described by a large size coupled with a desirable combination of sensory characteristics (i.e., taste, texture, colour, and firmness), which may differ between markets (Stefánsson et al., 2017). Also, each species vary greatly in terms of gonad size, colour, flavour, and other sensory attributes, that depend upon the interaction of environmental cues (temperature and photoperiod), annual reproductive cycle, sex (female or male; some cultures prefer one over the other) and the nutritional input (availability and quality of food).

As sea urchin stocks were exploited and overfished, leading to the collapse of native stocks, the continued growth of gonads consumption around the world boosted the interest in echinoculture to supply market demands (Sartori, Pellegrini, Macchia, & Gaion, 2016). Moreover, aquaculture practices allow for the production of seafood with a consistent quality along the year, which is a great advantage in the case of *P. lividus* that presents a seasonal quality variation, being only available for consumption during a short period of time (from November to March). During this period, gonads are mainly at the growing and early maturation stages of gametogenesis, holding the best nutritional and organoleptic features and consequently highest commercial value (Rocha et al., 2019). According to the literature, most formulated feeds for sea urchin species are able to promote gonad growth, but fail to meet important quality traits of the wild-caught sea urchins, such as colour, texture and flavour (Lourenço, Valente, & Andrade, 2019). Although aquaculture may accelerate the production of market-sized sea urchins, the market value and consumer acceptability of farmed urchins may be compromised by a poor gonad quality. Therefore, an integrated approach is needed to fill the gap between consumers' demands and aquaculture products. For instance, the sensory features sought by the consumer can be met with inclusion of new alternative additives in the aquafeed (Calanche, Beltrán, & Hernández Arias, 2019), but to achieve such goal those features must be clearly identified first.

Nowadays, wild-caught seafood is considered the quality reference for consumers, resulting in a critical limitation for the development and approval of farmed products (Claret et al., 2014). In this context, sensory properties are crucial for the acceptance and choice of consumers concerning seafood (Calanche et al., 2019). Appearance properties comprise different visual properties, including colour, shape and mode of presentation (Spence, 2015b). Indeed, these visual impressions are usually the first sensory stimuli that are presented to a consumer, raising expectations about the product (Reisfelt, Gabrielsen, Aaslyng, Bjerre, & Møller, 2009). The perceived properties may induce positive sensations leading to product acceptance, or negative sensations leading to its rejection (Spence, 2015b). Hence, visual features are essential regarding acceptance or rejection of a specific product (Cardello, 1996). Moreover, the colour of the plate may exert a significant effect on the perception of the majority of the sensory attributes of a food product under evaluation (Piqueras-Fiszman, Alcaide, Roura, & Spence, 2012), although with mixed results across different food matrices (Schifferstein, Howell, & Pont, 2017).

It is crucial to understand sea urchin sensorial attributes sought and preferred by consumers, before proceeding with the enhancement of the sea urchin gonads' quality features through dietary modulation. To assess the sensory properties of a product, descriptive sensory analysis arises as one of the most satisfactory and effective methods to analyse quality characteristics of seafood products, as it generates objective descriptions of the perceived sensory attributes of products (Massa, Manca, & Yeannes, 2012; Olafsdottir et al., 1997).

Projective mapping paired with Ultra Flash Profile have been previously used to assess *P. lividus* gonads' sensory properties (Baião et al., 2020), where a small experienced panel was able to discriminate sea urchin gonads according to sex. The odour and flavour attributes were also evaluated for other sea urchin species (*E. chloroticus*), in relation to sex and harvest location (Phillips et al., 2009; Phillips, Hamid, Silcock,

Sewell et al., 2010). They have shown that sex in *E. chloroticus* strongly influences sea urchin gonad taste. However, as far as we know, there are no previous studies addressing the liking or acceptance of *P. lividus* gonads, and studies on the impact of sex, harvest location and dish presentation on their sensory profile are scarce or even inexistent.

Thus, the main goal of the present work was to evaluate differences between sex, harvest location, as well as the impact of the presentation mode on the sensory profile, liking and consumer acceptance of *P. lividus* gonads. To accomplish this, a fast and user-friendly technique was used for the collection of sensory information deriving from consumers' perception, quicker and with a lower cost, when compared with the conventional profiling techniques (Varela & Ares, 2014). That is the case of the Check-All-That-Apply (CATA) tool, a profiling technique frequently used and considered appropriate for working with untrained panellists (Adams, Williams, Lancaster, & Foley, 2007; Meyners & Castura, 2014). This will act as a benchmark, providing a further selection of the best nutritional and feeding methodologies, able to have a positive impact on the final quality of farmed sea urchin, and ultimately result in the production of highly accepted sea urchin gonads by consumers.

2. Material and methods

2.1. Samples

Two different harvest locations along the Portuguese coast, with official reports concerning the existence of sea urchin fishing, (*Praia Norte*, Viana do Castelo, Northern of Portugal [41°41'49.8"N 8°51'04.5"W] and *Carreço*, Viana do Castelo, Northern of Portugal [41°44'32.5"N 8°52'39.7"W]) were selected for this study. Both areas are semi-exposed to ocean swells and the annual seawater temperature ranges from 12 to 20 °C. This study was divided in two sessions, with an interval of five days. To assure the required number of male and female specimens for each session, eighty fresh and alive *P. lividus* (with a minimum of 5 cm diameter and above 80 g), were simultaneously harvested from each of the selected places, the day before each session and kept alive at 6 °C until analysis. The specimens were harvested in February to assure that the gonads would be in pre-mature or early maturation period, providing the highest quality organoleptic features. At the beginning of the sensory evaluation test, 16 males and 16 females were identified from each location: live males presented a pale-yellow colour and oozed a white liquid through the gonads, while the live females presented a bright-orange colour and oozed an orange liquid through gonads. From each location, the sex and maturation stage of the sea urchins was later confirmed by histological examination of a small gonad slice fixed in 10% formalin in seawater, according to Barker, Keogh, Lawrence, and Lawrence (1998). Then the five gonads of each sea urchin were carefully removed using a spoon, washed in saline water (36‰) and placed in a petri dish at 6 °C, so that each of the first four gonads could be distributed to one panellist (Fig. 1). The remaining gonad of each individual was used for instrumental texture and colour analysis.

2.2. Sensory quality assessment

A sensory panel formed by 60 untrained panellists was recruited from the sensory evaluation company Sense Test's consumer database (Vila Nova de Gaia, Portugal). They were mainly residents in the Oporto metropolitan area, in the North of Portugal. All participants were selected through a larger seafood consumption questionnaire and were regular consumers of seafood and fish and received a small financial compensation for their participation. The company ensures the protection and confidentiality of data through the authorization 2063/2009 of the National Data Protection Commission, and following EU Regulation (EU 2016/679), as well as a longstanding internal code of conduct, assuring informed consent. Sensory evaluation was carried in individual

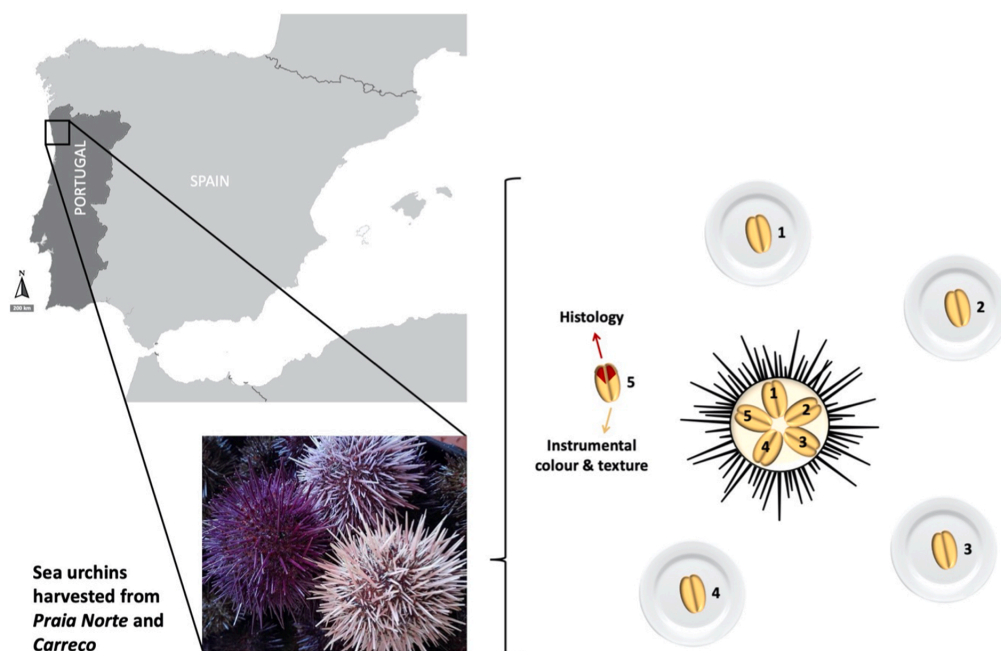


Fig. 1. Scheme of sampling procedures of sea urchin from both harvesting locations.

tasting booths at a special room equipped in accordance with ISO 8589:2007 – Sensory analysis – General guidance for the design of test rooms.

The samples were stored at 6 °C and were served at approximately 15 °C, in a small (top diameter = 6.0 cm) clear translucent glass bowl over a white plate (bowl presentation), corresponding to a more common sensory evaluation presentation over a neutral white background, or on small translucent plastic plate (used for hygienic purposes) placed inside a cleaned sea urchin test (gourmet presentation), improving the colour contrast and aiming for reinforced perception of the sea urchin gonads on a natural context (Baião, Moura et al., 2019), placed over a white plate (Fig. 2), blind-labelled with random three-digit codes. In the first session half of the untrained panel analysed the samples in the bowl presentation and the other half in the gourmet presentation; for the

second session the presentation modes were switched. In each session, every panellist analysed one male and one female gonad, from each harvesting location (total of four samples per panellist per session, eight in total). Panellists were provided with a porcelain spittoon, a glass of bottled natural water and unsalted crackers. All panellists were asked to chew a piece of cracker and to rinse the mouth with water before testing each sample. For each session, to compensate for eventual carry-over effects, each panellist received the set of four samples following a monadic and sequential order of presentation, with a balanced presentation order (MacFie, Bratchell, Greenhoff, & Vallis, 1989).

2.3. Overall liking and acceptance

For each sample, overall liking was evaluated using a 9-point scale,

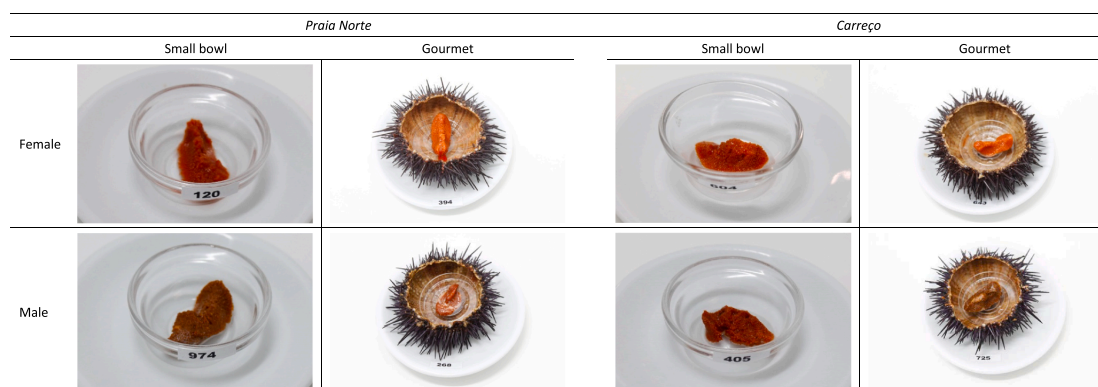


Fig. 2. Example of samples' presentation (small bowl or gourmet) to the panellists by harvest location (Praia-Norte or Carreço) and sex.

ranging from 1 – “dislike extremely” to 9 – “like extremely” (Peryam & Pilgrim, 1957). The Food Action Rating Scale (FACT), developed by Schutz (1965) was used to evaluate gonads acceptance. This is a 9-point successive-category food action rating scale, where: 9 – “I would eat this every opportunity that I had”, 8 – “I would eat this very often”, 7 – “I would frequently eat this”, 6 – “I like this and would eat it now and then”, 5 – “I would eat this if available but would not go out of my way”, 4 – “I do not like this but would eat this on an occasion”, 3 – “I would hardly ever eat this”, 2 – “I would eat this if there were no other food choices”, 1 – “I would eat this only if forced”. For each sample, overall liking and acceptance were immediately followed by the evaluation of the sensory profile, through Check-All-That-Apply (CATA) methodology, as suggested by Ares and Jaeger (2013) and King, Meiselman, and Carr (2013) to reduce bias.

2.4. CATA (Check-All-That-Apply)

2.4.1. Development of the CATA ballot

The list of terms included on the CATA ballot were generated by the research team, combining previous published literature from qualitative consumer studies with *E. chloroticus* (Phillips et al., 2009; Phillips, Hamid, Silcock, Delahunty et al., 2010; Phillips, Hamid, Silcock, Sewell et al., 2010; Phillips, Niimi et al., 2010) and *P. lividus* (Baião et al., 2020) sea urchin gonads, which were later refined and modified based on tasting sessions. During the initial sessions, the research team members individually tasted several samples of gonads from specimens of different sexes and from different locations, and discussed the attributes until a consensus was reached. Moreover, despite the research team experience, both with sea urchins and with sensory evaluation of foods with trained and naïve panels, a pilot test (with 15 consumers with no training on sensory description) was conducted to confirm the applicability of the attributes to describe the test samples and also to assure that they were easily understood by the consumers. This was done by discussing with participants if they recognized the attributes and asking them how they describe them.

A final ballot of 38 sensory attributes was obtained, with attributes organized by sensory dimension in order to reduce the cognitive effort required by the participants (Ares et al., 2013).

2.4.2. CATA evaluation

The untrained panellists were asked to perform a CATA evaluation with a list of 38 sensory attributes divided into four labelled dimensions (Table 1): appearance (9), odour (8), texture (7) and taste (14), launched in Sense Gest, a proprietary software for sensory evaluation management and data acquisition. While fixing the presentation order of the

four major dimensions, within each dimension the terms were presented in a randomized order, different for each participant and each product (Ares et al., 2014). The enquiry for CATA evaluation “Please select out of the following list of terms those that characterized the observed sample” was answered in a multiple-choice response scale, indicating if they recognized the presence or absence of such attributes. This option increased the focus of respondents on each attribute (Jaeger et al., 2014).

2.5. Gonad colour and texture

Gonad colour and texture were measured as previously described by Baião, Rocha et al. (2019). Gonad colour was assessed instrumentally using a CR-400 colorimeter (Konica Minolta) with a standard illuminant D₆₅, using the $L^*a^*b^*$ scale, from which additional measures of Chroma (C^*) and hue (h^*) were derived.

Instrumental evaluation of firmness and resilience properties was performed in sea urchin gonads using a TA.XT.plus texture analyser, fitted with a 5 kg load cell and controlled by Exponent v6 software (Stable Micro systems, UK). Evaluations were performed individually in a single freshly extracted gonad and was run without replicates, due to the limited amount of sample. Both parameters were calculated using the Stable Micro Systems software. Compression test was performed using a 35 mm cylinder probe, according to McBride, Price, Tom, Lawrence, and Lawrence (2004). Firmness was defined as the force (grams) required to compress the sample at 25% of its original height (at a 1.7 mm/s) and was calculated in accordance with the AACC standard firmness method (AACC, 1999). Resilience was defined as the capacity of a sample to recover its original height after a compression/distension cycle and was calculated as Resilience (%) = (post-test height/original height) × 100. After the determination of firmness, the probe continued to compress the gonad up to 60% of its original height (40% strain) and held that position for 2 s. Then, the probe was withdrawn and after 15 s the post-test height was taken.

2.6. Determination of gonad's sex and maturation stage

Gonads were fixed in formaldehyde during 24 h and then conserved in alcohol 70%. Fixed samples were dehydrated, embedded in paraffin and sectioned transversely to the length axis, according to standard histological procedures. Two paraffin sections (7 µm thickness) of each gonad were stained with haematoxylin and eosin (H/E) method and observed under a binocular microscope (CX22LED, Olympus) for confirmation of the sea urchin's sex. The gametogenic stages of ovary and testes were identified following the six stages of maturation described by Byrne (1990): recovery (stage I), with primary gametes (oocytes and spermatocytes) and nutritive phagocytes; growing (stage II) with clusters of primary gametes and packed nutritive phagocytes; premature (stage III) with gametes at all stages of development and reduced amount of nutritive phagocytes; mature (stage IV) with mature gametes (ova and spermatozoa) and few nutritive phagocytes; partly spawned (stage V) with loosely packed gametes and depletion of nutritive phagocytes; and spent (stage VI) with gonads empty of gametes.

2.7. Statistical analysis

2.7.1. Overall liking, acceptance, colour and texture

Data analysis was performed using XLSTAT 2019® system software (Addinsoft, USA). To evaluate the results of the overall liking, food action rating scale and colour and texture instrumental analysis, descriptive statistics (mean and standard deviation) were used, and a three-way ANOVA (sex, harvest location and presentation method) with interaction was applied. When significant effects, at a 95% confidence level, were identified, a Least Significant Difference (LSD) multiple comparison test was applied.

Table 1
List of 38 sensory attributes divided into four dimensions (original in Portuguese).

Sensory dimension	Descriptor
Appearance (Aparência)	firm (<i>firme</i>)*; milky white fluid (<i>exsudado branco</i>); grainy (<i>granuloso</i>)*; appealing (<i>apelativo</i>)*; not appealing (<i>não apelativo</i>)*; soft/slimy (<i>suave/viscoso</i>)*; yellow (<i>amarelo</i>)*; orange (<i>laranja</i>)*; brownish (<i>acastanhado</i>)*.
Odour (Odor)	sea smell (<i>maresia</i>)*; sweet (<i>doce</i>)*; fresh (<i>fresco</i>)*; tropical (<i>tropical</i>)*; pleasant (<i>agradável</i>); unpleasant (<i>desagradável</i>); earthy (<i>terra</i>)*; sulfur (<i>sulfúrico/ enxofre</i>)*.
Texture/Mouth-Feel (Textura)	soft (<i>suave</i>)*; creamy (<i>cremoso</i>)*; firm (<i>firme</i>)*; juicy (<i>suculento</i>)*; grainy (<i>granuloso</i>)*; dry (<i>seco</i>)*; astringent (<i>adstringente</i>)*.
Taste (Sabor)	salty (<i>salgado</i>)*; tropical (<i>tropical</i>)*; sweet (<i>doce</i>)*; sea taste (<i>mar</i>)*; intense (<i>intenso</i>)*; soft (<i>suave</i>); pleasant (<i>agradável</i>)*; cloying (<i>enjoativo</i>)*; unpleasant (<i>desagradável</i>)*; earthy (<i>terra</i>); sour (<i>azedo</i>)*; bitter (<i>amargo</i>)*; metallic (<i>metálico</i>)*; prolonged aftertaste (<i>sabor residual longo</i>).

* Adapted from Baião et al. (2020).

Adapted from Phillips et al. (2009).

Table 2

Results from the three-way ANOVA relating liking and attitudes (FACT-Food Action Rating Scale) to *P. lividus* gonads, according to sex, place of origin (source) and type of presentation: small bowl and gourmet.

Factors	Levels	Liking ^a	<i>F</i> (<i>df</i> _{level, error}); <i>p</i> -value	FACT [†]	<i>F</i> (<i>df</i> _{level, error}); <i>p</i> -value
Source	<i>Praia Norte</i> <i>Carreço</i>	7.20 (±1.42) 6.44 (±1.85)	<i>F</i> (1, 364) = 1.091; <i>p</i> = 0.297	7.09 (±1.41) 6.26 (±1.71)	<i>F</i> (1, 364) = 1.898; <i>p</i> = 0.169
Sex	Male Female	7.07 (±1.39) 7.23 (±1.45)	<i>F</i> (1, 364) = 2.189; <i>p</i> = 0.140	6.25 (±1.76) 6.46 (±1.80)	<i>F</i> (1, 364) = 2.807; <i>p</i> = 0.095
Presentation	Bowl Gourmet	7.14 (±1.39) 7.15 (±1.45)	<i>F</i> (1, 364) = 0.008; <i>p</i> = 0.931	6.34 (±1.74) 6.36 (±1.83)	<i>F</i> (1, 364) = 0.035; <i>p</i> = 0.852
Source × sex	–	–	<i>F</i> (1, 364) = 3.030; <i>p</i> = 0.083	–	<i>F</i> (1, 364) = 0.500; <i>p</i> = 0.480
Source × presentation	–	–	<i>F</i> (1, 364) = 0.273; <i>p</i> = 0.602	–	<i>F</i> (1, 364) = 0.035; <i>p</i> = 0.852
Sex × presentation	Male × bowl Male × gourmet Female × bowl Female × gourmet	7.24 ^{a,b} (±1.28) 6.90 ^c (±1.47) 7.05 ^{b,c} (±1.49) 7.41 ^a (±1.39)	<i>F</i> (1, 364) = 10.37; <i>p</i> = 0.001	6.35 (±1.73) 6.14 (±1.80) 6.33 (±1.77) 6.58 (±1.83)	<i>F</i> (1, 364) = 3.329; <i>p</i> = 0.069
Source × sex × presentation	–	–	<i>F</i> (1, 364) = 1.091; <i>p</i> = 0.297	–	<i>F</i> (1, 364) = 0.733; <i>p</i> = 0.392

Values are presented as mean (±standard deviation), *n* = 212.

^a Evaluated in a 9-point hedonic scale, ranging from 1 – “dislike extremely” to 9 – “like extremely” (Peryam and Pilgrim, 1957).

[†] Evaluated in a 9-point successive-category food action rating scale, ranging from 1 – “I would eat this only if forced” to 9 – “I would eat this every opportunity that I had” (Schutz, 1965).

^{a,b,c} Homogeneous groups, according to the LSD test at a 95% confidence level.

2.7.2. Check-All-That-Apply (CATA)

For the CATA evaluations, the frequency of use of each attribute was determined, calculating the number of panellists who have used each attribute to describe the samples and the Cochran test was applied to identify which attributes were discriminating among samples (Parente, Manzoni, & Ares, 2011). Over the frequency matrix of the discriminating terms, a correspondence analysis (CA) was applied to obtain a two-dimensional representation of the sample's terms. Such analysis provides a sensory map of the samples, allowing the perception of the similarities and differences between samples and their sensory characteristics (Ares, Barreiro, Deliza, Giménez, & Gámbaro, 2010; Ares, Deliza, Barreiro, Giménez, & Gámbaro, 2010; Ares, Varela, Rado, & Giménez, 2011). Based on the outcomes of the CA, a multi-dimensional alignment (MDA) evaluation (Meyners, Castura, & Carr, 2013) was also performed to determine the correlation between the attributes and the samples vectors in the full-dimensional space of CA, providing complete information about the relationship between products and attributes. From this analysis, one can understand in a more detailed way the differences between samples, namely their correlation with the attributes. The reversed scale on the horizontal axis allows an easier interpretation of values to the right of the centre (90° representing orthogonal vectors, i.e. no correlation between sample and attribute) indicating positive correlation, and those to the left indicate negative correlation. Vertical dashed lines indicate the significance cut-offs of 45° and 135°, respectively (Meyners et al., 2013).

2.7.3. Multiple factor analysis (MFA)

The MFA was applied to assess the consensus between the different table variables analysed: colour parameters ($L^*a^*b^*$, C^* and h^*), texture features (resilience and firmness), CATA, with overall liking and FACT scale as supplementary data. This statistical methodology allows the analysis of the consensus between different variables that differ in number and data nature and is an improved PCA, analysing individual differences instead of average differences (Louw et al., 2013; Nestrud & Lawless, 2008).

3. Results and discussion

3.1. Gonad sensory quality

3.1.1. Overall liking and acceptance

From a three-way ANOVA, no significant effect of sex, harvest location and presentation were identified for both overall liking and FACT ($p > 0.05$), although a significant effect of the interaction between presentation and sex was identified for liking ($p = 0.001$): females with gourmet presentation were rated higher than females presented in the small bowl; males presented in the small bowl were favoured in relation to gourmet presentation; males presented in the small bowl and females with gourmet presentation were equally accepted (Table 2).

3.1.2. Check-All-That-Apply (CATA)

Fig. 3 shows the configuration of the samples in the first and second

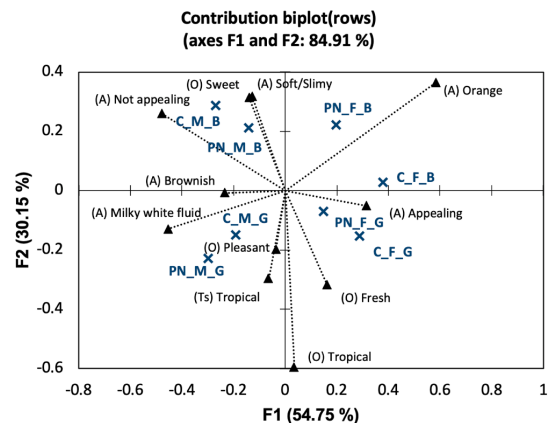


Fig. 3. Biplot with dimensions 1 and 2 of correspondence analysis of CATA (Check-All-That-Apply) data from the sensory profiling of sea urchin gonads. Abbreviations are C = *Carreço* beach, PN = *Praia Norte* beach, M = male, F = female, B = small bowl presentation, and G = gourmet presentation.

dimensions of correspondence analysis applied to the CATA data of sea urchin gonads, which explained 84.9% of the total variance of the experimental data. The representation shows that consumers of sea urchin gonads clustered the samples in four different groups. Individuals were discerned in relation to sex and presentation method. Differences in the sensory quality of gonads have already been correlated with sex by Baião et al. (2020) in *P. lividus* and by Phillips et al. (2009) in *E. chloroticus*. Baião et al. (2020) have demonstrated that male gonads have a sweet taste, soft texture and a pale-yellow colour, while female gonads reveal an intense sweet tropical taste and a bright-orange colour. Moreover, Phillips et al. (2009) have shown that male gonads had a sweet taste and dairy flavour, while female gonads have a bitter and sour taste and metallic flavour. In our study, females were strongly associated with a fresh odour and an orange and appealing appearance, while males had a brownish and milky white fluid appearance. Indeed, intrinsic cues inherent to the sea urchin – appearance, taste, flavour and odour – have been suggested to potentially be species-specific (Phillips, Hamid, Silcock, Delahunty et al., 2010). Moreover, Niimi, Leus, Silcock, Hamid, and Bremer (2010) and Phillips, Niimi et al. (2010) have demonstrated that location had a large influence on taste, flavour and texture qualities of *E. chloroticus*. So, it is expected that a sea urchin species endemic to New Zealand with different genetics, environmental cues (water temperature, photoperiod and density) and food availability and quality, would present different organoleptic features compared with sea urchins from the Atlantic Ocean.

Moreover, within species, the gonadal quality varies along the annual reproductive cycle, mainly due to water temperature, photoperiod and density (Boudouresque & Verlaque, 2020), food availability (Livore & Connell, 2012) and quality (Gago, Range, & Luis, 2003) and, also, gonads maturation stage (Walker, Lesser, & Unuma, 2020), as early stages of gametogenesis are associated with large and firm gonads (Agatsuma, Sato, & Taniguchi, 2005). However, sampled gonads were predominantly in stage III (pre-mature and early maturation stage) and did not present differences in size amongst the groups (data not shown), so maturity and size did not appear to have affected the variance between sea urchin gonads sensory characterization. Further research into the effect of reproductive stage on *P. lividus* gonads sensory quality

would be helpful to determine the optimal time for yield in aquaculture, as well as to obtaining high-quality gonads.

Additionally, there are no sensory studies neither in *P. lividus* nor in other sea urchin species where the impact of the presentation method has been evaluated. The majority of the studies used a white ceramic plate (Phillips et al., 2009; Takagi, Murata, Inomata, Aoki, & Agatsuma, 2019), however it is well-known that the appearance properties, as well as the mode of presentation are key factors in food choice (Spence, 2015b). From Fig. 4, one can depict in a more detailed way that the small bowl presentation, with the white ceramic background, favoured male gonads when compared to the gourmet presentation. Interactions with the participants at the end of the tasting sessions revealed a negative impact of the white exudate (data not presented). It is clear from Table 2 that the gourmet presentation intensifies the presence of the white exudate, while this is masked in the bowl presentation due to the white background. For isolated male gonads, the gourmet presentation has a detrimental effect on liking. On the other hand, female gonads were significantly more liked when delivered with the gourmet presentation, mainly because of the overall more appealing appearance. Indeed, the mode of presentation can enhance or deteriorate the appearance properties leading to a better or worse acceptance of the product, respectively (Spence, 2015b). It is also possible that one might be facing an assimilation/contrast effect (Davidenko et al., 2015), since the female gonads were considered as appealing and the gourmet presentation enhanced the attractiveness perception (assimilation effect), while males stood out negatively in a more gourmet and attractive presentation, most probably through the reported increased in the contrast between the white exudate and the darker test (contrast effect). Choosing the right format and colour of plate for a specific dish can be very important (Spence, Piqueras-Fiszman, Michel, & Deroy, 2014). Plating and the sight of food stimulates several anticipatory physiological responses, for instance the emotional responses such as the desire to eat (Ouweland & Papies, 2010), as well as the cognitive expectations linked to our previous experiences and familiarity with the food (Berthoud & Morrison, 2008; Shin, Zheng, & Berthoud, 2009). Moreover, as shown in Fig. 3, sea urchin gonads with a gourmet presentation demonstrated to have a strong association with a “tropical taste” and a “pleasant, tropical

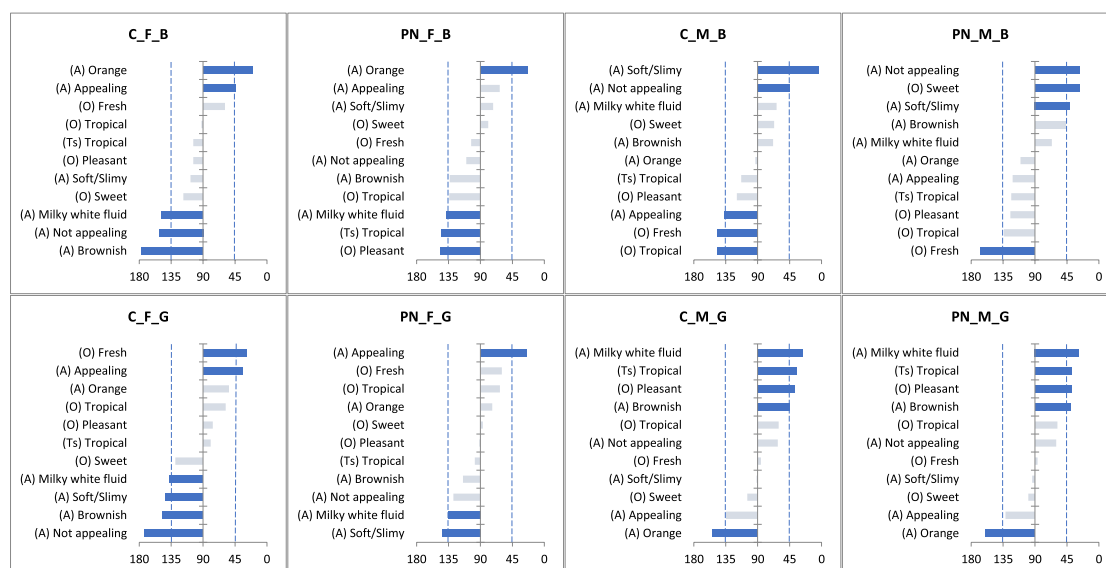


Fig. 4. Associations between attributes and products based on the vectorial angle (0–180°) between samples and attributes, based on the MDA. Abbreviations are C = Carreço beach, PN = Praia Norte beach, M = male, F = female, B = small bowl presentation, and G = gourmet presentation.

Table 3
Results from the three-way ANOVA relating instrumental colour (L^* , a^* , b^* scale) and texture (firmness and resilience) measurements to *P. lividus* gonads, according to sex, place of origin (source) and type of presentation: small bowl and gourmet.

Factors	Levels	L^*	$F(df_{level, error}); p$ -value	a^*	$F(df_{level, error}); p$ -value	b^*	$F(df_{level, error}); p$ -value	Firmness (g)	$F(df_{level, error}); p$ -value	Resilience (%)	$F(df_{level, error}); p$ -value
Source	<i>Praia Norte</i>	41.68 (± 4.69)	$F(1, 120) = 1.424$; $p = 0.235$	21.20 (± 5.12)	$F(1, 120) = 2.340$; $p = 0.129$	37.60 (± 7.27)	$F(1, 120) = 0.760$; $p = 0.385$	24.92 (± 9.34)	$F(1, 120) = 0.153$; $p = 0.697$	80.52 (± 3.57)	$F(1, 120) = 0.053$; $p = 0.818$
	<i>Curtejo</i>	42.60 (± 4.08)		20.44 (± 4.66)		38.40 (± 5.52)		25.54 (± 8.98)		80.38 (± 3.17)	
Sex	Male	43.26 (± 5.05)	$F(1, 120) = 8.590$; $p = 0.004$	16.77 (± 2.66)	$F(1, 120) = 267.7$; $p < 0.001$	33.74 (± 5.44)	$F(1, 120) = 80.09$; $p < 0.001$	27.78 (± 9.30)	$F(1, 120) = 9.774$; $p = 0.002$	80.06 (± 3.40)	$F(1, 120) = 1.529$; $p = 0.219$
	Female	41.02 (± 3.32)		24.87 (± 2.88)		42.00 (± 4.50)		22.77 (± 8.32)		80.83 (± 3.31)	
Presentation	Bowl	42.45 (± 4.38)	$F(1, 120) = 0.655$; $p = 0.420$	20.67 (± 5.06)	$F(1, 120) = 0.375$; $p = 0.542$	37.96 (± 6.38)	$F(1, 120) = 0.009$; $p = 0.923$	26.37 (± 8.49)	$F(1, 120) = 2.144$; $p = 0.146$	80.20 (± 2.74)	$F(1, 120) = 0.680$; $p = 0.411$
	Gourmet	41.83 (± 4.44)		20.97 (± 4.75)		38.04 (± 6.55)		24.02 (± 9.68)		80.71 (± 3.91)	
Source $\times$ sex		-	$F(1, 120) = 0.799$; $p = 0.373$	-	$F(1, 120) = 1.218$; $p = 0.272$	-	$F(1, 120) = 2.237$; $p = 0.137$	-	$F(1, 120) = 0.080$; $p = 0.777$	-	$F(1, 120) = 0.000$; $p = 0.993$
Source $\times$ presentation		-	$F(1, 120) = 0.669$; $p = 0.415$	-	$F(1, 120) = 0.751$; $p = 0.388$	-	$F(1, 120) = 0.000$; $p = 0.982$	-	$F(1, 120) = 0.853$; $p = 0.357$	-	$F(1, 120) = 0.077$; $p = 0.782$
Sex $\times$ presentation		-	$F(1, 120) = 1.026$; $p = 0.313$	-	$F(1, 120) = 1.042$; $p = 0.309$	-	$F(1, 120) = 0.036$; $p = 0.849$	-	$F(1, 120) = 1.304$; $p = 0.256$	-	$F(1, 120) = 0.935$; $p = 0.336$
Source $\times$ sex $\times$ presentation		-	$F(1, 120) = 0.006$; $p = 0.936$	-	$F(1, 120) = 0.002$; $p = 0.962$	-	$F(1, 120) = 0.046$; $p = 0.831$	-	$F(1, 120) = 3.056$; $p = 0.083$	-	$F(1, 120) = 0.588$; $p = 0.445$

Values are presented as mean ($\pm$ standard deviation), n = 60.

Table 4

RV coefficients resulting from multiple factor analysis applied to CATA, gonad instrumental colour and texture values and acceptance and FACT evaluations (p -value is shown in brackets).

	CATA	Colour	Texture	Liking	FACT	MFA
CATA	1.000	–	–	–	–	–
Colour	0.808 (0.002)	1.000	–	–	–	–
Texture	0.411 (0.109)	0.458 (0.065)	1.000	–	–	–
Liking	0.205	0.178	0.350	1.000	–	–
FACT	0.414	0.359	0.532	0.831	1.000	–
MFA	0.892	0.894	0.812	0.241	0.398	1.000

and fresh odour”, suggesting that the use of fresh sea urchin test was able to enhance the aroma of the gonads from both sexes. In fact, “fresh” and “tropical” odours are clearly associated with this mode of presentation. Females were characterized by an orange colour and appealing appearance, while males had a brownish colour and white fluid exudate that was negatively emphasized by the background of the sea urchin test (Fig. 2), with these being consistent with the “sex $\times$ presentation” interaction effect on overall liking (Table 2). In fact, colour is the most important product-intrinsic sensory feature when it comes to setting the consumers’ expectations regarding the likely taste and flavour of a specific food (Spence, 2015a, 2015b). The perception of positive properties, as an orange colour, may lead to acceptance of the product, whilst negative features, as the white exudate or brownish colour, might lead to its rejection (Cardello, 1996; Spence, 2015b).

3.1.3. Gonad colour expression and texture

Colour is the first quality attribute used by consumers, impacting the visual assessment and freshness perception of sea urchin gonads (from the species *Strongylocentrotus droebachiensis*), which are key purchase determinants (Robinson, 2004). Colour parameters L^* , a^* and b^* of gonads were not affected by the harvesting location (Table 3). However, differences were found between sex, with females exhibiting less luminous (lower L^* values), redder (higher a^*) and yellower (higher b^*) gonads than males. These data are in accordance with Rocha et al. (2019) that demonstrated that males had brighter gonads than females, while females had more reddish and yellowish gonads than males.

Gonad firmness was higher in males than in females (Table 3), however, in other studies with the same species, there were no statistical differences between sexes (Baião, Rocha et al., 2019; Rocha et al., 2019). Resilience was not affected by the source nor the sex of sea urchins.

3.1.4. Multiple Factor Analysis (MFA)

Multiple factor analysis was performed to compare results from sea urchin gonads’ instrumental colour and texture features, CATA, overall liking and FACT evaluations. Table 4 shows the RV coefficients resulting from MFA. For colour, texture and CATA, the RV values in the MFA configuration were very high and closer to the unit: 0.894, 0.812, 0.892, respectively. These results show that the projection patterns of the samples in all parameters subspaces were similar. When individually comparing the other RV values, one can note that the association between variables is weak, except for the comparison between colour and CATA in which the RV value is higher (0.808) and significant ($p < 0.01$).

The MFA plot, shown in Fig. 5, provides the first two dimensions of the MFA consensus, explaining 74.3% of the variability of the experimental data. The representation shows that participants clustered the samples in four different groups. Gonads were discerned in relation to sex and presentation method. Male sea urchins were negatively loaded along F1 and were associated with a “soft”, “not appealing”, “milky” and “brownish” appearance and a “sweet aroma”, as well as with high values of brightness (L^*) and firmness. On the other hand, female gonads were positively loaded along F1, and were associated with an “appealing” appearance, “orange” colour, and also with yellowness (b^*), redness (a^*) and resilience. A closer look into Fig. 5 indicates that there was a positive correlation between yellowness, redness and resilience with the “appealing” appearance, whereas the “orange” colour values were intrinsically related with consumer acceptance and overall liking. Contrarily, in Phillips, Hamid, Silcock, Delahunty et al. (2010) male gonads were rated higher than females for colour appearance, but *E. chloroticus* is a sea urchin species endemic to New Zealand. It is relevant to note that both for Japanese and New Zealander consumers, gonads with a vibrant yellow colour are more liked and preferred (Stefánsson et al., 2017).

Colour has an important influence on the consumer’s psychological perception and acceptability of a specific product (Spence, 2015b). It is

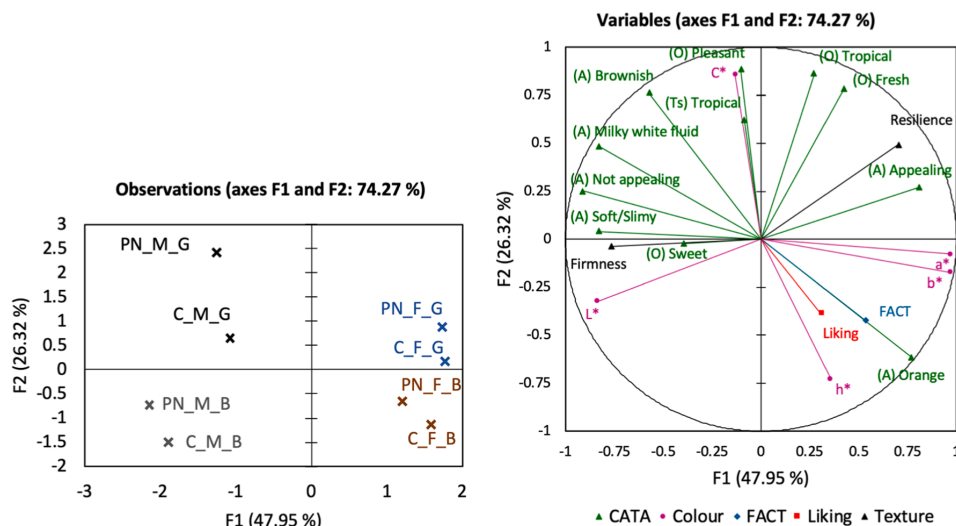


Fig. 5. Samples (left side) and attributes (right side) projections of dimension 1 and 2 from multiple factor analysis applied to sea urchin gonads data from instrumental colour (purple lines), texture features (black lines), CATA profiling (green lines), acceptance (red lines) and FACT (blue lines). Abbreviations are C = Carreço beach, PN = Praia Norte beach, M = male, F = female, B = small bowl presentation, G = gourmet presentation, (A) = appearance, (O) = odour and (Ts) = Taste. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

clear that when consumers recognise that the colour they see is inappropriate, it seems to be influenced in an automatic manner (Zampini, Sanabria, Phillips, & Spence, 2007). Nonetheless, the colour perception can be distinct among consumers of different regions. For example, high-priced sea urchins in Japan are represented by a yellowish colour as well as a sweet taste and firm texture (Barker, 2015; Stefánsson et al., 2017), whereas in Mediterranean countries the preference is represented by a bittersweet flavour, firm texture, reddish-orange colour (Sartori & Gaion, 2016). Therefore, the selection of the most appropriate diet for sea urchin should take into consideration the reference market. Moreover, as the sensory profile of sea urchin gonads seem to be linked to the sea urchin sex, dedicated diets for each sex might also be envisaged in the future.

4. Conclusions

One of the main barriers forming a reliable export market for sea urchin gonads has been the seasonal and sex variability in product quality. Aquaculture could hence be explored as a means of contributing to the uniformity of gonad properties, such as colour, taste and texture, once these properties impact the visual assessment and freshness perception of sea urchin gonads, which are key purchase determinants. This could be achieved through the modulation of abiotic (e.g. temperature, photoperiod) and dietary factors. Delaying the gametogenesis of the gonads, keeping them in pre-matured stage, and increasing the size and improving the quality of the gonads are all crucial appraised prerequisites to add economic value to this product. This study demonstrates that despite the lack of differences in overall liking and acceptance in relation to sex, consumers identified differences that go beyond liking, more specifically in the sensory profile of male and female gonads. An orange and appealing sea urchin gonad, presenting a fresh odour is preferable. This information will allow future nutritional research efforts to be focused on the enhancement of these *P. lividus* gonads attributes set. Moreover, for the foodservice industry, this work shows that the presentation mode may have a significant impact on both liking, acceptance and sensory profiling of sea urchin gonads.

CRedit authorship contribution statement

Luís F. Baião: Investigation, Formal analysis, Writing - original draft. **Célia Rocha:** Investigation, Formal analysis. **Rui C. Lima:** Methodology, Funding acquisition. **Alexandra Marques:** Investigation, Formal analysis. **Luisa M.P. Valente:** Writing - review & editing, Funding acquisition. **Luís M. Cunha:** Conceptualization, Methodology, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2020.109873>.

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Chapter VII.

Development of a Rate-All-That-Apply (RATA) ballot for sensory profiling of sea urchin (*Paracentrotus lividus*) gonads

by

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Abstract

Paracentrotus lividus gonads are receiving increasing interest for their unique sensory attributes. However, the sensory profile of this highly demanded gourmet product has not yet been well described, and it may potentially benefit the gonads' marketability and acceptability. The main goal of this study was to build a sensory profile of sea urchin (*Paracentrotus lividus*) gonads, to evaluate differences between sex and harvest location. Eighty untrained panellists (regular consumers of seafood) were asked to evaluate four samples of raw sea urchin gonads (inside a sea urchin test), divided by sex and harvest location. The panellists evaluated overall liking and acceptance (Food Action Scale), followed by a Rate-All-That-Apply (RATA) ballot with a list of 22 sensory attributes divided into four dimensions: appearance (6), odour (6), texture (4) and taste (6). From a three-way ANOVA, no significant effect of sex and harvest location were identified for liking, however a significant effect of the sex for FACT was identified. Sensory profiling clearly separates gonads according to sex, with females being more appealing than males. Females were strongly correlated with a pleasant and intense tropical taste, as well as an overall appealing and grainy appearance and a pleasant tropical odour, while males had a slimy appearance and a milky white exudate. The use of the RATA approach revealed an increase in discrimination power, as it allowed to separate female gonads according to their origin, with those from Praia Norte being firmer, more resilient and more appealing than the ones from Carreço. This study concluded that the female sea urchin with intense and pleasant tropical taste and a pleasant tropical odour are more appealing and accepted among panellists, which may contribute valuable information for future nutritional aquaculture studies aiming to enhance sea urchin gonads' attributes and, subsequently, their market valorisation.

Keywords: ballot development; Rate-All-That Apply (RATA); Food Action Rating Scale (FACT); *Paracentrotus lividus*; sea urchin; gonads

1. Introduction

Paracentrotus lividus gonads are considered a prized and gourmet seafood delicacy, due to their unique flavour, making their commercialization highly profitable, mainly in Mediterranean countries. The natural stocks of *P. lividus* found in France, Italy, Spain, Ireland and Portugal are considered overexploited, meaning its fishery is not sustainable, leading to the collapse of wild stocks (Boudouresque & Verlaque, 2020). The reduction of wild stocks driven by a high market demand generated a worldwide interest in developing sea urchin aquaculture for the production of high-quality gonads, able to supply the expanding markets (Sartori, Pellegrini, Macchia, & Gaion, 2016). Moreover, aquaculture practices may allow for the production of gonads with consistent high-quality along the year.

This would be a huge advantage in the case of *P. lividus*, as the species presents a strong seasonal quality variation, being only available for consumption from November to March (Rocha et al., 2019). In nature, during this period, gonads are mainly at the growing and early maturation stages of gametogenesis, presenting the best nutritional and organoleptic features and consequently highest commercial value (Rocha et al., 2019). The aim of sea urchin aquaculture is to increase gonad yields, enhance gonad sensory traits such as colour, taste, and texture and modulate the reproductive cycle to allow for the marketing of sea urchins throughout the year. Overall, sea urchin aquaculture is a challenging endeavour, as most formulated feeds for sea urchin species are able to promote gonad growth, but important quality traits, such as colour, texture and flavour of urchin gonads from cultured urchins has so far failed to properly meet the demands of this high-end market (Lourenço, Valente, & Andrade, 2019). Consequently, it is important to progress to an integrated approach between consumers' demands and aquaculture products. This could be achieved by including new alternative additives in the aquafeed to meet the sensory qualities sought by the consumer (Calanche, Beltrán, & Hernández Arias, 2019); however, those features must be clearly pointed-out first.

Currently, wild-caught seafood is considered the reference for naïve consumers (Claret et al., 2014), their sensory properties being crucial for the acceptance and choice of consumers (Calanche et al., 2019). Specifically, visual impressions, that comprise different appearance properties (e.g., colour, shape, texture, etc) (Spence, 2015b), are usually the first sensory stimuli presented to consumers, raising expectations about the product (Reisfelt, Gabrielsen, Aaslyng, Bjerre, & Møller, 2009). These properties might induce positive sensations leading to product acceptance, or negative sensations leading to its rejection (Spence, 2015b). Therefore, it is vital to identify sea urchins' sensory characteristics sought by consumers, before the improvement of the sea urchin gonads' quality features through dietary modulation. To evaluate the sensory properties of a product, descriptive sensory analysis is one of the most effective methods to evaluate sensory characteristics of seafood products, as it generates objective descriptions of the perceived sensory attributes of products (Massa, Manca, & Yeannes, 2012; Olafsdottir et al., 1997). Moreover, sensory evaluation often requires the use of a trained sensory panel, requiring deep knowledge, continuous training, and considerable financial investment, which could be a determinant limitation (Gacula, 1997). The use of naïve panellists (consumers) has gained increased importance, as they are a source of valuable information. During the last few years, several methodological developments have allowed for the collection of sensory information deriving from consumers' perception of a specific product. Baião, Oliveira, et al. (2020) have previously used projective mapping paired with the Ultra Flash Profile to assess *P. lividus* gonads' sensory properties, where a small experienced panel was able to

discriminate sea urchin gonads according to sex. Phillips et al. (2009) and Phillips, Hamid, Silcock, Sewell, et al. (2010) have also assessed the sensory attributes for another sea urchin species (*Evechinus chloroticus*), in relation to sex and harvest location, and have shown that gonad taste was discriminated according to sex. Recently, a study by Baião, Rocha, et al. (2020), using the Check-All-That Apply (CATA) technique, demonstrated a separation according to sex and presentation method. Female gonads were perceived as being more appealing than males that presented a milky white fluid. Moreover, the gourmet presentation was correlated with a tropical taste and fresh, tropical and pleasant odour, suggesting that the use of fresh sea urchin tests (outer shell) was able to enhance the aroma and affect taste perception of the gonads. The use of CATA questions (Check-All-That-Apply) is gaining popularity as a profiling technique, frequently used and considered appropriate for working with untrained panellists (Adams, Williams, Lancaster, & Foley, 2007; Meyners & Castura, 2014) to obtain a sensory product characterization (Ares & Jaeger, 2015). These questions consist of a list of terms from which consumers have to select all those terms that they consider applicable to describe the sample. Although the simplicity of CATA questions is considered an advantage able to provide valid and reliable data (Ares et al., 2015; Meyners & Castura, 2014), it is also a limitation. The CATA format does not allow a direct measurement of the intensity of the perceived sensory attributes, which could mask a potential discrimination between products that have similar sensory profiles, while varying in the intensity of those features (Ares, Bruzzone, et al., 2014; Meyners, Jaeger, & Ares, 2016; Vidal, Ares, Hedderley, Meyners, & Jaeger, 2018). To overcome this drawback, the use of attribute intensity measurements as a variant to CATA questions has been recommended by Ng, Chaya, and Hort (2013), to enhance sample description and discrimination. In this context, Ares, Bruzzone, et al. (2014) introduced the Rate-All-That-Apply (RATA) concept. In this alternative approach, incorporating intensity-based responses using a CATA-style question, consumers are asked to indicate which terms are applicable to describe the sample, and for those selected, to rate their intensity using a 3 or 5-point scale.

Thus, the main goal of the present work was to evaluate differences between the sensory profile, liking and consumer acceptance of *P. lividus* gonads with a gourmet presentation, according to sex and harvest location. This will act as a benchmark, supporting the identification of gonads with a given attribute set, that may be classified as a premium gonad. This sensory profile, will further support the selection of the best nutritional and feeding methodologies, able to positively impact the final nutritional and sensory quality of farmed sea urchin. Lastly, this may result in the production of sea urchin gonads that are highly accepted by consumers.

2. Material and methods

2.1. Samples

Two different harvest locations along the Portuguese coast, with official reports concerning the existence of sea urchin fishing (*Praia Norte*, Viana do Castelo, Northern Portugal [41°41'49.8"N 8°51'04.5"W] and *Carreço*, Viana do Castelo, Northern Portugal [41°44'32.5"N 8°52'39.7"W]), were selected for this study. Both areas are semi-exposed to ocean swells and the annual seawater temperature ranges from 12 to 20 °C. To assure the required number of male and female specimens for each session, eighty fresh and live *P. lividus* (with a minimum of 5 cm diameter and above 80 g), were simultaneously harvested from each of the selected locations, on the day before the sensory evaluation session and kept alive at 6 °C until analysis. The specimens were harvested in February to assure that the gonads would be in pre-mature or early maturation period, providing the best organoleptic features. At the beginning of the sensory evaluation test, 20 males and 20 females were identified from each location: live males presented a pale-yellow colour and oozed a white liquid through the gonads, while the live females presented a bright-orange colour and oozed an orange liquid through the gonads. From each location, the sex and maturation stage of the sea urchins was later confirmed by histological examination of a small gonad slice fixed in 10% formalin in seawater, according to Barker, Keogh, Lawrence, and Lawrence (1998). Then the five gonads of each sea urchin were carefully removed using a spoon, washed in saline water (36 ‰) and placed in a petri dish at 6 °C, so that each of the first four gonads could be distributed to a panellist (Figure 1). The fifth gonad of each collected urchin was used for instrumental texture, colour analysis and histology, as described above.

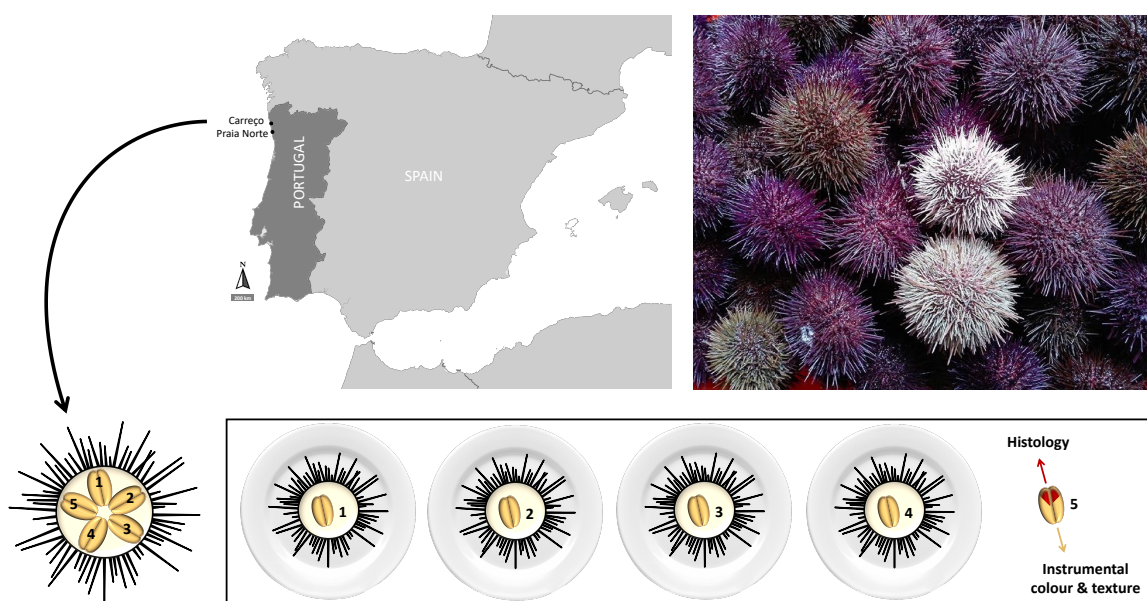


Figure 1 - Scheme of sampling procedures of sea urchin from both harvesting locations.

2.2. Sensory quality assessment

A sensory panel with 80 untrained panellists was recruited from the sensory evaluation company Sense Test's consumer database (Vila Nova de Gaia, Portugal). They were mainly residents in the Oporto metropolitan area, in the North of Portugal. All participants were regular consumers of seafood and fish and received a small financial compensation for their participation. The company ensures the protection and confidentiality of data through the authorization 2063/2009 of the National Data Protection Commission, and following the EU General Data Protection Regulation (EU 2016/679), as well as a longstanding internal code of conduct, assuring informed consent. Sensory evaluation was carried out in individual tasting booths at a special room equipped in accordance with ISO 8589:2007 - Sensory analysis - General guidance for the design of test rooms.

The samples were stored at 6 °C and served at approximately 15 °C, in a small translucent plastic plate (used for hygienic purposes), placed inside a cleaned sea urchin test (gourmet presentation) (Baião, Rocha, et al., 2020), placed over a white porcelain plate, blind-labelled with three-digit random codes. During the session, every panellist analysed one male and one female gonad, from each harvesting location (a total of four samples per panellist). Panellists were provided with a porcelain spittoon, a glass of bottled natural water and unsalted crackers. All panellists were asked to chew a piece of cracker and to rinse their mouth with water before testing each sample. For each session, to compensate for eventual carry-over effects, each panellist received the set of four samples following a monadic and sequential order of presentation, with a balanced presentation order (MacFie, Bratchell, Greenhoff, & Vallis, 1989).

2.3. Overall liking and acceptance

For each sample, overall liking was evaluated using a 9-point hedonic scale, ranging from 1 - "dislike extremely" to 9 - "like extremely" (Peryam & Pilgrim, 1957). The Food Action Rating Scale (FACT), developed by Schutz (1965) was also used to evaluate gonads acceptance. This is a 9-point successive-category food action rating scale, where: 9 - "I would eat this every opportunity that I had", 8 - "I would eat this very often", 7 - "I would frequently eat this", 6 - "I like this and would eat it now and then", 5 - "I would eat this if available but would not go out of my way", 4 - "I do not like this but would eat this on an occasion", 3 - "I would hardly ever eat this", 2 - "I would eat this if there were no other food choices", 1 - "I would eat this only if forced". For each sample, overall liking and acceptance were immediately followed by the evaluation of the sensory profile, through Rate-All-That-Apply (RATA) methodology, as suggested by Ares and Jaeger (2013) and King, Meiselman, and Carr (2013), to reduce bias.

2.4. RATA (Rate-All-That-Apply)

2.4.1. Development of the RATA ballot

The list of terms included on the RATA ballot were generated by the research team, combining previous published studies from qualitative consumer studies with *P. lividus* gonads (Baião, Oliveira, et al., 2020; Baião, Rocha, et al., 2020), which were later refined and modified based on group discussion among the research team. The team members individually tasted samples of gonads from sea urchins of both sex and from different locations and discussed the attributes until a consensus was reached. Moreover, taking into account the consumers feedback and results from the previous CATA study (Baião, Rocha, et al., 2020), a final ballot of 22 sensory attributes was obtained, with attributes organized by sensory dimension to reduce the cognitive effort required by the participants (Ares et al., 2013) (Table 1).

Table 1 - List of 22 sensory attributes divided into four dimensions (*original in Portuguese*). All attributes were rated as absent or present and if present, rated using a 5-point structured scale (from 1 = “slightly applicable” to 5 = “very applicable”).

Sensory dimension	Descriptor
Appearance (Aparência)	milky white fluid (<i>exsudado branco</i>); grainy (<i>granuloso</i>); appealing (<i>apelativo</i>); soft/slimy (<i>mole/viscoso</i>); orange (<i>laranja</i>); clay colour (<i>cor de tijolo</i>).
Odour (Odor)	sea smell (<i>maresia</i>); sweet (<i>doce</i>); fresh (<i>fresco</i>); tropical (<i>tropical</i>); pleasant (<i>agradável</i>); sulfur (<i>sulfúrico/enxofre</i>).
Texture/Mouth-Feel (Textura)	soft (<i>suave</i>); creamy (<i>cremoso</i>); juicy (<i>suculento</i>); grainy (<i>granuloso</i>)
Taste (Sabor)	tropical (<i>tropical</i>); sweet (<i>doce</i>); sea taste (<i>mar</i>); intense (<i>intenso</i>); pleasant (<i>agradável</i>); prolonged aftertaste (<i>sabor residual longo</i>).

Adapted from Baião et al (2021).

2.4.2 RATA evaluation

The untrained panellists were asked to perform a RATA evaluation with a list of 22 sensory attributes divided into four dimensions (Table 1): appearance (6), odour (6), texture (4) and taste (6). While fixing the order of the sensory dimensions, individual attributes within each sensory dimension were presented in a randomized order of presentation, different for each participant and each product (Ares, Etchemendy, et al., 2014). Panellists were asked to check the terms they considered applicable for describing samples and then to rate the intensity of each selected attribute, using a 5-point structured scale (from 1 = “slightly applicable” to 5 = “very applicable”) (Ares, Bruzzone, et al., 2014; Meyners et al., 2016).

2.5. Gonad colour and texture

Gonad colour and texture were measured as previously described by Baião, Rocha, et al. (2019). Gonad colour was assessed instrumentally using a CR-400 colorimeter (Konica Minolta) with a standard illuminant D65, using the $L^*a^*b^*$ scale. From a^* and b^* values the hue angle ($h^* = \tan^{-1} b^* / a^*$) and the chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) were calculated according to (Valente et al., 2015).

Instrumental evaluation of firmness and resilience properties of sea urchin gonads was obtained using a TA.XT.plus texture analyser, fitted with a 5 kg load cell and controlled by Exponent v6 software (Stable Micro systems, UK). Evaluations were performed individually on a single freshly extracted gonad and was run without replicates, due to the limited amount of samples. Both parameters were calculated using the Stable Micro Systems software. Compression test was performed using a 35 mm cylinder probe, according to McBride, Price, Tom, Lawrence, and Lawrence (2004). Firmness was defined as the force (grams) required to compress the sample at 25% of its original height (at 1.7 mm/s) and was calculated in accordance with the AACC standard firmness method (AACC, 1999). Resilience was defined as the capacity of a sample to recover its original height after a compression/distension cycle and was calculated as Resilience (%) = (post-test height / original height) x 100. After determination of firmness, the probe continued to compress the gonad up to 60% of its original height (40% strain) and held that position for 2 s. Then, the probe was withdrawn and after 15 s the post-test height was taken (Rocha et al., 2019).

2.6. Determination of gonad's sex and maturation stage

A small tip of each selected gonad was fixed in formaldehyde during 24 h and then conserved in alcohol 70%. Fixed samples were dehydrated, embedded in paraffin and sectioned transversely to the length axis, according to standard histological procedures. Two paraffin sections (7 µm thickness) of each gonad were stained with haematoxylin and eosin (H/E method) and observed under a binocular microscope (CX22LED, Olympus) for confirmation of the sea urchin's sex. The gametogenic stages of ovary and testes were identified following the six stages of maturation described by Byrne (1990): recovery (stage I), with primary gametes (oocytes and spermatocytes) and nutritive phagocytes; growing (stage II) with clusters of primary gametes and packed nutritive phagocytes; premature (stage III) with gametes at all stages of development and reduced amount of nutritive phagocytes; mature (stage IV) with mature gametes (ova and spermatozoa) and few nutritive phagocytes; partly spawned (stage V) with loosely packed gametes and depletion of nutritive phagocytes; and spent (stage VI) with gonads empty of gametes.

2.7. Statistical analysis

2.7.1. Overall liking and acceptance scores

Data analysis was performed using XLSTAT 2020® system software (Addinsoft, USA). To evaluate the results of the overall liking and food action rating scale, descriptive statistics (mean and standard deviation) were used, and a three-way mixed effects ANOVA, with consumers as a random factor and sex and harvest location, with interaction, as fixed factors, was applied. When significant effects, at a 90% confidence level, were identified, a Least Significant Difference (LSD) multiple comparison test was applied.

2.7.2. Rate-All-That-Apply (RATA)

From the RATA evaluation, a data set was generated with the RATA applicability (1 = “slightly applicable” to 5 = “very applicable”) that were analysed following the recommendations of Ares, Bruzzone, et al. (2014) and Meyners et al. (2016). For each sample and term, RATA scores were calculated by summing up the scores provided by the consumers who selected that term as applicable for describing that sample. The effect of sex and harvest location on the RATA scores for the sensory descriptors of the product was tested by a two-way ANOVA to identify which attributes were discriminating among samples ($p < 0.10$), followed by a pairwise multiple comparison test (Tukey HSD). Correspondence Analysis (CA) was performed, considering Hellinger’s distances (Meyners, Castura, & Carr, 2013), on the sum of scores given by all consumers to each term for describing each sample (RATA scoring) (Ares, Bruzzone, et al., 2014). Such analysis provides a sensory map of the samples, allowing the perception of the similarities and differences between samples and their sensory characteristics (Ares, Barreiro, Deliza, Giménez, & Gámbaro, 2010; Ares, Deliza, Barreiro, Giménez, & Gámbaro, 2010; Ares, Varela, Rado, & Giménez, 2011). Based on the outcomes of the CA, a multi-dimensional alignment (MDA) evaluation (Meyners et al., 2013) was also performed, to determine the correlation between the attributes and the samples vectors in the full-dimensional space of CA, providing complete information about the relationship between products and attributes. From this analysis, one can understand in a more detailed way the differences between samples, namely their correlation with the attributes. The reversed scale on the horizontal axis allows an easier interpretation of values to the right of the centre (90° representing orthogonal vectors, i.e., no correlation between sample and attribute), indicating positive correlation, and those to the left indicate negative correlation. Vertical dashed lines indicate the significance cut-offs of 45° and 135° , respectively (Meyners et al., 2013).

2.7.3. Multiple factor analysis (MFA)

The MFA was applied to assess the consensus between the different table variables analysed: colour parameters ($L^*a^*b^*$, C^* and h^*), texture features (resilience and firmness) and RATA; with overall liking and FACT scale as supplementary data. This statistical methodology allows the analysis of the consensus between different variables that differ in number and nature and is an improved PCA, analysing individual differences instead of average differences (Louw et al., 2013; Nestrud & Lawless, 2008).

3. Results and discussion

3.1. Gonad sensory quality

3.1.1. Overall liking and acceptance

From the three-way mixed effects ANOVA analysis, no significant effect of sex and harvest location were identified for both overall liking and FACT ($p > 0.05$), although a significant effect of the sex for FACT at $p < 0.10$ was identified. Females presented a higher value of FACT when compared to males, suggesting that female gonads were more accepted among panellists (Table 2). A similar result was found in Baião, Rocha, et al. (2020), where the gourmet presentation had also favoured female gonads and undervalued male gonads.

Table 2 - Results from the three-way mixed effects ANOVA, relating liking and attitudes (FACT-Food Action Rating Scale) to *P. lividus* gonads, according to the fixed factors sex and place of origin (source).

Factors	Levels	Liking*	F (df _(level, error)); p-value	FACT [‡]	F (df _(level, error)); p-value
Source	Carreço	7.51 (±1.19)	F (1,296) = 0.416;	6.95 ± (1.66)	F (1,296) = 0.079;
	Praia Norte	7.41 ± (1.31)	p=0.520	6.90 ± (1.62)	p=0.778
Sex	Female	7.56 ± (7.09)	F (1,296) = 1.910;	7.09 ± (1.57)	F (1,296) = 2.861;
	Male	7.36 ± (1.27)	p=0.168	6.77 ± (1.69)	p=0.092
Source × sex		--	F (1,296) = 2.453;	--	F (1,296) = 2.190;
			p=0.118		p=0.140

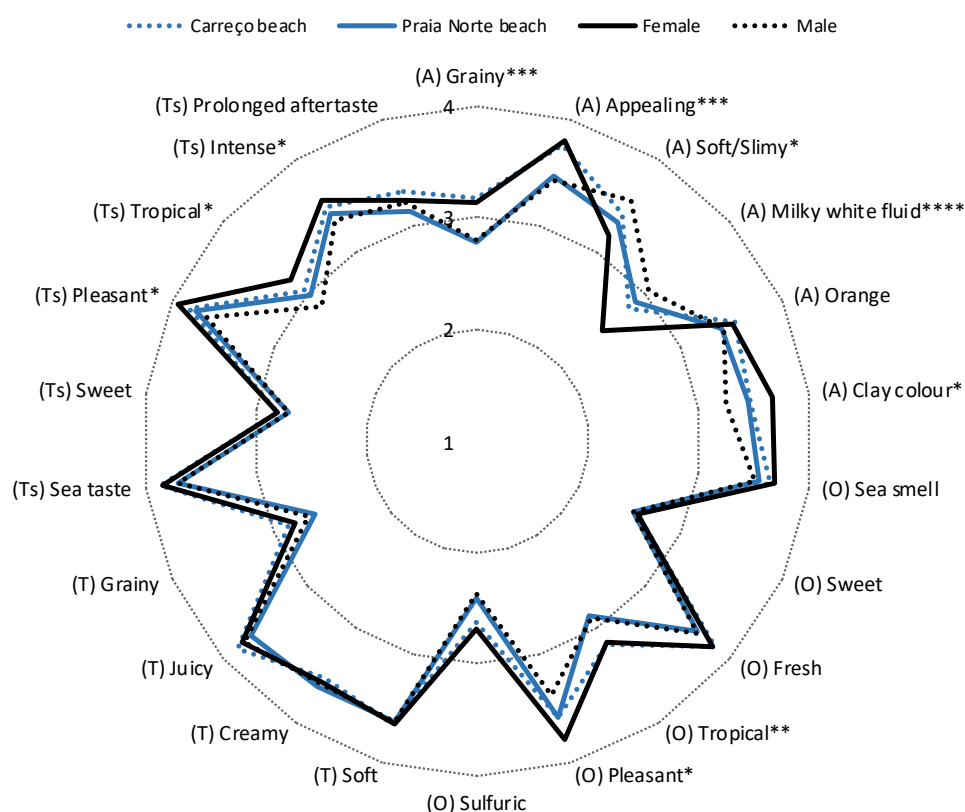
Values are presented as mean (± standard deviation), n = 150.

* Evaluated in a 9-point hedonic scale, ranging from 1 – “dislike extremelly” to 9 – “like extremelly” (Peryam and Pilgrim, 1957).

‡ Evaluated in a 9-point successive-category food action rating scale, ranging from 1 – “I would eat this only if forced” to 9 – “I would eat this every opportunity that I had” (Schutz, 1965).

3.1.2 Rate-All-That-Apply (RATA)

Figure 2 represents a radar chart in which sex and origin are compared regarding the sensory attributes on a two-dimensional plane. From the 22 sensory terms used in this study, only ten showed significant differences ($p < 0.10$) (Table 3). All significant appearance terms (grainy, appealing, slimy, milky white fluid, clay colour) were affected by the sex ($p < 0.05$), but only the grainy and appealing appearance were also affected by the harvest location ($p < 0.05$). The pleasant odour and the pleasant and tropical taste were also affected by the sex ($p < 0.05$). Moreover, the tropical odour and intense taste showed significant differences regarding the source and sex, respectively ($p < 0.10$).



*differences between sex at a significance level of 10%
 **differences between harvest locations at a significance level of 10%
 ***differences between sex and harvest locations at a significance level of 10%
 ****differences between sex x harvest locations at a significance level of 10%

Figure 2 – Radar plot with mean ratings of the sensory attributes (on a scale from 1 to 5, with 1 = “slightly applicable” to 5 = “very applicable”, if considered applicable) of the gonads, according to sex (female/male) and place of origin (*Carreço* and *Praia Norte*) of the sea urchins. Sensory attributes ordered by sensory dimension: A - appearance, O – odour, T – texture and Ts – taste).

Figure 3 shows the configuration of the samples in the first and second dimensions of correspondence analysis applied to the RATA data of sea urchin gonads, which explained 96.3% of the total variance of the experimental data. The representation shows that consumers of sea urchin gonads discriminated the samples in relation to sex. In our study, females were characterized by a pleasant and intense tropical taste, as well as an overall appealing and grainy appearance and a pleasant odour (Figure 3). From Figure 4, one can depict in a more detailed way that the females from *Carreço* were also described as having an attractive clay colour and pleasant tropical odour, the later with significant differences regarding the harvest location. Males had a slimy appearance and were characterized by a white fluid exudate (Figure 3 and 4) that was negatively emphasized by the background of the sea urchin test, with these being consistent with the sex effect on FACT (Table 2) and with the participants feedback at the end of the tasting sessions that revealed a negative impact of the white exudate on product presentation (data not presented). Differences in the sensory quality of gonads have already been correlated with sex by Baião, Oliveira, et al. (2020) and Baião, Rocha, et al. (2020) for *P. lividus* and by Phillips et al. (2009) for *E. chloroticus*. Baião, Oliveira, et al. (2020) have demonstrated that

male gonads have a sweet taste, soft texture and a pale-yellow colour, while female gonads reveal an intense sweet tropical taste and a bright-orange colour. Baião, Rocha, et al. (2020) demonstrated that females were more appealing than males that presented a milky white fluid. Females were associated with a fresh odour and an appealing orange appearance, while males had a brownish appearance and a milky white fluid. Furthermore, in their study, the gourmet presentation had a crucial positive effect on overall appearance, taste and odour of the product, suggesting that the mode of presentation can enhance or deteriorate the sensory properties leading to a better or worse acceptance of the product respectively (Spence, 2015b). Moreover, Phillips et al. (2009) have shown that *E. chloroticus* male gonads had a sweet taste and dairy flavour, while female gonads have a bitter and sour taste and metallic flavour. However, as already suggested by Phillips, Hamid, Silcock, Delahunty, et al. (2010), intrinsic features of the sea urchin (appearance, taste, flavour and odour) might be species-specific. Moreover, Niimi, Leus, Silcock, Hamid, and Bremer (2010) and Phillips, Niimi, et al. (2010) have discussed the impact of location on the quality features of *E. chloroticus*. Therefore, it is predictable that a sea urchin species endemic to New Zealand with different genetics, environmental features, such as water temperature, photoperiod and density, and food availability and quality, may have different organoleptic features compared with sea urchin species from the Atlantic Ocean and/or Mediterranean Sea. Furthermore, within distinct species, the gonadal quality varies along the annual reproductive cycle, mainly due to water temperature, photoperiod and density (Boudouresque & Verlaque, 2020), food availability (Livore & Connell, 2012) and quality (Gago, Range, & Luis, 2003), and also the gonads' maturation stage (Walker, Lesser, & Unuma, 2020) - as early stages of gametogenesis are associated high-quality features, for instance the size, firmness and flavour (Agatsuma, Sato, & Taniguchi, 2005). However, sampled gonads were predominantly in stage III and IV (pre-mature and early maturation stage) and did not present differences in size amongst the groups (data not shown), so maturity and size did not appear to have affected the variance in sensory characteristics of sea urchin gonads.

Table 3 - Results from the two-way ANOVA relating *P. lividus* gonads attributes with statistically significant differences, according to sex and place of origin (source).

Factors	Levels	(A) Grainy	<i>F</i> (df _(level, error)); <i>p</i> -value	(A) Appealing	<i>F</i> (df _(level, error)); <i>p</i> -value	(A) Soft/Slimy	<i>F</i> (df _(level, error)); <i>p</i> -value	(A) Milky white fluid	<i>F</i> (df _(level, error)); <i>p</i> -value	(A) Clay colour	<i>F</i> (df _(level, error)); <i>p</i> -value
Source	<i>Carreço</i>	3.17 ± (1.23)	F (1,296) = 5.553; p=0.019	3.75 ± (1.06)	F (1,296) = 4.925; p=0.027	3.42 ± (1.28)	F (1,296) = 0.446; p=0.505	2.79 ± (1.28)	F (1,296) = 0.284; p=0.594	3.48 ± (1.32)	F (1,296) = 0.020; p=0.888
	<i>Praia Norte</i>	2.77 ± (1.29)		3.47 ± (1.10)		3.33 ± (1.20)		2.88 ± (1.27)		3.46 ± (1.31)	
Sex	Female	3.14 ± (1.29)	F (1,296) = 4.209; p=0.041	3.79 ± (0.95)	F (1,296) = 8.111; p=0.005	3.19 ± (1.28)	F (1,296) = 6.455; p=0.012	2.49 ± (1.19)	F (1,296) = 9.260; p=0.003	3.67 ± (1.29)	F (1,296) = 6.889; p=0.009
	Male	2.79 ± (1.24)		3.43 ± (1.19)		3.56 ± (1.17)		3.04 ± (1.29)		3.26 ± (1.31)	
Source × sex	<i>Carreço</i> x Female (n=75)	3.38 ± (1.14)		3.84 ± (0.95)		3.24 ± (1.37)		2.63 ± (1.18) ^{ab}		3.75 ± (1.27)	
	<i>Carreço</i> x Male (n=75)	2.97 ± (1.27)	F (1,296) = 0.123; p=0.726	3.66 ± (1.16)	F (1,296) = 1.818; p=0.179	3.61 ± (1.15)	F (1,296) = 0.001; p=0.971	2.89 ± (1.33) ^{ab}	F (1,296) = 3.223; p=0.074	3.18 ± (1.31)	F (1,296) = 1.133; p=0.288
	<i>Praia Norte</i> x Female (n=75)	2.92 ± (1.37)		3.73 ± (0.94)		3.14 ± (1.18)		2.36 ± (1.17) ^b		3.58 ± (1.31)	
	<i>Praia Norte</i> x Male (n=75)	2.61 ± (1.18)		3.20 ± (1.18)		3.51 ± (1.20)		3.20 ± (1.22) ^a		3.33 ± (1.29)	

Factors	Levels	(O) Tropical	<i>F</i> (df _(level, error)); <i>p</i> -value	(O) Pleasant	<i>F</i> (df _(level, error)); <i>p</i> -value	(Ts) Pleasant	<i>F</i> (df _(level, error)); <i>p</i> -value	(Ts) Tropical	<i>F</i> (df _(level, error)); <i>p</i> -value	(Ts) Intense	<i>F</i> (df _(level, error)); <i>p</i> -value
Source	<i>Carreço</i>	3.16 ± (1.24)	F (1,296) = 3.252; p=0.072	3.59 ± (1.15)	F (1,296) = 0.013; p=0.908	3.83 ± (0.96)	F (1,296) = 0.249; p=0.618	3.06 ± (1.27)	F (1,296) = 0.255; p=0.614	3.48 ± (1.01)	F (1,296) = 0.272; p=0.603
	<i>Praia Norte</i>	2.87 ± (1.24)		3.58 ± (1.08)		3.77 ± (0.96)		2.98 ± (1.25)		3.42 ± (1.03)	
Sex	Female	3.14 ± (1.21)	F (1,296) = 2.224; p=0.137	3.80 ± (0.95)	F (1,296) = 10.689; p=0.001	3.95 ± (0.86)	F (1,296) = 6.484; p=0.011	3.20 ± (1.24)	F (1,296) = 4.986; p=0.026	3.56 ± (0.94)	F (1,296) = 3.361; p=0.068
	Male	2.90 ± (1.28)		3.38 ± (1.22)		3.66 ± (1.04)		2.83 ± (1.26)		3.34 ± (1.08)	
Source × sex	--	--	F (1,296) = 0.069; p=0.793	--	F (1,296) = 2.528; p=0.113	--	F (1,296) = 0.626; p=0.429	--	F (1,296) = 0.508; p=0.477	--	F (1,296) = 0.002; p=0.967

Values are presented as mean (± standard deviation), n = 150.

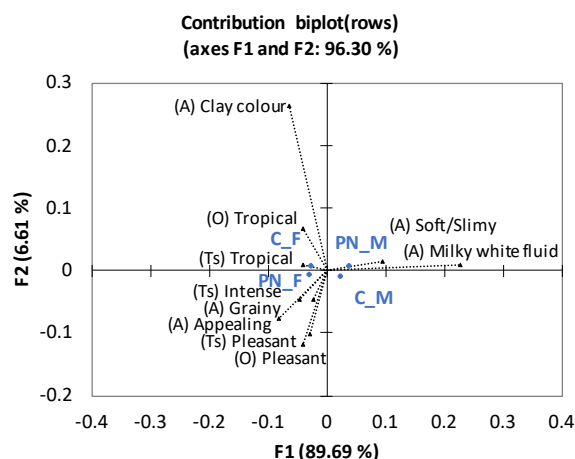


Figure 3 - Biplots with dimensions 1 and 2 of correspondence analysis of RATA (Rate-All-That-Applies) data from the sensory profiling of sea urchin gonads. A - Correspondence analysis performed on the sum of scores given by all consumers to each term for describing each sample (RATA scoring); and B - Correspondence analysis performed on the frequency table containing the number of panellists who used each term for describing each sample. Abbreviations are C = *Carreço* beach, PN = *Praia Norte* beach, M = male, and F = female.

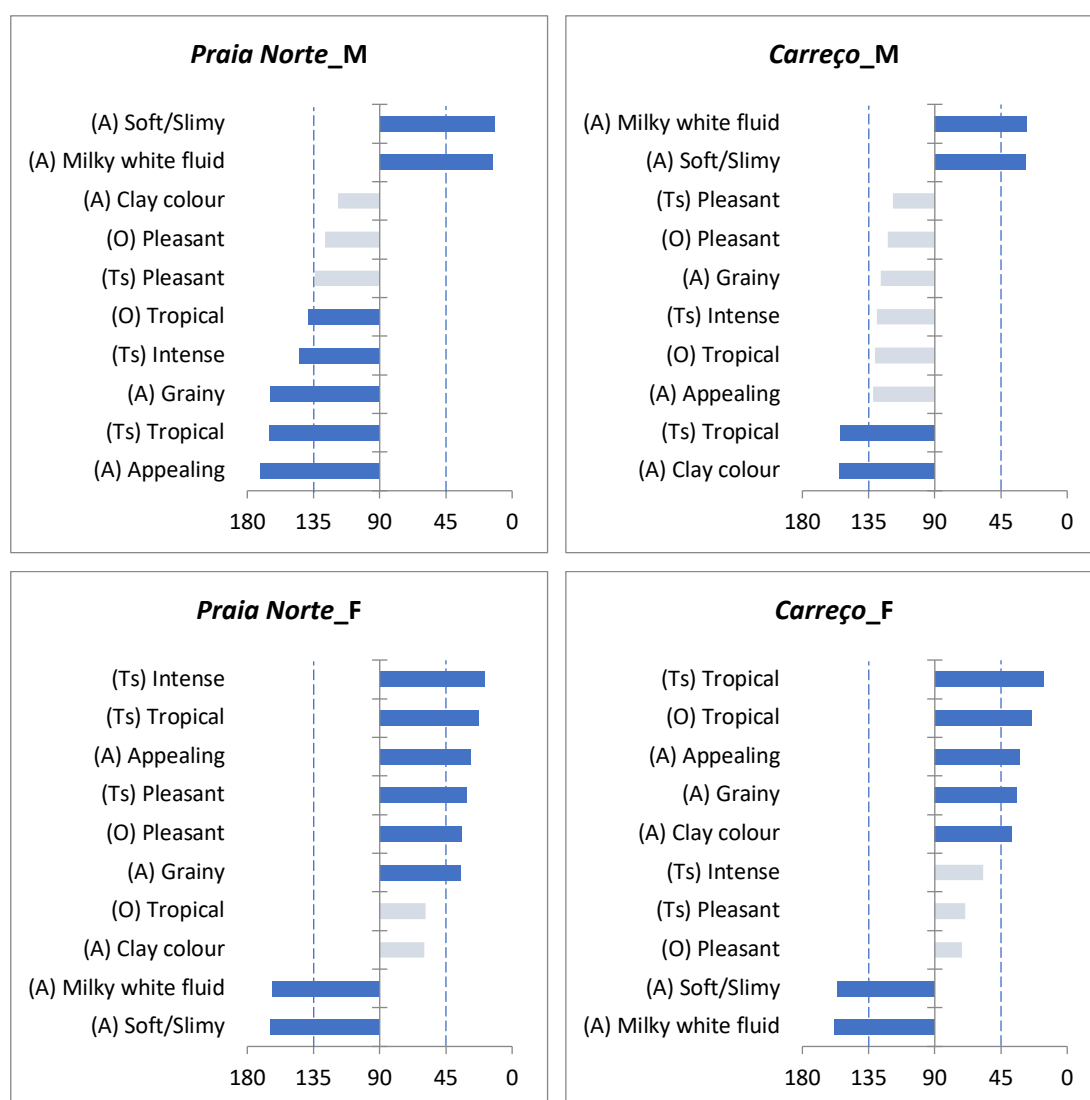


Figure 4 - Associations between attributes and products based on the vectorial angle (0 to 180°) between samples and attributes, based on the MDA. A - Performed using data from the correspondence analysis on the sum of scores given by all consumers to each term for describing each sample; and B - Performed using data from the correspondence analysis on the frequency table containing the number of panellists who used each term for describing each sample. Abbreviations are M = male, F = female.

3.1.3 Gonad colour expression and texture

Colour is the first visual feature used by consumers for the visual assessment and freshness perception of sea urchin gonads, impacting the choice and purchase decisions (Robinson, 2004). Colour parameters, except for a^* ($p = 0.01$), as well as texture parameters were not affected by the harvesting location ($p > 0.05$) (Table 3). However, differences were found between sex, with females exhibiting less luminous (lower L^* values), redder (higher a^*) and yellower (higher b^*) gonads than males. These data are in accordance with Rocha et al. (2019) and Baião, Rocha, et al. (2020) that demonstrated that *P. lividus* males had brighter gonads than females, while females had more reddish and yellowish gonads than males. In fact, colour is the most important product-intrinsic sensory feature when it comes to setting the consumers' expectations regarding the likely taste and flavour of a specific food (Spence, 2015a, 2015b). The perception of positive properties, such as their orange (Baião, Rocha, et al., 2020) or clay colour in this study, may lead to a better acceptance of the product, whilst negative features, as the white exudate or dark coloration (Baião, Rocha, et al., 2020), might lead to its rejection (Cardello, 1996; Spence, 2015b).

Gonad resilience was higher in females than in males (Table 3), however, in other studies with the same species, there were no statistical differences between sexes (Baião, Rocha, et al., 2019; Rocha et al., 2019). Firmness was not affected by the source nor the sex of sea urchins.

3.1.4 Multiple factor analysis (MFA)

Multiple factor analysis was performed to compare results from sea urchin gonads' instrumental colour and texture features, RATA, overall liking and FACT evaluations. Table 4 shows the RV coefficients resulting from MFA. For colour, texture and RATA, the RV values in the MFA configuration were very high and close to unit: 0.937, 0.853, 0.923, respectively. These results show that the projection patterns of the samples in all parameter subspaces were similar. When individually comparing the other RV values, one can note that the association between colour and RATA presented a significantly high RV value (0.935; $p < 0.05$).

Table 4 - Results from the two-way ANOVA relating instrumental colour ($L^*a^*b^*$ scale) and texture (firmness and resilience) measurements to *P. lividus* gonads, according to sex and place of origin (source).

Factors	Levels	L^*	F (df _(level, error)); p -value	a^*	F (df _(level, error)); p -value	b^*	F (df _(level, error)); p -value	Firmness (g)	F (df _(level, error)); p -value	Resilience (%)	F (df _(level, error)); p -value
Source	Carreço	43.99 ± (4.52)	F (1,76) = 2.816; p =0.097	23.81 ± (4.49)	F (1,76) = 6.665; p =0.012	40.61 ± (6.17)	F (1,76) = 0.694; p =0.407	41.28 ± (18.39)	F (1,76) = 0.545; p =0.463	77.71 ± (3.17)	F (1,76) = 2.297; p =0.134
	Praia	45.47 ± (5.03)		22.12 ± (4.66)		39.39 ± (7.38)		44.25 ± (17.35)		78.88 ± (3.72)	
	Norte										
Sex	Female	41.92 ± (3.28)	F (1,76) = 40.594; p <0.001	26.55 ± (2.78)	F (1,76) = 120.950; p <0.001	42.26 ± (5.07)	F (1,76) = 9.550; p =0.003	39.80 ± (15.39)	F (1,76) = 2.091; p =0.152	79.10 ± (3.89)	F (1,76) = 4.321; p =0.041
	Male	47.55 ± (4.50)		19.38 ± (3.16)		37.74 ± (7.57)		45.61 ± (19.71)		77.49 ± (2.84)	
Source × sex	--	--	F (1,76) = 0.440; p =0.509	--	F (1,76) = 0.771; p =0.383	--	F (1,76) = 0.804; p =0.373	--	F (1,76) = 2.216; p =0.141	--	F (1,76) = 0.804; p =0.373

Values are presented as mean (± standard deviation), n = 40.

Table 5 - RV coefficients resulting from multiple factor analysis applied to RATA, gonad instrumental colour and texture values and acceptance and FACT evaluations (p -value is shown in brackets, for the correlation between multidimensional variables).

	RATA	Colour	Texture	Liking	FACT	MFA
RATA	1.000	-	-	-	-	-
Colour	0.935 (0.04)	1.000	-	-	-	-
Texture	0.600 (0.50)	0.634 (0.25)	1.000	-	-	-
Liking	0.673	0.476	0.207	1.000	-	-
FACT	0.746	0.573	0.356	0.954	1.000	-
MFA	0.923	0.937	0.853	0.479	0.601	1.000

The MFA plot, shown in Figure 5, presents the first two dimensions of the MFA consensus, explaining 92.1% of the variability of the experimental data. It shows that participants clustered the samples into three different groups. Sea urchin gonads were discerned in relation to sex and females were further discriminated regarding their source. Male sea urchins were negatively loaded along F1 and were associated with a “slimy” and “milky” appearance, as well as with high values of brightness (L^*) and firmness. On the other hand, female gonads were positively loaded along F1, and were associated with an “appealing” appearance, “clay” colour and an “intense” but “pleasant” “tropical” taste and odour. Furthermore, females were correlated with high values of yellowness (b^*) and redness (a^*). A closer look into Figure 5 indicates that there was a positive correlation between yellowness, redness, resilience, and the “appealing” appearance and the “pleasant” “tropical” taste, that were intrinsically associated with consumer acceptance and overall liking. This is in accordance with the previous CATA study (Baião, Rocha, et al., 2020) that has demonstrated a strong correlation between yellowness, redness, resilience, “appealing” appearance and “orange” colour values, with consumer acceptance and overall liking. Contrarily, in Phillips, Hamid, Silcock, Delahunty, et al. (2010) male gonads were rated higher than females for colour appearance. However, these results result from a different species (*E. chloroticus*), endemic to a different region: New Zealand. Indeed, the perception of appearance can be distinct among consumers of different regions. For example, Japanese and New Zealander consumers favour a yellowish colour as well as a sweet taste and firm texture (Barker, 2015; Stefánsson, Kristinsson, Ziemer, Hannon, & James, 2017), whereas Mediterranean consumers rather prefer a bittersweet flavour, firm texture and a reddish-orange colour (Sartori & Gaion, 2016).

Visual features impact the visual assessment and freshness perception of sea urchin gonads and consequently raise positive or negative expectations about the product, leading to their acceptance or rejection respectively (Reisfelt et al., 2009; Spence, 2015a). Therefore, future research for the selection of the most suitable diet for *P. lividus* should take into consideration the reference market. Likewise, the latent characteristic of the discrimination of the sensory profile of sea urchin gonads regarding the sex, may lead to dedicated diets for each sex or production of monosexual populations in the future. Alternatively, aquaculture may focus on the production of good quality gonads with a mixture of individuals from both sexes, and the gastronomic exploitation of mixed gonads may be explored, as suggested by Baião, Moura, Lima, Valente, and Cunha (2019).

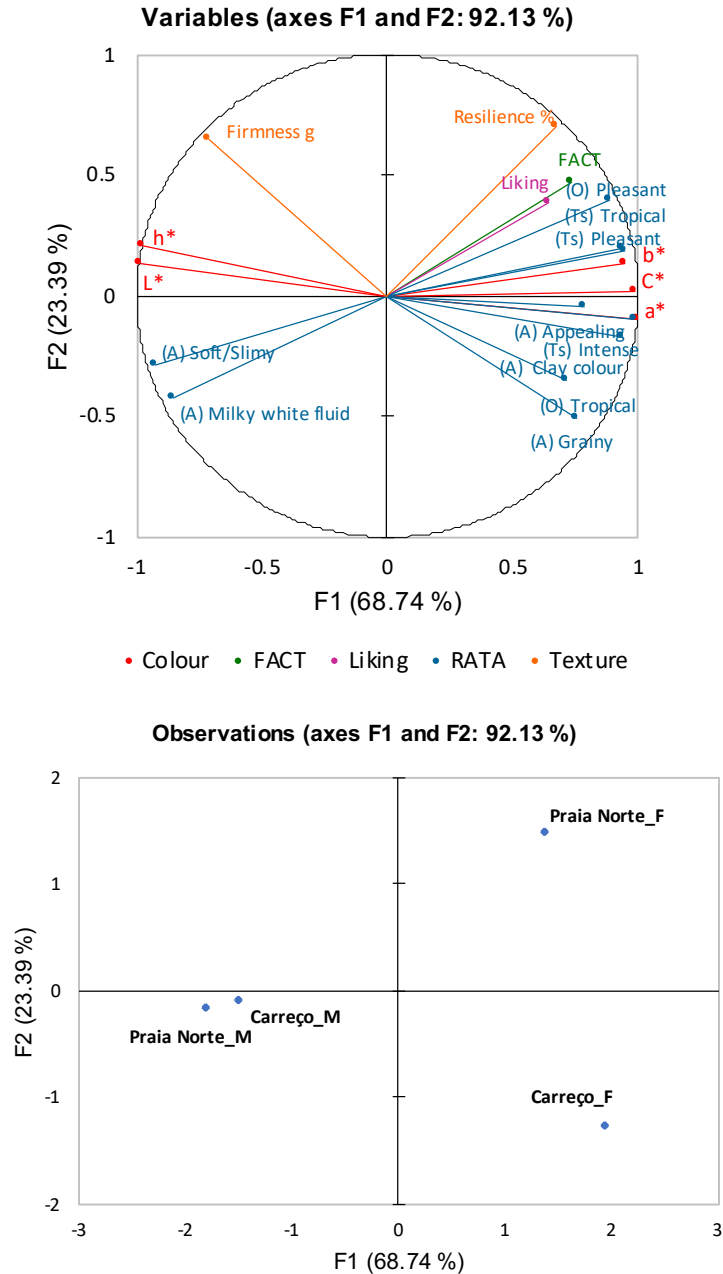


Figure 5 - Samples (down side) and attributes (top side) projections of dimension 1 and 2 from multiple factor analysis applied to sea urchin gonads data from instrumental colour (red lines), texture features (orange lines), RATA profiling (blue lines), liking (purple lines) and FACT (green lines). Abbreviations are M = male, F = female, (A) = appearance, (O) = odour and (Ts) = Taste.

4. Conclusions

Despite the lack of differences in overall liking and acceptance in relation to sex, this study identified differences in the sensory profile of male and female gonads, clearly going beyond liking in the evaluation of sea urchin gonads. Female had a more appealing gonad, presenting an intense but pleasant tropical flavour that was preferable among consumers.

The use of the RATA approach revealed an increase in discrimination power, as it allowed to separate female gonads according to their origin, with those from *Praia Norte* being firmer, more resilient and more appealing than the ones from *Carreço*.

This information will allow future nutritional research efforts to be focused on the enhancement of this *P. lividus* gonads attributes set. However, as the sensory profile of sea urchin gonads seems to be linked to the sea urchin's sex, dedicated diets for each sex might also be envisioned in the future. On the other hand, processing methodologies (garnishes, sauces, and pates) for animals with low gonad quality or mixture with the higher quality ones, might also be solutions to add economic value to this product.

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Chapter VIII.

Effect of protein and lipid levels in diets for adult sea urchin *Paracentrotus lividus*

by

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Effect of protein and lipid levels in diets for adult sea urchin *Paracentrotus lividus* (Lamarck, 1816)

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ABSTRACT

Paracentrotus lividus gonads are considered a prized seafood delicacy due to its organoleptic features. The increasing market demand for sea urchin depleted several wild stocks, thus aquaculture can play an important role in supplying high-quality animals. Nevertheless, the success of this activity largely depends on the development of nutritionally balanced diets capable of producing excellent gonads. In this study, four extruded diets formulated with low or high protein (30 and 50% dry matter (DM), LP and HP, respectively) and lipid (6 and 11% DM, LL and HL, respectively) levels were fed to wild-caught individuals (4.5 cm diameter) for 15 weeks. Twelve homogeneous groups of 15 individuals were randomly distributed by mesh cages (45 urchins/m²) within 250 L tanks in a recirculating aquaculture system (RAS) at 18 °C and a 12 L:12 D photoperiod regime. Sea urchins fed the LP/LL diet were compared to wild specimens with similar maturation stage.

All experimental diets were well accepted by sea urchins and resulted in similar final body weight (42–43 g). No significant differences were observed in specific growth rate or protein efficiency ratio among diets. The HP diets led to a decrease of dry matter and energy intake resulting in the lowest feed conversion ratio (FCR). Weight gain, whole body composition and nutrient gain remained similar among dietary treatments. The gonad somatic index (GSI) varied between sex but was similar among dietary treatments (19–23%) and was higher than values observed in wild specimens (6–7%). Females had significantly higher total concentration of carotenoids than males, irrespectively of the dietary treatments. Dietary treatments and sex did not affect gonad texture (firmness and resilience) or colour parameters (brightness, redness, yellowness, chroma or hue angle), but affected gonad composition. Total carotenoid content in gonads was not affected by diets and resulted in lower values compared to wild specimens. These had higher levels of echinenone, β-cryptoxanthin and β-carotene and showed higher redness and firmness values when compared to the LP/LL diet, irrespectively of the sex. Gonad lipid content and n-3 PUFA were higher in female than in male, whilst protein content showed an opposite trend, suggesting some sex related differences in the metabolic use of nutrients.

This study clearly shows that it is possible to enhance sea urchin gonad yield in relation to wild animals in a RAS at 18 °C. All diets were effective in promoting gonad growth, but the diet with the lowest protein (30% DM) and lowest lipid (6% DM) level resulted in the highest gonad yield in both sexes.

1. Introduction

Sea urchin gonads are prized seafood products in several parts of the world, especially in Asia and Europe. Although all echinoids can be

considered edible (Lawrence, 2013), only a few species are harvested. *Loxechinus albus* and *Strongylocentrotus* spp. are the most harvested species worldwide, whereas *Paracentrotus lividus* is the most exploited and commercially important species in the Atlantic and Mediterranean

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coasts, where its gonads are considered a delicacy.

The increasing demand for urchins' gonad, its intensive harvest, as well as the global warming, have resulted in a drastic reduction of natural stocks (Bertocchi et al., 2018; Ghisaura et al., 2016; Ouréns et al., 2015; Yeruham et al., 2015). This generated a worldwide interest in developing sea urchin aquaculture for the production of high-quality gonads, able to supply the expanding markets, filling the gap between the growing demand and decrease of natural supply (Phillips et al., 2010). Although the sea urchin life cycle is already controlled under captivity, echinoculture is still largely dependent on wild-caught sea urchins, as growing out from small-sized juveniles in aquaculture is faster and less cost-intensive (Phillips et al., 2010). Different factors can influence both the yield and quality of roe, such as abiotic factors (e.g. temperature, salinity, photoperiod), nutritional factors (e.g. feed ingredients and chemical composition) and rearing conditions (e.g. stock density, water circulation) (Lourenço et al., 2018b; Rocha et al., 2018; Rocha et al., 2019). The quality of gonads is mainly determined by its size, taste, colour and consistency, which are influenced by the protein, lipid, carbohydrate and carotenoid content of diets (Cuesta-Gomez and Sánchez-Saavedra, 2017; Taylor et al., 2017). It has become clear that the success and sustainability of commercial sea urchin aquaculture, its economic viability and market acceptance strongly depend on the development of suitable, cost effective and nutritionally balanced diets capable of producing high-quality gonads for the caviar-like industry (Eddy et al., 2012; Pearce et al., 2002; Woods et al., 2008).

Dietary protein is an important macronutrient that provides essential amino acids and energy used in several biological processes, including maintenance, growth and reproduction of sea urchins (Hammer et al., 2012). However, protein is one of the costliest nutrients in aquafeeds and should be spared from excessive catabolism and mainly used for tissue growth (Hefflin et al., 2012b). To date, only a few studies have evaluated different protein levels in formulated diets for *P. lividus* (Carboni et al., 2015; Prato et al., 2018b; Zupo et al., 2019) without clearly identifying the species requirement for maximal growth and gonad yield. Recently, Lourenço et al. (2018a) evaluated six practical extruded diets with increasing protein levels (20–45% of dry matter, DM) in adult specimens, observing that a 30% DM dietary protein with 7% lipids concomitantly induced high nutrient utilization and promoted highest gonadosomatic index (GSI). Moreover, according to Zupo et al. (2019), the protein content of diets for *P. lividus* is strongly correlated with the GSI and should be incorporated up to 40–47%, keeping low lipid levels (< 9%), to assure the highest GSI and theca diameter. Most studies in sea urchin showed that dry formulated diets with a protein content varying between 15 and 50% DM were able to simultaneously promote somatic and gonad growth (Lourenço et al., 2018b). In *Strongylocentrotus franciscanus*, McBride et al. (1998) reported highest gonad production at protein levels of 40% DM, but in *L. variegatus* a minimum protein level of 20–21% seems optimal for growth and feed efficiency, in both juvenile and adults (Hammer et al., 2004, 2006a). In *Strongylocentrotus purpuratus*, diets that contained three protein (17%, 23% and 30%) and two carbohydrate (42% and 50%) levels were all effective in significantly increasing GSI, but individuals fed the 30% protein and 50% carbohydrate diet had the highest gonad index (Cuesta-Gomez and Sánchez-Saavedra, 2018). Previous studies have shown that sea urchins may adjust feed intake to satisfy energy requirements (principally carbohydrates), regardless other nutrient levels (Hammer et al., 2006b; Taylor, 2006). However, in adult *Lytechinus variegatus*, Hefflin et al. (2016b) have recently shown that regardless of dietary macronutrient combination, sea urchins maintained an average dietary protein intake of 0.047–0.061 g/d, suggesting that this species regulates feed intake to satisfy its protein requirement. However, according to Schlosser et al. (2005) besides protein, the energy content of formulated diets seems a limiting factor for *P. lividus* gonad growth and development.

Many studies evaluated the effects of dietary protein and carbohydrate levels in sea urchin species (Eddy et al., 2012; Fernandez, 1997;

Hammer et al., 2006b, 2012; Hefflin et al., 2012a, 2012b; Schlosser et al., 2005; Woods et al., 2008), but the effects of dietary lipid levels have been scarcely reported. Gibbs et al. (2009) observed that diets with 7–11% lipids resulted in a higher deposition of fat in the gonads of *Lytechinus variegatus* males compared to a diet with 3% lipids, but no differences were observed in females, suggesting distinct metabolic pathways between sexes. Moreover, while dietary lipid level can be crucial for normal metabolic functioning, the quality of lipids should also be considered. Although sea urchins can synthesize long chain-polyunsaturated fatty acids (LC-PUFA), for instance 20:4n-6 (arachidonic acid, ARA) and 20:5n-3 (eicosapentaenoic acid, EPA), from their precursors, the linoleic (18:2n-6, LA) and linolenic (18:3n-3, ALA) fatty acids (FA), respectively, all these essential fatty acids (EFA) must be supplied in the diet to improve growth (Carboni et al., 2015; Castell et al., 2004; González-Durán et al., 2008). In fact, *Strongylocentrotus droebachiensis* fed diets deficient in n-3 and n-6 PUFA showed poor growth (González-Durán et al., 2008). Dietary FA composition is reflected in sea urchin tissues (Castell et al., 2004; González-Durán et al., 2008; Liyana-Pathirana et al., 2002), for example, dietary phospholipids seem to be mainly metabolized and stored as neutral lipids in the gut and gonads, affecting gonad quality (Gibbs et al., 2009). The increase of n-3 LC-PUFA in sea urchin gonads is important from a consumer's perspective, since these FA have been associated to a decreased risk of cardiovascular diseases (Givens, 2009).

This study was designed to evaluate the effects of different dietary protein: lipid ratios on *P. lividus* growth and gonad quality, under a recirculating aquaculture system (RAS). Four extruded diets were formulated with two protein (30 and 50%) and two lipid (6 and 11%) levels and fed to wild-caught adult sea urchins. After 15 weeks, biometric parameters (weight, test diameter and gonad yield) and gonad quality (maturation stage, nutritional value, texture and colour) were analysed in both female and male urchins. At the end of the growth trial, the gonads with highest yield were compared to those of wild specimens at similar maturation stage.

2. Material and methods

Experiments were previously approved by the CIIMAR ethical committee for Managing Animal Welfare (ORBEA), in compliance with the European Union Directive 2010/63/EU and the Portuguese Decree Law n° 113/2013 on "the protection of animals used for scientific purposes". Wild sea urchins were collected after authorization from the national authorities: the Portuguese maritime authority (Captaincy of the Port of Viana do Castelo) and the Institute for Nature Conservation and Forests (ICNF) following the Nagoia protocol.

2.1. Experimental diets

In this study, four extruded diets were formulated to contain 30 or 50% DM protein (P), designated by low (LP) or high (HP) protein level, and 6 or 11% DM lipid (L) designated by low (LL) or high (HL) lipid levels. This resulted in four experimental diets: LP/LL, LP/HL, HP/LL and HP/HL. The ingredients and chemical composition of the experimental diets are shown in Table 1. Diets were manufactured by SPAROS, Lda (Portugal). All ingredients were grounded in a micro-pulverizer hammer mill (Hosokawa Micron, SH1, Germany) and subsequently mixed according to each specific formulation, in a paddle mixer (Mainca RM90, Spain). This mixture was subsequently humidified with water (250 g kg⁻¹) and agglomerated by low shear extrusion (Italpast P55, Italy). Upon extrusion, the resulting feed was dried in a convection oven (OP 750-UF, LTE Scientifics, UK) for 3 h at 70 °C and dried feed pellets were subsequently sieved to retrieve the desired particle size (around 2.6 cm). Diets stability was consistent among diets over 48 h.

Table 1

Formulation and proximate composition of the experimental diets with different dietary protein/lipid levels (P/L).

	Dietary treatments			
	LP		HP	
	LL	HL	LL	HL
Ingredients %				
Squid meal ^a	5.0	5.0	5.0	5.0
Fish gelatin ^b	7.5	7.5	7.5	7.5
Pea protein concentrate ^c	3.5	3.8	14.0	14.0
Wheat gluten ^d	3.5	3.8	14.0	14.5
Corn gluten ^e	3.5	3.8	14.8	15.0
Wheat meal ^f	38.2	33.2	6.2	1.2
Laminaria powder ^g	5.0	5.0	5.0	5.0
Macroalgae mix ^h	15.0	15.0	15.0	15.0
Krill oil ⁱ	1.0	1.0	1.0	1.0
Linseed oil ^j	1.0	1.0	1.0	1.0
Soy lecithin ^k	3.3	7.6	3.0	7.3
Binder ^l	5.0	5.0	5.0	5.0
Antioxidant powder ^m	0.5	0.5	0.5	0.5
Mono calcium phosphate ⁿ	1.0	1.0	1.0	1.0
Calcium carbonate ^o	5.0	5.0	5.0	5.0
Vit & Min Premix ^p	2.0	2.0	2.0	2.0
Chemical composition (% DM)				
Dry matter	96.9	95.5	95.3	97.7
Crude protein	30.2	30.2	51.8	50.2
Crude fat	6.6	11.3	6.2	11.7
Carbohydrates ^q	43.3	39.0	22.0	18.0
Starch	21.7	21.6	6.8	5.4
Gross energy (kJ/g DM)	16.8	17.3	18.5	19.1
Ash	19.6	19.5	20.0	20.1
Protein: energy ratio (g kJ ⁻¹)	1.8	1.7	2.8	2.6
Carotenoids (µg/g DM)				
Total carotenoid	29.9	31.4	27.1	33.0
450 nm	7.3	7.8	7.7	9.6
Fatty acids (mg/g DM)				
14:0	1.3	1.7	1.1	1.6
16:0	13.3	23.0	11.8	23.0
18:0	2.1	3.5	2.0	3.8
20:0	0.1	0.2	0.1	0.2
22:0	0.2	0.3	0.2	0.3
Σ SFA ^r	17.4	29.7	15.7	29.8
16:1n-7	0.7	0.9	0.6	0.8
18:1n-9	11.3	18.8	11.8	23.0
18:1n-7	1.3	2.1	1.1	2.0
20:1n-9	0.3	0.5	0.3	0.4
22:1n-9	0.1	0.1	0.1	0.1
Σ MUFA ^s	14.0	22.6	13.9	25.1
18:2n-6 (LA)	22.3	42.4	22.4	45.6
18:3n-3 (ALA)	6.2	9.3	5.0	9.0
18:4n-3	0.2	0.3	0.2	0.3
20:4n-6 (ARA)	0.2	0.2	0.1	0.2
20:5n-3 (EPA)	1.2	1.7	0.9	1.4
22:6n-3 (DHA)	0.7	1.1	0.6	0.9
EPA + DHA ^t	1.9	2.9	1.4	2.3
Σ PUFA ^u	31.1	55.5	29.4	57.8
Σ n-6	22.6	42.7	22.6	45.9
Σ n-3	8.5	12.6	6.7	11.8
n-6/n-3	2.7	3.4	3.4	3.9

The abbreviations for the experimental diets stand for: HP - High protein (51%); LP - Low protein (30%); HL - High lipids (11%) and LL - Low lipids (6%).

^a Super prime without guts: 82% CP, 3.5% CF, Sopropêche, France.

^b Pharma grade bloom 240: 92% CP, LAPI Gelatine SPA, Italy.

^c Lysamine GP: 78% CP, 8% CF, ROQUETTE, France.

^d Wheat gluten composition: DM: 901 g kg⁻¹; protein: 838 g kg⁻¹ DM; lipids: 5 g kg⁻¹.

^e Corn gluten feed: 61% CP, 6% CF, COPAM, Portugal.

^f Micronized wheat composition; DM: 876 g kg⁻¹; protein: 144 g kg⁻¹ DM; lipids: 24 g kg⁻¹.

^g Algover®^h, *Laminaria digitata*: 4.7–12.1% CP, 0.7–1.6% CF, Setalg, France.

^h Ocean feed shrimp - Seaweed meal™, Ocean Harvest Technology, Ireland.

ⁱ SuperbaKrill™ oil, Solchem, Spain.

^j Henry Lamotte Oils GmbH, Germany.

^k Lecico P700IPM, LECICO GmbH, Germany.

^l Kielseguhr (natural zeolite): LIGRANA GmbH, Germany.

^m Antioxidant powder, Paramega PX, Kemin Europe NV, Belgium.

ⁿ Monocalcium phosphate: 22% phosphorus, 16% calcium, Fosfitalia, Italy.

^o PREMIX Lda, Portugal.

^p Premix PV01 for marine fish, PREMIX Lda, Portugal. Vitamins (IU or mg/kg diet): DL-alpha tocopherol acetate, 100 mg; sodium menadione bisulphate, 25 mg; retinyl acetate, 20,000 IU; DL cholecalciferol, 2000 IU; thiamin, 30 mg; riboflavin, 30 mg; pyridoxine, 20 mg; cyanocobalamin, 0.1 mg; nicotinic acid, 200 mg; folic acid, 15 mg; ascorbic acid, 1000 mg; inositol, 500 mg; biotin, 3 mg; calcium panthotenate, 100 mg; choline chloride, 1000 mg, betaine, 500 mg. Minerals (g or mg/kg diet): cobalt carbonate, 0.65 mg; copper sulphate, 9 mg; ferric sulphate, 6 mg; potassium iodide, 0.5 mg; manganese oxide, 9.6 mg; sodium selenite, 0.01 mg; zinc sulphate, 7.5 mg; sodium chloride, 400 mg; calcium carbonate, 1.86 g; excipient wheat middlings.

^q Estimated by difference: Carbohydrates = 100 - (Crude protein + Crude fat + Ash).

^r SFA, Saturated fatty acids.

^s MUFA, Monounsaturated fatty acids.

^t EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid.

^u PUFA, Polyunsaturated fatty acids.

2.2. Growth trial

The growth trial was conducted in the Fish Culture Experimental Unit of CIIMAR, Matosinhos, Portugal, with adults of *P. lividus* with test diameter of 44.80 ± 0.35 mm, collected in a rocky intertidal shore along the Praia Norte in Viana do Castelo, Portugal [41°41'57"N 8°51'13"W]. Sea urchins were kept in quarantine to adjust to laboratory conditions for 4 weeks prior to the beginning of the experiment and were fed with the control diet.

After acclimatization, sea urchins were fasted for 6 days before being individually weighed (mass, g) and measured (test diameter, cm). Twelve homogeneous groups of 15 individuals (initial weight of 34.92 ± 0.66 g per urchin; diameter of 4.48 ± 0.35 cm) were randomly distributed by square plastic mesh cages (45 urchins/m²) within 12 tanks of 250 L in a RAS with both mechanical and biological filtration and UV sterilization. Water was supplied to each tank at 4 L/min (water exchange rate of 100% per hour) and rearing conditions were maintained at 35–37‰ of salinity, 18 °C water temperature, 12 L:12 D photoperiod and 8–10 mg/L of oxygen saturation through the experiment. Nitrogenous compounds and pH were regularly monitored during the trial and kept at levels recommended for marine species (ammonia < 0.05 mg/L, nitrite < 0.5 mg/L, pH between 8 and 8.5).

Each one of the four diets was randomly assigned to triplicate tanks and hand-fed to urchins every two days. The feed ration started at 0.5% of total biomass and after 3 consecutive meals, the ration was adjusted: feed ration was increased or decreased in one pellet (5–10%) depending on the absence or presence of non-ingested feed, respectively. Any uneaten pellet was collected and weighed.

The experiment was conducted for 15 weeks and sea urchins were bulk-weighed in the middle of the trial to monitor weight gain and feed consumption. At the beginning of the trial, the whole body content (gut and gonads) of six sea urchins was collected and kept at –20 °C, until initial whole body composition was analysed. At the end of the 15-week period, each sea urchin was individually weighed (mass, g) and measured (test diameter, cm). The whole body content of four urchins per tank was collected and kept at –20 °C until analysis of whole body composition. From each tank, the gonads of four females and four males were individually weighed for GSI determination, and one portion of a gonad per individual was collected for histological analysis. One gonad from each individual was used for sensory parameters analysis (gonad colour and texture), and the remaining gonads were pooled from each tank for determination of gonad composition. All gonads were then frozen with liquid nitrogen and kept at –80 °C until analysis.

2.3. Proximate analysis of feed and whole body

Sea urchins feed and whole body content (gut and gonads) were freeze-dried, ground and homogenized before proceeding with the analysis. All chemical analyses followed AOAC (2006) methods and were performed in duplicate. Samples were analysed for DM (105 °C for 24 h), ash by combustion in a muffle furnace (Nabertherm L9/11/B170, Bremen, Germany; 500 °C for 5 h), crude protein (N x 6.25) using a Leco nitrogen analyser (Model FP-528, Leco Corporation, St. Joseph, USA), crude lipid content by petroleum ether extraction (40–60 °C) using a Soxtec™ 2055 Fat Extraction System (Foss, Hilleroed, Denmark) and gross energy using an adiabatic bomb calorimeter (Werke C2000, IKA, Staufen, Germany). Also, fatty acid methyl esters (FAME) of dietary lipids were prepared by one-step extraction using 10% HCl in methanol (Sukhija and Palmquist, 1988).

2.4. Gonad quality

2.4.1. Gonad chemical composition

Samples collected for gonad composition were ground in liquid nitrogen, pooled, and moisture was determined (105 °C for 24 h). Afterwards, gonads pools were freeze-dried before proceeding with previously described chemical analyses.

Total lipids in sea urchins gonads were extracted and quantified gravimetrically from freeze-dried tissue samples by the method described by Folch et al. (1957) using dichloromethane:methanol (2:1) instead of chloroform:methanol (2:1). Afterwards, gonad lipids were transesterified into fatty acid methyl ester (FAME) by acidic catalysis, as already reported by Campos et al. (2017). The internal standard was C23:0 at 1 mg/mL hexane (Matreya LLC, USA). FAME were separated by gas chromatography with flame-ionization detector using a Shimadzu GC-2010 Plus gas chromatograph (Shimadzu Europe GmbH, Germany) equipped with an Omegawax 250 capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness; Supelco, Bellefonte, USA), under previously described conditions (Campos et al., 2017). Identification of FAME was obtained by comparison of retention times with those of authentic standards (FAME mix 37 components from Supelco Inc., Bellefont, PA, USA; PUFA-1, PUFA-2, PUFA-3, Bacterial Acid Methyl Esters CP and GLC-110 Mixtures from Matreya LLC, Pleasant Gap, USA). Dietary and gonad FA were quantified as % of total FA and mg/g of gonad wet weight, respectively.

2.4.2. Total carotenoids content analysis

Carotenoid extraction was performed according to Symonds et al. (2007) with some modifications. In order to minimize sample oxidation, the carotenoids extraction was carried out as rapidly as possible under reduced light conditions. Briefly, freeze-dried gonad tissue was homogenized using a pestle and mortar. Carotenoids from homogenized tissue (0.4–0.5 g) were extracted, in duplicate, in 100% acetone (5:1 v:w), centrifuged at 3500 x g for 10 min at 8 °C and the supernatant collected. Then, the pellet was re-suspended in acetone and the extraction procedure was repeated several times until obtaining a colourless supernatant (three extractions were usually sufficient). Samples were evaporated to dryness under a constant stream of oxygen-free nitrogen to eliminate all acetone solvent. Extracts were re-suspended in 100% n-hexane and filtered with a 0.2 µL syringe filter, to obtain a clear extract for further chromatography analysis. Total carotenoid content in the gonads was determined in a spectrophotometer (Shimadzu UV-1603, Japan), using the maximum absorption in hexane and the extinction coefficient ($E_{1\text{cm}}^{1\%}$) of 2500 (Tsushima et al., 1995), based in the equation describe by Pocock et al. (2004):

$$C = (E \times V \times 1000) / [100 \times G \times (E_{1\text{cm}}^{1\%})]$$

where,

C = concentration of carotenoid (mg)/ wet weight (g).

E = maximal absorption of a known volume of carotenoid solution read at the wavelength of maximum absorption.

V = sample final volume (mL).

$E_{1\text{cm}}^{1\%}$ = the extinction coefficient of a 1% (w/v) solution in a 1 cm cuvette at a defined wavelength.

G = sample weight (g).

Samples were then evaporated to dryness under a constant stream of nitrogen and kept at –80 °C until further carotenoid identification by high-performance liquid chromatography analysis (Waters Alliance Series 600, Mildford, MA, USA). To assess the qualitative and quantitative profile of carotenoid pigments, samples from the same sex of LP/LL treatment were pooled. The carotenoid extract from each sample was suspended in 1 mL of n-hexane and duplicate injections of 20 µL were performed for each sample. The separation of carotenoid pigments was performed in a reverse phase Acclaim™ C30 LC column (250 × 4.6 mm i.d., 5 µm particle size, Thermo Scientific) with a mixture of acetonitrile, methanol, dichloromethane, hexane and ammonium acetate (55:22:11.5:11.5:0.02 v:v:v:v:w) under isocratic conditions at 1.0 mL/min flow rate, for 25 min, at 25 °C. Detection was achieved by a diode array detector (Waters 996 PDA, USA) at wavelengths ranging from 200 to 600 nm. Absorbance was measured at 454 nm. Retention times and spectra of compounds were analysed by comparison with pure standards and quantification was performed by calibration curves of α-carotene, β-carotene, echinenone, (Sigma, Portugal), lutein and β-cryptoxanthin (Extrasynthèse, France). Data were analysed using the Waters Empower™ 2 software and expressed as µg/g of fresh biomass.

2.4.3. Gonad colour and texture

Gonad colour measurements were done with a CR-400 colorimeter (Konica Minolta) with an aperture of 8 mm, at standard illuminant D65 using the CIE 1976 (L^* , brightness; a^* , redness; b^* , yellowness) as previously described for sea urchins (Robinson et al., 2002). Initially, the apparatus was calibrated with a white plate reference standard (Minolta Co., Ltd., Osaka, Japan). Three replicate measurements were taken for each gonad sample and averaged to determine the colour parameters. Colour parameters were measured by applying the colorimeter onto the gonad. After flashing, L^* , a^* and b^* reflected light values were recorded. From a^* and b^* values the hue angle ($h^* = \tan^{-1} b^* / a^*$) and the chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) were calculated according to Valente et al. (2015).

An instrumental evaluation of firmness and resilience properties was performed in sea urchin gonads using a TA.XT.plus texture analyser, fitted with a 5 kg load cell and controlled by Exponent v6 software (Stable Micro systems, UK). Tests were performed individually in a single portion of freshly dissected gonad and was run without replicates, due to the limited amount of sample. Both parameters were calculated using the Stable Micro Systems software. Compression test was performed using a 35 mm cylinder probe, according to McBride et al. (2004). Firmness was defined as the force (grams) required to compress the sample at 25% of its original height (at a 1.7 mm/s) and was calculated in accordance with the AACC standard firmness method (AACC, 1999). Resilience was defined as the capacity of a sample to recover its original height after a compression and was calculated as Resilience (%) = (post-test height / original height) × 100. After firmness determination, the probe continued to compress the gonad up to 60% of its original height (40% strain) and held that position for 2 s. Then, the probe was withdrawn and after 15 s the post-test height was taken.

2.5. Gonad maturation

Sea urchins' maturation stage and sex were determined by histological examination of the gonads. Sex determination was also made on freshly dissected specimens by examining the gonads for extruded

gametes but was further confirmed by histological analysis. Gonads were fixed in formaldehyde during 24 h and then conserved in alcohol 70%. Fixed samples were dehydrated, embedded in paraffin and sectioned transversely to the length axis, according to standard histological procedures. Two paraffin sections (7 µm thickness) of each gonad were stained with haematoxylin and eosin (H/E) method and observed under a binocular microscope (CX22LED, Olympus). The gametogenic stages of ovary and testes were identified following the six stages of maturation described by Byrne (1990): recovery (stage I), with primary gametes (oocytes and spermatocytes) and nutritive phagocytes; growing (stage II) with clusters of primary gametes and packed nutritive phagocytes, premature (stage III) with gametes at all stages of development and reduced amount of nutritive phagocytes; mature (stage IV) with mature gametes (ova and spermatozoa) and few nutritive phagocytes; partly spawned (stage V) with loosely packed gametes and depletion of nutritive phagocytes and spent (stage VI) with gonads empty of gametes.

2.6. Calculations

Average body weight (ABW) = (final body weight + initial body weight)/2; FCR = dry feed intake/weight gain; SGR = $(\text{LN}(\text{final body weight}) - \text{LN}(\text{initial body weight}))/\text{days} \times 100$; Protein efficiency ratio (PER) = weight gain/crude protein intake; GSI = gonad weight/total weight $\times 100$; Dry matter (DM), protein (P), lipids (L) or energy (E) intake = (dry feed consumption $\times$ DM, P, L (%) or E (kJ/g) in the diet/ABW/days); DM, P, L or E gain = (final gonad DM, P, L or E content – initial gonad DM, P, L or E content)/ABW/days; DM, P, L or E retention = (DM, P, L or E gain/DM, P, L or E intake) $\times 100$.

2.7. Statistical analysis

Results are expressed as mean $\pm$ standard deviation. Data were tested for normality and homogeneity of variances by Shapiro-Wilk test and Levene's test, respectively, and were log-transformed whenever necessary.

Dietary protein and lipid were considered the main factors and the tank was the experimental unit. The trial data were submitted to a two-way ANOVA to determine significant differences ($p < .05$), followed by a pairwise multiple comparison test (Tukey HSD) with Bonferroni correction using the SPSS (IBM SPSS Statistics, IL, USA). Comparisons between individuals fed LP/LL diet and wild specimens followed the same procedures but considered sex and rearing conditions as main factors and followed the same tests.

3. Results

All the experimental diets were well accepted by the sea urchins and induced similar growth. Final body weight remained similar among dietary treatments (42–43 g) (Table 2). No significant differences were obtained in SGR and PER among experimental diets ($p > .05$). Increasing dietary protein level from 30 to 50% significantly decreased DM and energy intake, and decreased FCR from 3 to 1.6–1.7. Lipid intake was also affected by the experimental diets ($p < .001$, Table 2).

Final whole body composition did not vary among dietary treatments (19% DM, 12% wet weight (WW) protein, 3% WW crude lipids, 35 kJ/g WW energy). Energy and protein retention remained unaffected by the experimental diets, however, DM and lipid retention were highest in diets with HP and LL, respectively ($p < .05$). Nutrient gain was similar in all diets (Table 2).

The GSI was generally high and similar among dietary treatments (~21%), however, the highest values were observed in urchins fed the LP/LL diet, independently of the sex. Females presented higher gonad weight and GSI than males (Table 3), and also a higher percentage of lipid (2 vs 3–4% WW) and DM than males, but a lower percentage of protein (17–18 vs 13% WW). Total lipids in gonads were significantly

increased in sea urchins fed the HP/HL diet compared to all other diets ($p < .05$). Total carotenoid concentrations in gonads were similar in all dietary treatments, but significantly higher in females than in males (15–17 vs 12–14 µg/g WW). Colour parameters (brightness, redness, yellowness, chroma and hue angle) were neither affected by dietary treatments nor by sex ($p > .05$). However, males tended to have higher values of brightness (L^*) and hue angle (h^*), and lower values of redness (a^*) and yellowness (b^*), when compared to females. The tested diets and sex did not affect gonad texture: firmness and resilience ($p > .05$).

The FA composition of gonads from reared *P. lividus* is presented in Table 4. PUFA comprised the most abundant FA group (2.7–5.9 mg/g WW), followed by saturated FA (SFA) (1.3–3.3 mg/g WW) and mono-unsaturated FA (MUFA) (0.9–2.4 mg/g WW). The concentration of the majority of individual FA was significantly higher in ovaries than in testis ($p < .01$), except for 18:0, 20:3n-6, 20:3n-3 and 22:6n-3 (DHA) that were similar ($p > .05$) between sexes. The main SFA for both sexes was the 16:0 that varied between 0.7 and 1.9 mg/g WW in males and females, respectively. The major MUFA was the 20:1n-9 in males (0.3–0.4 mg/g WW) and the 18:1n-9 in females (0.6–0.8 mg/g WW). In males, the major PUFA were the ARA and EPA that ranged between 0.7 and 0.8 and 0.6–0.7 mg/g WW, respectively. In females the major PUFA was the 18:2n-6 (LA) that ranged between 1.5 and 2.2 mg/g WW, followed by ARA and EPA with 1.0–1.4 and 0.9–1.0 mg/g WW, respectively. Conversely, DHA was one of the less abundant FA (0.1 mg/g WW), irrespectively of the dietary treatment or sex. Although the FA profile did not differ among experimental diets ($p > .05$), significant effects of both sexes ($p < .001$) and diet ($p = .001$) were found for the n-6/n-3 PUFA ratio. In fact, the ratio was lowest in males fed LP/LL (1.2 mg/g WW) and highest in females fed LP/HL, HP/HL or HP/LL diets (1.8–1.9 mg/g WW).

At the end of the trial, the predominant maturation stage was the mature stage (IV and V). As the LP/LL diet resulted in similar growth, but in the highest gonad yield and lowest productions costs, their gonad quality traits were correlated with wild samples, of the same maturation stage, that were harvested at the end of the trial in the same beach where sea urchins from the growth trial were collected. The GSI of reared sea urchins was significantly higher (20–23%) than that of wild harvested animals (6–7%). Animals fed with LP/LL diet showed higher levels of protein and energy, and lower levels of lipids when compared to wild animals (Table 5). Results in Table 5 also show that males presented higher levels of protein (16–18 vs 13%) and lower levels of lipids (2.2–2.6 vs 3.3–4.0%) and DM (20.1–20.9 vs 21.1–21.7%) than females, irrespectively of rearing conditions.

Total carotenoids in wild sea urchins had significantly higher values (87–89 vs 12–15%) than those fed the LP/LL diet, irrespectively of the sex (Table 5). Moreover, the presence of five carotenoid pigments was identified in all individuals: α - and β -carotene, echinenone, lutein and β -cryptoxanthin. Levels of individual pigments varied significantly with sex and rearing conditions. Wild individuals, both males and females, had significantly higher levels of all the five carotenoids identified, when compared to urchins fed LP/LL diet. Echinenone was the dominant carotenoid in gonads of all sea urchin, irrespectively of sex or rearing conditions, however males showed higher levels of echinenone (22–93 µg/g WW in individuals fed LP/LL and wild, respectively) compared to females (8–27 µg/g WW in individuals fed LP/LL and wild, respectively). β -cryptoxanthin was the second most abundant pigment in all sea urchins, and the males fed with LP/LL diet had significantly higher levels of this carotenoid than females (5.1 vs 4.6 µg/g WW), although in wild specimens no sex differences occurred. Females always showed a significantly higher amount of lutein than males.

Concerning gonads colour, brightness and hue angle values were highest in male individuals cultivated indoor (59 vs 38–48 and 74 vs 53–62, respectively), but redness was highest in wild females (25 vs 7–18). In addition, yellowness and chroma were also higher in females when compared to males (Table 5). Wild specimens have shown higher

Table 2

Growth performance, feed intake, final whole body composition, nutrient gain and retention of *Paracentrotus lividus* fed different dietary protein/lipid levels (P/L) over 106 days.

	Dietary treatments ¹				Two-way ANOVA P-value		
	LP		HP		Protein	Lipid	P x L
	LL	HL	LL	HL			
Growth							
FBW ² (g)	43.18 ± 1.79	42.39 ± 1.31	43.41 ± 2.40	43.03 ± 0.72	0.66	0.56	0.84
Final test diameter (cm)	4.86 ± 0.08	4.85 ± 0.10	4.97 ± 0.03	4.83 ± 0.13	0.41	0.19	0.27
SGR ³	0.21 ± 0.03	0.17 ± 0.03	0.21 ± 0.04	0.20 ± 0.00	0.25	0.13	0.34
FCR ⁴	2.92 ± 0.60 ^a	3.42 ± 0.47 ^a	1.63 ± 0.26 ^b	1.76 ± 0.09 ^b	< 0.001	0.21	0.44
PER ⁵	1.16 ± 0.21	0.98 ± 0.14	1.21 ± 0.20	1.14 ± 0.06	0.32	0.22	0.56
Intake (g or kJ/kg ABW ⁶ /day)							
Dry matter	5.90 ± 0.61 ^a	5.65 ± 0.27 ^a	3.37 ± 0.01 ^b	3.47 ± 0.21 ^b	< 0.001	0.72	0.40
Protein	1.78 ± 0.19	1.71 ± 0.08	1.75 ± 0.01	1.74 ± 0.11	0.98	0.58	0.61
Lipid	0.39 ± 0.04 ^{ab}	0.64 ± 0.03 ^a	0.21 ± 0.00 ^{bb}	0.40 ± 0.02 ^{ba}	< 0.001	< 0.001	0.13
Energy	98.86 ± 10.18 ^a	97.72 ± 4.57 ^a	62.21 ± 0.16 ^b	66.21 ± 3.93 ^b	< 0.001	0.69	0.47
Final body composition ⁷ (% WW)							
Dry matter	19.02 ± 1.31	18.93 ± 0.36	18.30 ± 1.01	18.26 ± 1.85	0.36	0.93	0.97
Crude protein	12.38 ± 0.58	12.86 ± 1.05	11.72 ± 0.63	11.75 ± 1.64	0.19	0.69	0.73
Crude fat	3.14 ± 0.46	2.98 ± 0.15	2.93 ± 0.24	2.75 ± 0.31	0.26	0.37	0.94
Gross energy (kJ/g)	3.86 ± 0.40	3.70 ± 0.13	3.46 ± 0.29	3.58 ± 0.55	0.26	0.92	0.54
Gain (g or kJ/kg ABW ⁶ /day)							
Dry matter	0.58 ± 0.13	0.50 ± 0.08	0.51 ± 0.14	0.49 ± 0.19	0.62	0.54	0.77
Protein	0.42 ± 0.06	0.42 ± 0.14	0.35 ± 0.10	0.34 ± 0.17	0.34	0.98	0.91
Lipid	0.12 ± 0.05	0.09 ± 0.01	0.10 ± 0.04	0.07 ± 0.03	0.33	0.20	1.00
Energy	9.44 ± 4.19	6.38 ± 2.00	5.48 ± 4.30	6.24 ± 5.56	0.42	0.65	0.46
Retention (% of intake)							
Dry matter	10.03 ± 3.29 ^b	8.91 ± 1.35 ^b	15.25 ± 4.12 ^a	14.14 ± 5.56 ^a	0.04	0.63	1.00
Protein	23.87 ± 6.00	24.83 ± 7.72	20.39 ± 5.42	19.97 ± 9.86	0.36	0.95	0.88
Lipid	30.86 ± 15.64 ^A	14.10 ± 1.80 ^B	46.67 ± 15.70 ^A	18.29 ± 7.74 ^B	0.18	0.01	0.42
Energy	9.89 ± 5.43	6.54 ± 2.06	8.80 ± 6.88	9.48 ± 8.20	0.80	0.71	0.58

Values are presented as mean ± standard deviation (n = 3). ^{A,B} Uppercase superscripts indicate differences between levels of lipids. ^{a,b} Lowercase superscripts indicate differences between levels of protein (P < .05).

¹ HP, high protein; LP, low protein; HL, high lipid; LL, low lipid diets.

² Final body weight.

³ Specific growth rate.

⁴ Feed conversion ratio.

⁵ Protein efficiency ratio.

⁶ Average body weight.

⁷ Initial body composition (% WW): dry matter, 16.77 %; protein, 10.43 %; lipids, 2.52 %; energy, 3.68 kJ/g; ash, 0.41 %

values of firmness and resilience than individuals fed with the LP/LL diet, notwithstanding only firmness values varied significantly between sexes, being highest in males.

The comparison of FA composition of gonads of wild and reared sea urchins is portrayed in Table 6. The majority of individual FA concentrations was significantly higher in ovaries than in testis (p < .05), except for 18:1n-9 concentration that was higher in wild males than females. On the other hand, wild specimens had higher levels of most FA compared to sea urchins fed LP/LL diet, except for LA, ALA, 20:3n-6 and DHA that were higher in individuals fed the LP/LL diet. Wild females had higher concentrations of 14:0, 20:1n-9, 22:1n-9 and 18:4n-3, while LP/LL females had higher concentrations of LA and ALA. The most abundant SFA for both sexes was the 16:0, that was higher in females' gonads when compared to males. MUFA varied with both sexes and rearing condition, being highest in wild females mainly due to a higher level of 20:1n-9 (1.2 vs 0.4–0.7). PUFA and the sum of EPA and DHA were significantly higher in females, particularly in wild individuals. Moreover, the n-6 /n-3 PUFA ratio was higher in females fed LP/LL diet than in other animals (1.5 vs 0.6–1.2).

4. Discussion

The present study shows that all extruded diets were well accepted by animals and that it is possible to enhance wild sea urchins' gonad

yield under a RAS system. Dietary protein was the major factor affecting feed intake. DM and energy intake were significantly lower in diets with a high-protein level, irrespectively of the dietary lipid level. This is consistent with previous findings of Lourenço et al. (2018a) also reporting decreased DM and energy intake with increasing dietary protein levels. According to Schlosser et al. (2005) besides protein, the energy content of formulated diets could also be a limiting factor for *P. lividus* gonad growth and development, but this could not be confirmed in the present study. Indeed, we observed a similar dietary protein intake, independently of the crude protein or protein: energy ratio of experimental diets. Likewise, Heflin et al. (2016b) suggested that regardless of dietary macronutrient combination, adult *L. variegatus* regulates feed intake to satisfy its protein requirement.

P. lividus fed the HP diets had a lower FCR (1.7) when compared to those fed the LP diets (3.2), what is consistent with previous data in several species where lower FCR values were typically associated with high protein diets (Cuesta-Gomez and Sánchez-Saavedra, 2016; Heflin et al., 2012b). The observed wide range of FCR values in our study were also reported by Shpigel et al. (2004) in *P. lividus* (1.8–2.6), fed a low protein diet at varying temperatures and photoperiods. Although feed was formulated and extruded to have a good stability in water, feeding took place every 2 days. Therefore, and taking into account that sea urchins are grazers and have a passive feeding behaviour (Marsh et al., 2013), some nutrient leaching and consequently an overestimation of

Table 3
Final gonad composition (% WW), gonad yield, total carotenoids (µg/g WW), colour and texture of male and female *Paracentrotus lividus* fed different dietary protein/lipid levels (P/L).

	Dietary treatments ¹				Two-way ANOVA			
	Male		Female		P-value			
	LP/LL	LP/HL	HP/LL	HP/HL	LP/LL	LP/HL	HP/LL	HP/HL
Final gonad composition (% WW)								
Dry matter	20.12 ± 0.15 ^{ab}	19.79 ± 0.53 ^{ab}	19.35 ± 0.67 ^b	20.56 ± 1.31 ^a	21.63 ± 0.54 ^{ab}	20.77 ± 0.73 ^{ab}	20.92 ± 0.47 ^b	21.97 ± 0.28 ^a
Crude protein	17.82 ± 0.40	17.19 ± 0.29	17.14 ± 0.54	18.02 ± 0.24	13.39 ± 0.27	12.75 ± 0.46	13.00 ± 0.58	13.49 ± 0.90
Total lipids	2.19 ± 0.14 ^a	2.24 ± 0.12 ^a	2.12 ± 0.13 ^a	2.39 ± 0.23 ^a	3.33 ± 0.39 ^b	3.47 ± 0.24 ^b	3.08 ± 0.26 ^b	4.16 ± 0.34 ^a
Gross energy (kJ/g)	4.67 ± 0.22	4.37 ± 0.15	4.36 ± 0.15	4.60 ± 0.13	4.64 ± 0.12	4.61 ± 0.17	4.51 ± 0.13	4.83 ± 0.32
Yield								
Gonad weight (g)	9.69 ± 0.52	8.62 ± 0.64	9.64 ± 0.94	8.22 ± 0.33	11.02 ± 2.02	9.26 ± 0.80	9.47 ± 1.07	11.06 ± 1.31
GSI ² (%)	20.39 ± 1.74	20.05 ± 0.45	19.47 ± 0.79	19.07 ± 0.67	22.97 ± 3.19	19.84 ± 2.03	20.70 ± 1.52	22.53 ± 1.39
Carotenoids, colour and texture								
Total carotenoid	11.88 ± 3.88	13.61 ± 4.14	11.95 ± 3.43	14.09 ± 1.20	15.31 ± 0.98	19.21 ± 2.09	15.50 ± 2.69	17.08 ± 5.02
L*	58.99 ± 6.61	56.11 ± 8.00	51.21 ± 5.28	52.17 ± 5.40	46.27 ± 3.84	46.97 ± 11.14	50.63 ± 4.38	51.83 ± 5.02
a*	7.13 ± 3.18	12.41 ± 7.22	16.63 ± 2.66	13.55 ± 10.62	18.15 ± 0.15	14.14 ± 6.11	16.06 ± 3.26	16.81 ± 4.51
b*	25.14 ± 4.02	31.40 ± 8.46	35.11 ± 4.08	32.10 ± 13.20	34.26 ± 3.29	30.71 ± 5.04	35.76 ± 3.30	33.35 ± 3.87
C*	26.2 ± 4.7	34.0 ± 10.3	39.1 ± 3.4	35.2 ± 16.0	38.9 ± 2.9	34.0 ± 6.7	39.3 ± 4.2	37.6 ± 5.3
H*	74.8 ± 5.0	70.2 ± 8.2	65.0 ± 4.7	71.2 ± 12.1	61.9 ± 2.8	66.1 ± 7.5	65.9 ± 2.9	65.5 ± 5.4
Resilience (%)	74.82 ± 6.56	74.87 ± 4.35	77.50 ± 6.11	82.32 ± 8.12	75.94 ± 2.53	74.30 ± 12.47	80.51 ± 5.76	78.27 ± 7.51
Firmness (g)	13.80 ± 0.85	15.31 ± 2.09	13.24 ± 1.79	13.47 ± 2.77	11.35 ± 0.86	14.51 ± 2.11	14.03 ± 1.11	12.33 ± 0.09
Sex								
Diet								
S x D								

Values are presented as mean ± standard deviation (n = 3). In each line, different superscript letters indicate significant differences (P < .05); differences between among diets are shown using a,b,* without significant differences after post-hoc analysis.

¹ HP/HL, high protein and high lipid; LP/LL, low protein and low lipid; LP/HL, low protein and high lipid; HP/LL, high protein and low lipid diets.

² Gonad somatic index.

Table 4Effect of different dietary protein and lipid levels on total fatty acid (FA) (mg/g WW) composition of gonads from *Paracentrotus lividus*.

	Dietary treatments ¹								Two-way ANOVA P-value		
	Male				Female				Sex	Diet	S x D
	LP/LL	LP/HL	HP/LL	HP/HL	LP/LL	LP/HL	HP/LL	HP/HL			
Fatty acids (mg/g WW)											
14:0	0.25 ± 0.02 ^b	0.23 ± 0.07 ^b	0.25 ± 0.17 ^b	0.24 ± 0.08 ^b	0.78 ± 0.12 ^a	0.70 ± 0.22 ^a	0.84 ± 0.33 ^a	0.72 ± 0.15 ^a	< 0.001	0.86	0.93
16:0	0.76 ± 0.04 ^b	0.73 ± 0.15 ^b	0.68 ± 0.35 ^b	0.82 ± 0.19 ^b	1.77 ± 0.17 ^a	1.82 ± 0.54 ^a	1.91 ± 0.76 ^a	1.89 ± 0.43 ^a	< 0.001	0.98	0.97
18:0	0.26 ± 0.03	0.27 ± 0.03	0.23 ± 0.07	0.32 ± 0.09	0.30 ± 0.02	0.31 ± 0.10	0.33 ± 0.12	0.30 ± 0.06	0.21	0.85	0.56
Σ SFA ²	1.39 ± 0.02 ^b	1.35 ± 0.23 ^b	1.27 ± 0.64 ^b	1.51 ± 0.30 ^b	3.05 ± 0.32 ^a	3.03 ± 0.92 ^a	3.30 ± 1.31 ^a	3.11 ± 0.69 ^a	< 0.001	1.00	0.94
18:1n-9	0.14 ± 0.01 ^b	0.19 ± 0.09 ^b	0.17 ± 0.14 ^b	0.19 ± 0.06 ^b	0.61 ± 0.04 ^a	0.76 ± 0.27 ^a	0.72 ± 0.25 ^a	0.77 ± 0.22 ^a	< 0.001	0.69	0.91
18:1n-7	0.13 ± 0.01 ^b	0.11 ± 0.02 ^b	0.11 ± 0.06 ^b	0.12 ± 0.04 ^b	0.28 ± 0.02 ^a	0.27 ± 0.07 ^a	0.29 ± 0.11 ^a	0.27 ± 0.05 ^a	< 0.001	0.95	0.94
20:1n-9	0.38 ± 0.02 ^b	0.32 ± 0.05 ^b	0.33 ± 0.12 ^b	0.40 ± 0.13 ^b	0.49 ± 0.06 ^a	0.46 ± 0.13 ^a	0.60 ± 0.26 ^a	0.50 ± 0.10 ^a	0.01	0.79	0.64
22:1n-9	0.13 ± 0.00 ^b	0.10 ± 0.03 ^b	0.11 ± 0.06 ^b	0.11 ± 0.05 ^b	0.23 ± 0.03 ^a	0.23 ± 0.07 ^a	0.27 ± 0.11 ^a	0.23 ± 0.05 ^a	< 0.001	0.89	0.85
Σ MUFA ³	0.98 ± 0.05 ^b	0.89 ± 0.18 ^b	0.90 ± 0.46 ^b	1.01 ± 0.30 ^b	2.06 ± 0.17 ^a	2.13 ± 0.66 ^a	2.35 ± 0.90 ^a	2.20 ± 0.50 ^a	< 0.001	0.97	0.93
18:2n-6 (LA)	0.45 ± 0.07 ^b	0.65 ± 0.22 ^b	0.49 ± 0.32 ^b	0.64 ± 0.27 ^b	1.52 ± 0.12 ^a	2.17 ± 0.76 ^a	1.88 ± 0.65 ^a	2.23 ± 0.67 ^a	< 0.001	0.32	0.78
18:3n-3 (ALA)	0.13 ± 0.02 ^b	0.15 ± 0.06 ^b	0.13 ± 0.11 ^b	0.13 ± 0.05 ^b	0.54 ± 0.03 ^a	0.59 ± 0.20 ^a	0.56 ± 0.20 ^a	0.55 ± 0.14 ^a	< 0.001	0.97	1.00
18:4n-3	0.05 ± 0.01 ^b	0.04 ± 0.02 ^b	0.06 ± 0.06 ^b	0.03 ± 0.02 ^b	0.17 ± 0.06 ^a	0.16 ± 0.03 ^a	0.19 ± 0.09 ^a	0.19 ± 0.01 ^a	< 0.001	0.91	0.90
20:2n-6	0.11 ± 0.01 ^b	0.12 ± 0.05 ^b	0.11 ± 0.03 ^b	0.11 ± 0.06 ^b	0.23 ± 0.02 ^a	0.16 ± 0.05 ^a	0.31 ± 0.17 ^a	0.15 ± 0.01 ^a	0.002	0.26	0.18
20:3n-6	0.08 ± 0.01	0.10 ± 0.04	0.10 ± 0.02	0.11 ± 0.04	0.13 ± 0.01	0.11 ± 0.03	0.11 ± 0.04	0.10 ± 0.02	0.34	1.00	0.55
20:4n-6 (ARA)	0.79 ± 0.03 ^b	0.74 ± 0.09 ^b	0.76 ± 0.30 ^b	0.81 ± 0.19 ^b	1.07 ± 0.09 ^a	1.06 ± 0.30 ^a	1.38 ± 0.55 ^a	1.06 ± 0.17 ^a	0.004	0.70	0.61
20:3n-3	0.13 ± 0.01	0.10 ± 0.02	0.11 ± 0.04	0.12 ± 0.03	0.14 ± 0.01	0.14 ± 0.04	0.11 ± 0.07	0.15 ± 0.02	0.20	0.58	0.92
20:5n-3 (EPA)	0.69 ± 0.07 ^b	0.62 ± 0.08 ^b	0.60 ± 0.20 ^b	0.61 ± 0.18 ^b	0.96 ± 0.11 ^a	0.89 ± 0.27 ^a	1.02 ± 0.44 ^a	0.85 ± 0.15 ^a	0.004	0.87	0.90
22:6n-3 (DHA)	0.10 ± 0.02	0.10 ± 0.01	0.08 ± 0.01	0.10 ± 0.03	0.11 ± 0.01	0.12 ± 0.05	0.07 ± 0.07	0.10 ± 0.03	0.69	0.24	0.90
EPA + DHA ⁴	0.79 ± 0.05 ^b	0.73 ± 0.08 ^b	0.68 ± 0.21 ^b	0.71 ± 0.21 ^b	1.07 ± 0.11 ^a	1.02 ± 0.32 ^a	1.08 ± 0.46 ^a	0.95 ± 0.18 ^a	0.006	0.91	0.94
Σ PUFA ⁵	2.89 ± 0.06 ^b	2.96 ± 0.28 ^b	2.73 ± 1.10 ^b	3.00 ± 0.79 ^b	5.17 ± 0.26 ^a	5.68 ± 1.81 ^a	5.90 ± 2.23 ^a	5.67 ± 1.23 ^a	< 0.001	0.97	0.94
Σ n-6	1.45 ± 0.06 ^b	1.64 ± 0.17 ^b	1.48 ± 0.65 ^b	1.69 ± 0.44 ^b	2.98 ± 0.10 ^a	3.54 ± 1.15 ^a	3.71 ± 1.39 ^a	3.57 ± 0.85 ^a	< 0.001	0.76	0.89
Σ n-3	1.19 ± 0.06 ^b	1.09 ± 0.15 ^b	1.05 ± 0.43 ^b	1.08 ± 0.31 ^b	2.01 ± 0.18 ^a	1.98 ± 0.61 ^a	2.03 ± 0.81 ^a	1.92 ± 0.35 ^a	< 0.001	0.98	0.99
n-6/n-3	1.23 ± 0.12 ^c	1.51 ± 0.15 ^b	1.41 ± 0.13 ^b	1.58 ± 0.08 ^b	1.49 ± 0.14 ^b	1.78 ± 0.05 ^a	1.85 ± 0.17 ^a	1.85 ± 0.12 ^a	< 0.001	0.001	0.54

Values are presented as mean ± standard deviation (n = 3). In each line, different superscript letters indicate significant differences (P < .05): differences between among diets are shown using a, b.

¹ HP/HL, high protein and high lipid; LP/LL, high protein and low lipid; LP/HL, low protein and high lipid; LP/LL, low protein and low lipid diets.

² SFA, Saturated fatty acids.

³ MUFA, Monounsaturated fatty acids.

⁴ EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid.

⁵ PUFA, Polyunsaturated fatty acids.

feed intake might have occurred, partially explaining the high FCR values. Moreover, high levels of dietary protein tended to increase the protein efficiency ratios in *P. lividus*, but without statistical significance. In *L. variegatus* and *S. purpuratus* juveniles, studies conducted by Heflin et al. (2012b) and Cuesta-Gomez and Sánchez-Saavedra (2017) reported that PER decreased with increasing protein levels, probably due to the protein catabolism as an energy source. This difference between studies can be attributed to species distinct protein requirements and/or considered age classes.

Globally, the sea urchin final body weight and growth performance were similar among experimental groups. Likewise, in a recent study, Lourenço et al. (2018a) observed that a protein content between 20 and 45% at a fixed lipid level (7%) did not affect *P. lividus* growth (SGR = 0.26–0.32% per day). But Zupo et al. (2019) reported that a high protein diet (47%) promoted higher size increments when compared to diets with 22–35% protein in this same species. There is no reference to previous studies concerning the effects of dietary lipids in *P. lividus*, but Gibbs et al. (2009); Hammer et al. (2010) have shown that in *L. variegatus* high dietary levels of lipids (> 9%) negatively influenced growth. In contrast, the present study showed that dietary lipid level of 11% did not affect sea urchins' growth nor gonad yield, but a protein sparing effect could not be observed either.

Proximate final whole body composition remained unaffected by the dietary treatments. Protein retention was highest in LP diets resulting in a higher protein gain in LP compared to HP, although without significant differences. Moreover, the lipid retention was highest in LL diets, but lipid gain was similar among diets. Lourenço et al. (2018a) also reported that DM, protein and energy gain did not vary in urchins fed distinct protein levels, but fat gain decreased with increasing dietary protein. These results suggest that once the species protein

requirements are met, dietary nutrients seem to be mainly used for metabolic process, such as gamete production in gonads, instead of being stored in the animal as protein accretion.

The quality of sea urchin gonads mainly depends on its size, colour, taste and firmness (Pearce et al., 2002; Prato et al., 2018a). It is also known that growth and nutritional composition of gonads depends on dietary regime (Cuesta-Gomez and Sánchez-Saavedra, 2016; Volpe et al., 2018), once nutritive phagocytes are the main nutrient storage in gonads (Carboni et al., 2015; Walker et al., 2015). In our experiment, the tested diets were equally effective in promoting the increase of gonad weight and GSI (3.7 g and 10% in initial samples, respectively). At the end of the trial, GSI ranged between 19% and 23%, irrespectively of the dietary treatment. These values are higher than those observed in previous studies with adult *P. lividus* reared in captivity (3–19%) (Prato et al., 2018c; Sartori and Gaion, 2016; Volpe et al., 2018). In the present study, *P. lividus* fed the diet LP/LL produced the highest GSI, and despite not being statistically significant, it might suggest that protein inclusion higher than 30% does not bring additional advantages in terms of gonad yield. Likewise, Fernandez and Pergent (1998) have demonstrated that increasing dietary protein from 29% to 47% did not affect GSI. Previous studies showed that dietary protein level was strongly correlated with gonad production (Heflin et al., 2012b; Heflin et al., 2016a; Zupo et al., 2019), but no relationship between dietary protein and GSI was found in the present study. Moreover, GSI in females was significantly higher than in males, irrespectively of diets. It is possible that *P. lividus* females might have a better efficiency in converting nutrients into gonad tissue compared to males.

Diets had a significant effect on sea urchin gonad composition. But, as already reported by Fernandez (1997), the dietary protein level did not influence the gonads' protein content. Conversely, Prato et al.

Table 5

Gonad yield, final gonad composition (% of WW), total carotenoids ($\mu\text{g/g}$ WW), color and texture of male and female *Paracentrotus lividus* fed low protein and low lipid diets (LP/LL) vs wild specimens harvested with similar maturation stage.

	Male		Female		Two-way ANOVA P-value		
	LP/LL ¹	Wild	LP/LL	Wild	Sex	RC ³	S x RC
Yield							
GSI ² (%)	20.39 $\pm$ 1.74	7.14 $\pm$ 2.15	22.97 $\pm$ 3.19	5.86 $\pm$ 0.75	0.61	< 0.001	0.16
Final gonad composition (% of WW)							
Dry matter	20.12 $\pm$ 0.15	20.89 $\pm$ 0.52	21.63 $\pm$ 0.54	21.13 $\pm$ 0.56	0.01	0.64	0.05
Crude protein	17.82 $\pm$ 0.40	15.63 $\pm$ 1.62	13.39 $\pm$ 0.27	12.86 $\pm$ 0.37	< 0.001	0.03	0.14
Total lipids	2.19 $\pm$ 0.14	2.64 $\pm$ 0.36	3.33 $\pm$ 0.39	4.00 $\pm$ 0.51	< 0.001	0.03	0.62
Gross energy (kJ/g)	4.67 $\pm$ 0.22	4.18 $\pm$ 0.10	4.64 $\pm$ 0.12	4.56 $\pm$ 0.23	0.12	0.02	0.08
Carotenoids ($\mu\text{g/g}$ WW)							
Total carotenoid	11.89 $\pm$ 3.88	89.05 $\pm$ 45.09	15.31 $\pm$ 0.98	86.64 $\pm$ 25.08	0.97	0.001	0.85
α -Carotene	0.15 $\pm$ 0.01 ^c	1.34 $\pm$ 0.05 ^a	0.18 $\pm$ 0.02 ^c	0.35 $\pm$ 0.02 ^b	< 0.001	< 0.001	< 0.001
β -Carotene	0.96 $\pm$ 0.02 ^c	19.35 $\pm$ 0.11 ^a	2.14 $\pm$ 0.01 ^c	10.05 $\pm$ 0.60 ^b	< 0.001	< 0.001	< 0.001
Echinenone	22.04 $\pm$ 0.13 ^c	93.77 $\pm$ 0.21 ^a	7.96 $\pm$ 0.04 ^d	26.52 $\pm$ 0.01 ^b	< 0.001	< 0.001	< 0.001
Lutein	0.17 $\pm$ 0.00 ^d	0.68 $\pm$ 0.01 ^c	1.75 $\pm$ 0.04 ^b	4.64 $\pm$ 0.04 ^a	< 0.001	< 0.001	< 0.001
β -Cryptoxanthin	5.06 $\pm$ 0.00 ^b	20.33 $\pm$ 0.01 ^a	4.59 $\pm$ 0.00 ^c	20.33 $\pm$ 0.01 ^a	< 0.001	< 0.001	< 0.001
Colour and texture							
L*	58.99 $\pm$ 6.61	47.82 $\pm$ 4.70	46.27 $\pm$ 3.84	38.15 $\pm$ 3.99	0.004	0.01	0.61
a*	7.13 $\pm$ 3.18	14.65 $\pm$ 2.9	18.15 $\pm$ 0.15	25.41 $\pm$ 3.16	< 0.001	0.001	0.94
b*	25.14 $\pm$ 4.02	25.09 $\pm$ 0.46	34.26 $\pm$ 3.29	34.32 $\pm$ 5.54	0.03	0.99	0.98
C*	26.21 $\pm$ 4.66	29.24 $\pm$ 1.25	38.90 $\pm$ 2.86	42.74 $\pm$ 6.23	0.001	0.19	0.87
h	74.81 $\pm$ 5.04	59.72 $\pm$ 5.44	61.88 $\pm$ 2.82	53.26 $\pm$ 1.62	0.003	0.001	0.20
Resilience (%)	74.82 $\pm$ 6.56	80.27 $\pm$ 4.29	75.94 $\pm$ 2.53	80.39 $\pm$ 0.62	0.80	0.07	0.84
Firmness (g)	13.80 $\pm$ 0.85	22.18 $\pm$ 6.80	11.35 $\pm$ 0.86	15.35 $\pm$ 1.49	0.04	0.02	0.31

Values are presented as mean $\pm$ standard deviation (n = 3). Mean values within a row unlike superscript letters showed a significant interaction between the two factors (Sex versus RC) (P < .05).

¹ LP/LL, low protein and low lipid diets; Wild, wild animals harvested with similar maturation stage.

² Gonad somatic index.

³ Rearing conditions.

(2018a) reported that increasing dietary protein from 18 to 27% resulted in increased protein and lipid levels in *P. lividus* gonads. Lipids are also an important storage nutrient for the development and maturity of sea urchin gonads (González-Durán et al., 2008) and in the present study were significantly increased in sea urchins fed the HP/HL diet compared to all other diets, in both male and female. Moreover, ovaries had a higher lipid and DM, but lower protein content than testis, suggesting that males and females differ in their ability to incorporate dietary nutrients, which might be related with differences in metabolic processes. There are no previous studies considering the effect of dietary protein and sex on gonad composition, as most nutritional studies did not discriminate samples between sex.

The FA profile of sea urchin gonads represents a good indicator of food quality and urchin well-being as it can affect survival, growth and development of determined tissues, as well as reproductive performance (Prato et al., 2018c). In the present study, gonad FA profile was similar among treatments, but the ratio n-6/n-3 was significantly affected by diet and sex. The concentration of ALA and LA was higher in diets than in gonads, suggesting its use as substrate for energy or for the synthesis of LC-PUFA. Indeed, Kabeya et al. (2017) have recently shown that *P. lividus* can perform the desaturation reactions required for the biosynthesis of n-3 and n-6 LC-PUFA. PUFA comprised the most predominant FA class, followed by SFA and MUFA. The 16:0 was the most predominant SFA, 18:1n-9 and 20:1n-9 comprised the majority of MUFA and LA, ARA and EPA were the most abundant PUFA in *P. lividus* gonads, confirming previous observations (Angioni and Addis, 2014; Barbera et al., 2011; Liyana-Pathirana et al., 2002; Volpe et al., 2018). Overall, SFA, MUFA and PUFA were higher in ovaries than in testis of cultured urchins. Higher levels of SFA, particularly 16:0 and 14:0, and some MUFA in ovaries have already been reported in literature (Hughes et al., 2005; Martínez-Pita et al., 2010) due to a possible higher accumulation of these FA in the neutral lipid fraction of eggs as an energy

reserve for embryo development (Kozhina et al., 1978; Mita et al., 1994). However, the higher accumulation of PUFA, particularly of ARA and EPA, in urchin ovaries contradicts previous reports that observed highest values of ARA (Hughes et al., 2005; Martínez-Pita et al., 2010) and EPA (Hughes et al., 2005) in testis. The discrepancies found might be explained by different efficiencies of incorporation of these FA from the diet (Mai et al., 1996) and synthesis of ARA and EPA from LA and ALA, respectively, in either female or male urchins. Also, distinct concentrations of dietary PUFA (Prato et al., 2018a) may contribute to the differences across studies.

In literature, most nutritional studies evaluating colour in sea urchin gonads refer to visual or instrumental determinations without sex differentiation (Pearce et al., 2004; Robinson et al., 2002; Shpigel et al., 2006; Symonds et al., 2007). In the present trial, we were able to instrumentally assess the colour and quantify the carotenoid profile, in both female and male, fed the experimental diets, but likewise Phillips et al. (2009), all gonads had similar L*, a* and b* values, irrespectively of dietary treatments or sex. Chroma (C*) and hue angle (h*) followed the same trend. Conversely, total carotenoid content was significantly higher in females than in males, although the diets had the same formulation and pigment levels. This suggests sex-specific pathways of carotenoids metabolism (Robinson et al., 2002). Moreover, and in agreement with a number of other studies, firmness and resilience of gonads were not affected by dietary treatments or sex (Pearce et al., 2002; Phillips et al., 2009; Senaratna et al., 2005).

The present results clearly show that all diets were effective in promoting gonad growth, suggesting that a 30% dietary protein with a low lipid level is not only able to sustain sea urchin growth, but even to promote the highest gonad yield in both sexes. The gonads quality of sea urchins fed this diet (LP/LL) were further compared to marketable wild specimens at similar maturation stage. It was clearly evidenced that the GSI of sea urchins fed the LP/LL diet was significantly higher

Table 6

Total fatty acids (mg/g WW) gonads composition of male and female *Paracentrotus lividus* fed low protein and low lipid diets (LP/LL) vs wild specimens harvested with similar maturation stage.

	Male		Female		Two-way ANOVA P-value		
	LP/LL	Wild	LP/LL	Wild	Sex	RC ⁵	S x RC
Fatty acids (mg/g WW)							
14:0	0.25 ± 0.02 ^b	0.49 ± 0.13 ^b	0.78 ± 0.12 ^b	2.39 ± 0.56 ^a	< 0.001	0.001	0.004
16:0	0.76 ± 0.04	1.24 ± 0.24	1.77 ± 0.17	3.53 ± 1.03	0.001	0.01	0.07
18:0	0.26 ± 0.03	0.32 ± 0.05	0.30 ± 0.02	0.39 ± 0.10	0.11	0.04	0.69
Σ SFA ¹	1.39 ± 0.02 ^b	2.25 ± 0.45 ^b	3.05 ± 0.32 ^b	6.80 ± 1.80 ^a	< 0.001	0.003	0.03
18:1n-9	0.14 ± 0.01 ^a	1.27 ± 0.05 ^a	0.61 ± 0.04 ^b	0.41 ± 0.08 ^c	< 0.001	0.01	0.01
18:1n-7	0.13 ± 0.01	0.25 ± 0.03	0.28 ± 0.02	0.68 ± 0.23	0.003	0.01	0.07
20:1n-9	0.38 ± 0.02 ^b	0.67 ± 0.05 ^b	0.49 ± 0.06 ^b	1.24 ± 0.28 ^a	0.004	< 0.001	0.03
22:1n-9	0.13 ± 0.00 ^b	0.23 ± 0.04 ^b	0.23 ± 0.03 ^b	0.72 ± 0.19 ^a	0.001	0.001	0.01
Σ MUFA ²	0.98 ± 0.05 ^b	2.19 ± 0.22 ^b	2.06 ± 0.17 ^b	5.31 ± 1.26 ^a	< 0.001	< 0.001	0.03
18:2n-6 (LA)	0.45 ± 0.07 ^b	0.05 ± 0.02 ^c	1.52 ± 0.12 ^a	0.20 ± 0.06 ^c	< 0.001	< 0.001	< 0.001
18:3n-3 (ALA)	0.13 ± 0.02 ^c	0.08 ± 0.02 ^c	0.54 ± 0.03 ^a	0.29 ± 0.08 ^b	< 0.001	0.001	0.01
18:4n-3	0.05 ± 0.01 ^b	0.14 ± 0.04 ^b	0.17 ± 0.06 ^b	0.59 ± 0.12 ^a	< 0.001	< 0.001	0.004
20:2n-6	0.11 ± 0.01 ^b	0.25 ± 0.04 ^a	0.23 ± 0.02 ^{ab}	0.24 ± 0.09 ^a	0.09	0.03	0.04
20:3n-6	0.08 ± 0.01	0.07 ± 0.01	0.13 ± 0.01	0.08 ± 0.02	0.003	0.004	0.12
20:4n-6 (ARA)	0.79 ± 0.03	1.47 ± 0.25	1.07 ± 0.09	1.81 ± 0.38	0.05	0.001	0.81
20:3n-3	0.13 ± 0.01	0.23 ± 0.05	0.14 ± 0.01	0.31 ± 0.07	0.10	0.001	0.23
20:5n-3 (EPA)	0.69 ± 0.07	1.48 ± 0.25	0.96 ± 0.11	2.25 ± 0.60	0.03	0.001	0.22
22:6n-3 (DHA)	0.10 ± 0.02	0.03 ± 0.01	0.11 ± 0.01	0.05 ± 0.02	0.15	< 0.001	0.45
EPA + DHA ³	0.79 ± 0.05	1.50 ± 0.25	1.07 ± 0.11	2.29 ± 0.61	0.03	0.001	0.22
Σ PUFA ⁴	2.89 ± 0.06	4.16 ± 0.70	5.17 ± 0.26	6.29 ± 1.54	0.002	0.04	0.89
Σ n-6	1.45 ± 0.06 ^c	1.90 ± 0.32 ^{bc}	2.98 ± 0.10 ^a	2.42 ± 0.57 ^{ab}	0.001	0.78	0.03
Σ n-3	1.19 ± 0.06	2.24 ± 0.39	2.01 ± 0.18	3.81 ± 0.95	0.004	0.002	0.25
n-6/n-3	1.23 ± 0.12 ^b	0.85 ± 0.07 ^c	1.49 ± 0.14 ^a	0.64 ± 0.01 ^c	0.65	< 0.001	0.002

Values are presented as mean ± standard deviation (n = 3). Mean values within a row unlike superscript letters showed a significant interaction between the two factors (Sex versus RC) (P < .05).

¹ SFA, Saturated fatty acids.

² MUFA, Monounsaturated fatty acids.

³ EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid.

⁴ PUFA, Polyunsaturated fatty acids.

⁵ Rearing conditions.

(20–23%) than those observed in the wild (6–7%). This support previous studies showing that sea urchins produce higher gonad growth with formulated diets compared to natural feed (Lawrence, 2013; Woods et al., 2008). Zupo et al. (2019) have already compared the effect of fresh and formulated diets on the sea urchin gonad growth. This author used *Ulva rigida* and *Posidonia oceanica* that characterize environments often populated with *P. lividus*. *Ulva rigida* contains up to 25% DM protein and up to 2.5% DM fat (Valente et al., 2006), while *Posidonia oceanica* has 30–40% DM protein and 1–3% DM fat (Zupo et al., 2019).

In relation to gonads nutritional value, protein level was highest in farmed sea urchins, but lipids were highest in gonads of wild animals. The 16:0, EPA and ARA were the most abundant FA, being higher in wild individuals. These data are consistent with the results from Volpe et al. (2018). It has been demonstrated that differences in the lipid composition of sea urchin gonads are strongly related to water temperature, food, season, growing stage and sampling area (Rocha et al., 2018; Rocha et al., 2019; Siliani et al., 2016; Zárate et al., 2016). Considering that the growing stage and sampling area were the same in the captured and reared urchins, external factors such as water temperature and food source might have been the principal causes for the differences in the FA concentrations between groups.

Previous studies have demonstrated that the dietary inclusion of carotenoid pigments is crucial to enhance the bright yellow/orange coloration of gonads sought by the market (Robinson et al., 2002; Shpigel et al., 2005). In this study, diets were not supplemented with carotenoids, resulting in gonads with lower redness compared to wild sea urchins. Echinenone, is the major carotenoid in both ovaries and testis (Griffiths and Perrott, 1976; Sartori and Gaion, 2016; Tsushima, 2007) being metabolized from dietary β-carotene (Symonds et al.,

2007), but both pigments showed the highest values in wild specimens. However, males (reared and wild) had lower redness and yellowness values and higher values of echinenone than females, which is not consistent with previous works that have shown a possible positive relationship between echinenone levels and the desirable yellow-orange coloration in sea urchin gonads (Griffiths and Perrott, 1976; Shpigel et al., 2006). Nonetheless, higher concentrations of echinenone may also originate more intense and darker coloration of gonads (Shpigel et al., 2005), which could suggest that males' gonads had darker coloration. Higher values of firmness were observed in wild individuals with access to natural food sources compared to those fed formulated diets, confirming previous studies demonstrating that kelp-fed and wild individuals had slightly better gonad firmness than those fed prepared diets (Pearce et al., 2004; Phillips et al., 2010; Siikavuopio et al., 2007). Pearce et al. (2004) have demonstrated that there is a relationship between the rate of gonad yield and firmness, suggesting that the faster the gonad yield the softer the gonads will become. The present results support such an observation as sea urchins fed the experimental diet had larger but less firm gonads.

5. Conclusions

This study clearly shows that it is possible to successfully enhance sea urchin gonad yield in relation to wild animals in a RAS at 18 °C. All diets were effective in promoting gonad growth, but the diet with the lowest protein (30% DM) and lowest lipid (6% DM) level resulted in the highest gonad yield in both sexes. Dietary treatments and sex did not affect gonad texture (firmness and resilience) or colour parameters (brightness, redness, yellowness, chroma or hue angle), but affected gonad composition. Total carotenoid content in gonads was not affected

by diets and resulted in lower values in reared compared to wild specimens. The wild urchins had higher levels of echinenone, β -cryptoxanthin and β -carotene and showed higher redness and firmness values compared to the urchins fed LP/LL diet, irrespectively of the sex.

Colour is the first quality attribute used by consumers, impacting the visual assessment and freshness perception of sea urchins' gonads, which are key purchase determinants. So, further nutritional strategies to improve the colour and texture of gonads are still required.

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Chapter IX.

Sensory profiling, liking and gonad composition of sea urchin gonads fed synthetic or natural sources of β -carotene enriched diets

by

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Abstract

Sea urchin gonads are considered a prized seafood product. Their market price depends on quality and appearance, mainly evaluated through colour. The desirable yellow-orange colour is determined by carotenoids deposition in the gonads, mainly the accumulation of echinenone, which depends on availability, uptake and bioconversion of β -carotene from dietary sources. Two β -carotene sources, a synthetic (SYN) and a natural (paprika, PAP) source, were tested at low (L) and high (H) incorporation target levels (100 and 250 mg β -carotene kg⁻¹ feed) in diets for *Paracentrotus lividus*. A non-supplemented diet was used as a control (CTRL) diet. All diets contained 30% protein and 6% fat and were extruded. Groups of 20 wild-harvested *P. lividus* (44 g; 4.7 cm diameter) were randomly distributed by plastic mesh cages within 50L tanks in a saltwater recirculation system (35 ‰, 18 °C) with a 12 h:12 h (light/dark) regime. After eight weeks of feeding, all diets effectively increased gonad weight and somatic index in both sexes, without significant differences between treatments. Total carotenoid content and profile, lightness (L^*) and texture of gonads did not vary significantly among dietary treatments. However, gonad redness (a^*) and yellowness (b^*) values were significantly higher in urchins fed the L_SYN diet than in those fed either with the CTRL or L_PAP diets; female displayed higher values than male. L_SYN also showed higher values of acceptance when compared to the other diets, whilst demonstrating significant differences only with L_PAP in females and H_SYN in males. Female displayed higher acceptance rates than male, probably due to the fact that females were more orange, appealing and pleasant compared with the yellow, grainy and not appealing males.

Male fed L_SYN diet exhibited similar colour and carotenoid content compared to wild animals in the same stage of gonad maturation (stage III), but female differed significantly; however, neither of the sex demonstrated acceptance differences in relation to their wild counterparts. Diets with the lower β -carotene inclusion induced changes in gonad colour but not in carotenoid pigments. This suggests that gonad colour is not exclusively modulated by dietary carotenoid supplementation and that it may be affected by other sex-dependent nutritional or metabolic factors. Further studies are required to fully understand the mechanisms involved in the modulation of gonad colour through dietary supplementation to enhance the production of desirable gonads in both sexes.

Keywords: Check-All-That-Apply; echinenone; echinoculture; gonad quality; sea urchin diet

1. Introduction

Sea urchin gonads are a prized delicacy worldwide, making their commercialisation highly profitable. Consequently, several sea urchin species have been overexploited, leading to the collapse of wild stocks with a significant reduction and fragmentation of sea urchin populations (Sun and Chiang, 2015). The increasing demand for sea urchin products boosted their production in aquaculture to fulfil the market demand. Indeed, echinoculture has gained extreme interest for commercial and scientific purposes (McBride, 2005; Sartori and Gaion, 2016). However, to maximise the economic viability of echinoculture production, sea urchins should be preferentially fed on formulated diets to meet the species' requirements and promote somatic growth and gonadal quality (Suckling et al., 2011). The quality of gonads is mainly determined by their size, taste, colour and consistency, mainly influenced by the dietary nutrient composition (Cuesta-Gomez and Sánchez-Saavedra, 2017; Taylor et al., 2017). However, according to the literature, most formulated feeds for sea urchin species can promote gonads' growth but cannot assure essential quality traits, such as colour and flavour, compared to their wild counterparts (Lourenço et al., 2019).

Paracentrotus lividus is the most abundant echinoid species in the Atlantic coast and the Mediterranean Sea and is mainly consumed and commercialised in Europe (Bertocci et al., 2014; Bertocci et al., 2018; Boudouresque and Verlaque, 2013; Yeruham et al., 2015). Artificial diets were shown to be effective in promoting *P. lividus* gonad size, albeit without clear identification of species' protein requirement for maximal growth and gonad yield (Cirino et al., 2017; Cook and Kelly, 2007; Prato et al., 2018a; Spirlet et al., 2000; Zupo et al., 2019). Recent studies demonstrated that an extruded diet with 30% crude protein and a low lipid content (6% DM) induced the highest gonad yield in *P. lividus*, but gonad colour, texture and carotenoid content still differed from wild urchins (Baião et al., 2019; Lourenço et al., 2020). Likewise, Prato et al. (2018b) highlighted significant differences in gonad colour and taste compared to wild populations. The main pigment accumulated in *P. lividus* gonads is echinenone (Rocha et al., 2019 a,b), obtained from the metabolic conversion of dietary β -carotene via β -isocryptoxanthin (Kelly and Symonds, 2013; Symonds et al., 2007). The concentration of echinenone in gonads is dependent on the dietary β -carotene concentration (Shpigel et al., 2006) and is strongly correlated with gonads colour intensity (Shpigel et al., 2005). The high dependency of gonads quality on its colour highlights the importance of formulating diets to improve sea urchins market acceptance.

Satisfactory improvement of gonad colour was obtained in several sea urchin species through dietary supplementation of pigments, using natural or synthetic sources of carotenoids, in particular, β -carotene (McBride et al., 2004; Prato et al., 2018b; Robinson et al., 2002; Shpigel et al., 2006). Robinson et al. (2002) compared several natural sources

of β -carotene (kelp, paprika, dulse and carrot) at 1 and 10% inclusion levels, reporting that either paprika or carrots at the highest dietary incorporation minimised the loss of the red and yellow colours of *Strongylocentrotus droebachiensis* gonads during a 6-weeks growth trial. However, the concentration of β -carotene in those natural products was still not enough to produce gonads of acceptable colour. Using a spectrophotometric system with CIE $L^*a^*b^*$ coordinates, Robinson et al. (2002) have further evidenced the effectiveness of concentrated commercial forms of β -carotene (100-250 mg β -carotene kg^{-1} dry weight of feed) in providing a commercially desirable colouration of *S. droebachiensis* gonads. In *P. lividus*, Shpigel et al. (2006) demonstrated that natural β -carotene isomers (66 mg kg^{-1} of diet DM) induced better gonads colouration than synthetic forms (25 mg kg^{-1} DM), but dietary pigment concentrations were not comparable.

Sensory properties are crucial for seafood acceptance and choice of consumers (Calanche et al., 2019). Appearance comprises different visual properties, including colour (Spence, 2015b). Indeed, visual impressions are usually the first sensory stimuli perceived by consumers, raising expectations about the product (Reisfelt, Gabrielsen, Aaslyng, Bjerre, & Møller, 2009). The perceived properties may induce positive sensations leading to product acceptance or negative sensations leading to its rejection (Spence, 2015b). Hence, visual features are essential regarding acceptance or rejection of a specific product (Cardello, 1996). In order to modulate sea urchin quality to meet consumer's expectations, it is of extreme relevance to use innovative methodologies to understand the factors that influence its sensory properties and overall quality. CATA (Check-All-That-Apply) is a fast and user-friendly sensory method to determine the best organoleptic characteristics of a food product that has gained increasing attention in recent years (Meyners et al., 2013). This method permits a visual and odour evaluation by a panel constituted by naïve consumers, allowing extrapolating consumers' perception and acceptance features regarding sea urchin gonad (Baião et al., 2021).

In this study, we have evaluated the potential of two β -carotene sources, a synthetic (SYN) and a natural (paprika, PAP) source, and two incorporation levels, low (L) and high (H) (100 and 250 mg β -carotene kg^{-1} feed) in extruded diets for *P. lividus* to produce gonads with acceptable colour. Biometric values (weight, test diameter and gonad yield), gonad quality (maturation stage, nutritional value, instrumental texture and colour) and consumer perception using a CATA methodology were analysed in both female and male sea urchins after feeding the experimental diets to wild-caught adult sea urchins for eight weeks.

2. Material and methods

The study was directed by accredited scientists in laboratory animal science by the Portuguese Veterinary Authority (1005/92, DGAV-Portugal) and conducted according to the

Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals for scientific purposes. Wild sea urchins were collected after authorisation from the national authorities: Portuguese maritime authority (Captaincy of the Port of Viana do Castelo) and the Institute for Nature Conservation and Forests, ICNF, following the Nagoya protocol.

2.1. Experimental diets

Four extruded diets containing 30% protein and 6% fat (DM basis) were supplemented with either a synthetic (SYN) or a natural source of β -carotene (paprika, PAP), at low (L, 100 mg β -carotene kg⁻¹ feed) and high (H, 250 mg β -carotene kg⁻¹ feed) target incorporation levels; the dietary treatments were identified as L_PAP, H_PAP, L_SYN and H_SYN. A non-supplemented diet was used as a control (CTRL) diet. The formulation and chemical composition of the experimental diets are shown in Table 1. SPAROS, Lda (Portugal) manufactured diets: all ingredients were grounded in a micropulverizer hammer mill (Hosokawa Micron, SH1, Germany), mixed in a paddle mixer (Mainca RM90, Spain) according to each specific formulation, then the mixture was humidified with water (250 g kg⁻¹) and agglomerated by low shear extrusion (Italpast P55, Italy). Upon extrusion, the resulting feed was dried in a convection oven (OP 750-UF, LTE Scientifics, UK) for three h at 70 °C, and dried feed pellets were subsequently sieved to retrieve the desired particle size (around 3 cm).

2.2. Experimental trial

The experiment was conducted in the CIIMAR aquaculture and animal experimentation facility, Matosinhos, Portugal (Figure 1). Adult *P. lividus* were randomly collected, by hand, in a rocky intertidal shore close to Praia do Homem do Leme, Porto, Portugal [41° 9' 49.378"N 8° 41' 14.449"W]. Harvesting of wild animals was performed in February before the onset of the spawning season, which occurs between spring and summer seasons (April to July) in the North coast of Portugal (Fernandez-Boo et al., 2018; Rocha et al., 2019). The harvested sea urchins were kept in quarantine for two weeks to adapt to the experimental conditions and fed the CTRL diet. After acclimatisation, animals were fasted for six days to empty the gut content thoroughly, and individual records of total body weight (g) and test diameter (cm) were registered before distribution among tanks. Gonad wet weight and the gonad somatic index (GSI = gonad weight (g)/whole body weight (g)*100) of eight females and nine males from the initial wild population were determined at the beginning of the experiment.

Twelve homogeneous groups of 20 individuals (initial body weight of 44.0 ± 0.8 g; diameter of 4.7 ± 0.1 cm) were randomly distributed by 55 L tanks and placed within plastic

mesh cages (25x30x15 cm; 60 urchins per m²). The cages were suspended and submerged inside the tanks, at a depth of 5 cm from the top. The experiment was conducted in a saltwater recirculation system (RAS) at the following conditions: 35-37 ‰, 18 °C, 8 mg L⁻¹ of dissolved oxygen, the water flow of 4 L min⁻¹ and photoperiod of 12 h:12 h (light/dark). The RAS was provided of both mechanical and biological filtration and UV sterilisation, being the nitrogenous compounds and pH of the water monitored weekly and kept at recommended levels for marine species. Due to a limited number of tanks, both the CRTL and L_SYN diet were tested in triplicate, while the remaining treatments were tested in duplicate. The trial was conducted for eight weeks, and sea urchins were hand-fed every two days until apparent satiation as previously described (Baião et al., 2019).

At the end of the trial, all sea urchins were individually weighted (whole body, g) and measured (test diameter, cm). Twenty-eight and thirty sea urchins were sampled for histological analysis and gonadal composition in treatments tested in duplicate and triplicate, respectively, and the remaining individuals were used for consumers' sensory evaluation. After dissection, gonads from all sampled sea urchins were weighed to determine the GSI. One gonad was collected for dry matter determination; one for colour and texture instrumental assessment; a small gonad portion was collected for histological evaluation of the gametogenic stages; and the remaining three gonads of each animal for subsequent chemical analyses (Figure 1.A). Gonads were snap-frozen and kept at -80 °C, except those for histologic evaluation that were immediately fixed in 4% buffered formalin for 24 h. Due to sample scarcity, prior chemical analyses, gonad samples were pooled per tank, according to sex, after confirmation by histological analysis. At the end of the experiment, eighteen wild animals were harvested in the same area of those used in the experiment, compared to sea urchins fed the experimental diets. Gonads from five males and five females were pooled before chemical analyses. For sensory evaluation, twelve to thirty sea urchins were collected from each dietary treatment to evaluate the sensory quality of gonads from both sexes. Sea urchin test was opened to identify male (pale-yellow colour) and female (bright-orange colour); the sex was later confirmed by gonad histological examination. The gonads were carefully removed using a spoon, washed in saline water (35 ‰), placed in a petri dish and maintained at 6° C until each gonad could be distributed to each panellist. One of the gonads of each specimen was used for instrumental texture and colour analysis.

Table 1 - Formulation and proximate composition of the experimental diets after extrusion.

Experimental diets	CTRL	L_SYN	H_SYN	L_PAP	H_PAP
β -carotene targets (mg kg ⁻¹ DM)	0	100	250	100	250
Ingredients (%)					
Squid meal ^a	5.0	5.0	5.0	5.0	5.0
Pea protein concentrate ^b	3.5	3.5	3.5	3.5	3.5
Plant based glutens ^c	7.0	7.0	7.0	7.0	7.0
Plant based meals ^d	38.2	37.2	37.9	38.0	37.6
Laminaria ^e	5.0	5.0	5.0	5.0	5.0
Macroalgae mix ^f	15.0	15.0	15.0	15.0	15.0
Krill oil ^g	1.0	1.0	1.0	1.0	1.0
Linseed oil ^h	1.0	1.0	1.0	1.0	1.0
Lecithin ⁱ	3.3	3.3	3.3	3.3	3.3
Vitamin and mineral premix ^j	2.0	2.0	2.0	2.0	2.0
Organic-based binder ^k	7.5	7.5	7.5	7.5	7.5
Mineral-based binders ^l	11.0	11.0	11.0	11.0	11.0
Antioxidant ^m	0.5	0.5	0.5	0.5	0.5
Synthetic β -carotene ⁿ	0.0	0.1	0.3	0.0	0.0
Paprika oleoresin ^o	0.0	0.0	0.0	0.2	0.6
Chemical composition (% DM)					
Dry matter	92.5	92.3	92.8	93.6	94.0
Crude protein (CP)	28.5	28.6	28.7	28.7	28.7
Crude fat	6.2	6.6	6.3	6.6	6.7
Gross energy (GE, kJ g ⁻¹ DM)	16.4	16.5	16.3	16.4	16.6
Ash	17.6	17.4	17.5	17.5	17.5
Carbohydrate ^p	47.7	47.4	47.5	47.2	47.1
CP:GE (mg kJ ⁻¹)	17.4	17.3	17.6	17.5	17.3
Carotenoids (mg kg⁻¹ DM)					
Lutein	0.0	2.3	2.3	0.9	0.9
Zeaxanthin	0.0	0.7	0.7	0.9	0.9
α -carotene	0.0	2.4	2.4	3.4	3.4
β -carotene	0.0	30.5	66.4	11.5	14.3
Total carotenenes ^q	0.0	32.9	68.8	14.9	17.7
Total carotenoids ^r	0.0	35.9	71.7	16.7	19.6
β -carotene degradation (%) ^s	n.a.	69.5	73.4	88.5	94.3

The abbreviations for the experimental diets stand for: CTRL - Control diet (not supplemented); LSYN – supplemented with 100 mg kg⁻¹ synthetic β -carotene; HSYN - supplemented with 250 mg kg⁻¹ synthetic β -carotene; LPAP – supplemented with 100 mg kg⁻¹ natural β -carotene (paprika); HPAP - supplemented with 250 mg kg⁻¹ natural β -carotene (paprika).

^a Super prime without guts: 82% CP, 3.5% CF, Sopropêche, France.

^b Lysamine GP: 78% CP, 8% CF, ROQUETTE, France.

^c Wheat gluten, 84% CP; 1.6 % CF; corn gluten 61% CP, 6% CF, COPAM, Portugal.

^d Micronized wheat 14.4% CP; 2.4% CF; corn grain 9.4% CP; 73.4% starch; 18.7 MJ kg⁻¹ GE, Ribeiros, Portugal.

^e Algovert®, *Laminaria digitata*: 4.7-12.1% CP, 0.7-1.6% CF, Setalg, France.

^f Ocean feed shrimp - Seaweed meal™, Ocean Harvest Technology, Ireland.

^g SuperbaKrill™ oil, Solchem, Spain.

^h Henry Lamotte Oils GmbH, Germany.

ⁱ Lecico P700IPM, LECICO GmbH, Germany

^j Premix PV01 for marine fish, PREMIX Lda, Portugal. Vitamins (IU or mg kg⁻¹ diet): DL-alpha tocopherol acetate, 100 mg; sodium menadione bisulphate, 25 mg; retinyl acetate, 20,000 IU; DL cholecalciferol, 2000 IU; thiamin, 30 mg; riboflavin, 30 mg; pyridoxine, 20 mg; cyanocobalamin, 0.1 mg; nicotinic acid, 200 mg; folic acid, 15 mg; ascorbic acid, 1000 mg; inositol, 500 mg; biotin, 3 mg; calcium panthotenate, 100 mg; choline chloride, 1000 mg, betaine, 500 mg. Minerals (g or mg kg⁻¹ diet): cobalt carbonate, 0.65 mg; copper sulphate, 9 mg; ferric sulphate, 6 mg; potassium iodide, 0.5 mg; manganese oxide, 9.6 mg; sodium selenite, 0.01 mg; zinc sulphate, 7.5 mg; sodium chloride, 400 mg; calcium carbonate, 1.86 g; excipiente wheat middlings.

^k Gelatin and plant gums, LAPi Gelatine SPA, Italy.

^l Zeolite, Kielseguhr, LIGRANA GmbH, Germany; monocalcium phosphate, Fosfitalia, Italy; calcium carbonate, PREMIX Lda, Portugal.

^m Antioxidant powder, Paramega PX, Kemine Europe NV, Belgium.

ⁿ β -carotene content: 87630 mg kg⁻¹, PREMIX Lda, Portugal.

^o Paprika extract (*Capsicum annuum* L.) and sunflower oil, β -carotene content: 43460 mg kg⁻¹, Frutarom, Spain.

^p Determined by difference: 100 - (crude protein %DM-crude fat %DM-ash %DM).

^q Sum of α -carotene and β -carotene.

^r Sum of Lutein, Zeaxanthin, α -carotene and β -carotene.

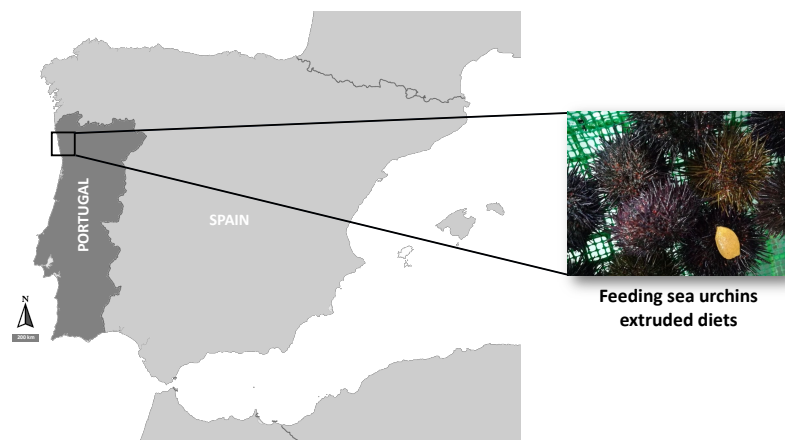
^s Determined as 100*(β -carotene target in the dietary mixture - Total carotenenes determined after extrusion)/ β -carotene target in the dietary mixture.

2.3. Histological analysis

Fixed gonad was dehydrated, embedded in paraffin, and transversely sectioned at 7 µm. Sections were stained with hematoxylin-eosin and observed under a microscope (CX22LED, Olympus). The gametogenic stages of both ovary and testes were identified according to Byrne (1990): recovery (stage I), with primary gametes and nutritive phagocytes; growing (stage II), with clusters of primary gametes and packed nutritive phagocytes; premature (stage III), with gametes at all stages of development and reduced amount of nutritive phagocytes; mature (stage IV), with mature gametes and few nutritive phagocytes; partly spawned (stage V), with loosely packed gametes and depletion of nutritive phagocytes, and spent (stage VI), with gonads empty of gametes.

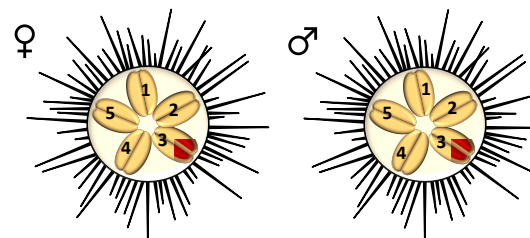
2.4. Chemical composition of diets and gonads

For dry matter determination, one fresh gonad was collected from each sea urchin and dried at 105 °C for 24 h (Figure 1.A). Two pooled samples (one with male and another with female) from each tank were freeze-dried, ground, and homogenised before proceeding with the analysis. The experimental diets were also ground and homogenised before analysis. All chemical analyses followed the AOAC methods and were performed in duplicate. Samples of pooled gonads and diets were analysed for: ash content by incineration in a muffle furnace at 500 °C for 6 h (Nabertherm L9/11/B170, Bremen, Germany); crude protein (N x 6.25) by a flash combustion technique using a Leco nitrogen analyser (Model FP-528, Leco Corporation, St. Joseph, USA) and gross energy using an adiabatic bomb calorimeter (Werke C2000, IKA, Staufen, Germany). Crude fat from diets was determined by petroleum ether extraction (40-60 °C) using a Soxtec™ 2055 Fat Extraction System (Foss, Hilleroed, Denmark). Total lipids were extracted and quantified gravimetrically by the method described by Folch et al. (1957), using dichloromethane-methanol (2:1).



A) Gonad quality

(maturation, chemical composition, colour and texture)



Procedure repeated for each specimen:

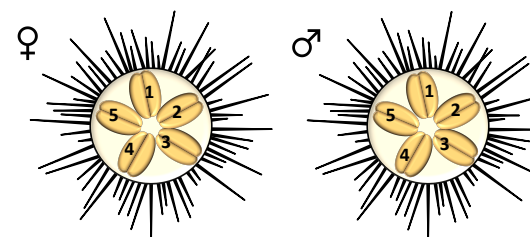
- 1: Dry matter
- 2: Instrumental colour and texture
- 3: Small portion to histology

Pools according to sex per tank:

- 3, 4, 5: Chemical analysis (total protein, total lipids, energy, ash, carotenoids)

B) Sensory evaluation

(overall liking with 9-point scale, Check-All-That-Apply)



Procedure repeated for each specimen:

- 1: Assessor 1
- 2: Assessor 2
- 3: Assessor 3
- 4: Assessor 4
- 5: Instrumental colour and texture

Figure 1 - Scheme of sampling procedures of sea urchins after 8 weeks trial.

2.5. Carotenoid analysis

Total carotenoid content was quantified in both gonad and diets. The extractions and carotenoid determinations were performed according to Rocha et al. (2019). The qualitative and quantitative profile of carotenoids in gonad and diet samples was performed by high-performance liquid chromatography analysis (Waters Alliance Series 600, Mildford, USA). Carotenoid extracts suspended in 1 ml n-hexane and 50 μ L were injected in a reverse phase Acclaim™ C18 LC column (250 x 4.6 mm i.d., 5 μ m particle size, Thermo Scientific) with a mixture of acetonitrile, methanol, dichloromethane, hexane and ammonium acetate (55:22:11.5:11.5:0.02, v:v:v:v:w) under isocratic conditions (1.0 ml min⁻¹, 25 min at 25 °C). Chromatograms were recorded through a diode array detector (Waters 996 PDA, USA), and absorbance was measured at 454 nm. Retention times and spectra of compounds were analysed by comparison with pure standards and quantification performed by the calibration curves of β -carotene, echinenone and zeaxanthin (Sigma, Portugal). Data were analysed using the Waters Empower™ 2 software and expressed as μ g g⁻¹ of gonad biomass.

2.6. Colour assessment

Gonad colour was assessed instrumentally using a CR-400 colourimeter (Konica Minolta) with an aperture of 8 mm, at standard illuminant D65 using the CIE 1976 (L^* , brightness; a^* , redness; b^* , yellowness) as previously described for sea urchins (Baião et al., 2019). Initially, the apparatus was calibrated with a white plate reference standard (Minolta Co., Ltd., Osaka, Japan). Three replicate measurements were taken for each gonad sample and averaged to determine the colour parameters. Colour parameters were measured by applying the colourimeter onto the gonad. After flashing, L^* , a^* and b^* reflected light values were recorded. From a^* and b^* values the hue angle ($h^* = \tan^{-1} b^* / a^*$) and the chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) were calculated according to (Valente et al., 2015). Total colour difference (ΔE_{76}) was used to compare L^* , a^* and b^* of sea urchins fed the experimental diets (2) with values from wild individuals (1), using the CIE 1976 formula:

$$\Delta E_{76} = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}.$$

2.7. Texture evaluation

Firmness and resilience were determined instrumentally in fresh gonads using a TA.XT.plus analyser, as Rocha et al. (2019) described previously.

2.8. Sensory quality assessment

An external sensory panel formed by 44 untrained panellists, all regular consumers of seafood and fish, was used. For each sample, overall liking was evaluated using a 9-point scale, going from 1- “dislike extremely” to 9- “like extremely” (Peryam and Pilgrim,

1957). To compensate for eventual carry-over effects, each panellist received a set of five samples following a monadic and sequential order of presentation, with a balanced presentation order (MacFie et al., 1989). Every panellist analysed one gonad from each dietary treatment of one of the sexes. Panellists were provided with a porcelain spittoon, a glass of bottled natural water and unsalted crackers. All panellists were asked to chew a piece of cracker and rinse the mouth with water before testing each sample. For each sample, overall liking was immediately followed by the evaluation of the sensory profile, through Check-All-That-Apply (CATA) methodology to reduce bias, following recent recommendations (Ares and Jaeger, 2013; King et al., 2013).

The untrained panellists were asked to perform a CATA question with a list of 16 sensory attributes divided into two dimensions: appearance (8), odour (8) (Table 2). This list of terms was created combining previously published data for sea urchin gonads (Niimi et al., 2010; Phillips et al., 2010a) with the descriptors generated by the trained panel in an ultra-flash profile study (Baião et al., 2020). This list was presented in a randomised order of presentation, different for each participant and each product (Ares et al., 2014). The CATA question “Please select out of the following list of terms those that characterised the observed sample”, indicating if they recognise the presence or absence of such attributes.

Table 2 - List of 16 sensory attributes divided into four dimensions (original in Portuguese), adapted from Baião et al. (2021).

Sensory dimension	Descriptor
Appearance (Aparência)	Firm (<i>firme</i>); grainy (<i>granuloso</i>); appealing (<i>apelativo</i>); not appealing (<i>não apelativo</i>); soft/slimy (<i>suave/viscoso</i>); yellow (<i>amarelo</i>); orange (<i>laranja</i>); brownish (<i>acastanhado</i>).
Odour (Odor)	Sea smell (<i>maresia</i>); sweet (<i>doce</i>); fresh (<i>fresco</i>); tropical (<i>tropical</i>); pleasant (<i>agradável</i>); unpleasant (<i>desagradável</i>); earthy (<i>terra</i>); sulfuric (<i>sulfúrico/enxofre</i>).

2.9. Statistical analysis

Data are presented as means and standard deviation. All variables were analysed using two-way analyses of variance (ANOVA), considering sex (male and female) and experimental diets (CTRL, L_SYN, H_SYN, L_PAP and H_PAP) as fixed factors. When significant effects, at a 95% confidence level, were identified, a Least Significant Difference (LSD) multiple comparison test was applied. Pearson's correlation was used to relate all parameters of each sex separately. In all cases, significant differences were considered when P values < 0.05. All statistical tests were conducted with the STATISTICA 6.4 package (StatSoft, Inc., Tulsa, OK, USA).

Sensorial data analyses were performed using XLSTAT 2019® system software (Addinsoft, USA). To evaluate the overall liking tests results, descriptive statistics (mean and standard deviation) were used, and a mixed three-way ANOVA with panellists as a

random factor and sex and diet as fixed factors was applied at a 95% confidence level. For CATA evaluations, the Cochran test was applied to identify which attributes discriminate among samples (Parente et al., 2011). Then, the frequency of use of each attribute was determined, calculating the number of panellists who have used each attribute to describe the samples. Over this frequency matrix, a correspondence analysis (CA) was applied to obtain a two-dimensional representation of the sample's terms. Multidimensional alignment (MDA) was also performed to determine the correlation between the attributes and the samples vectors in the full-dimensional space of CA, providing complete information about the relationship between products and attributes (Meyners et al., 2013). The reversed scale on the horizontal axis in Figure 3 and 4 warrants the usual interpretation of values to the right of the centre, indicating a positive correlation, and those to the left indicate a negative correlation. Vertical dashed lines indicate the cut-off of 45° and 135°, respectively. The multiple factor analysis (MFA) was applied to assess the consensus among the different variables analysed: carotenoids, colour parameters (L^* , a^* , b^* , C^* and h^*), gonad chemical composition, CATA and acceptance as supplementary data. This statistical methodology allows the analysis of the consensus between different variables that differ in number and data nature and is an improved PCA, which analyses the individual differences instead of average differences (Coelho et al., 2016).

3. Results

3.1. Dietary Carotenoid

The β -carotene levels determined in diets after extrusion were much lower than doses included in the mixture (targeted at either 100 or 250 mg kg⁻¹ DM, in L and H diets), as shown in Table 1. A high degradation (70-94%) was registered in all diets after extrusion. The use of synthetic β -carotene resulted in extruded diets with a 3x higher β -carotene content (30.5 and 66.4 μ g g⁻¹ DM) than that observed in extruded diets with paprika (11.5-14.3 μ g g⁻¹ DM) at equivalent inclusion levels (H and L). No β -carotene was detected in the CTRL diet.

3.2. Growth performance and gonad yield

All diets were well accepted by the sea urchins and induced similar weight gain and growth. No significant differences were obtained regarding weight gain, specific growth rate, voluntary feed intake and feed conversion ratio among experimental diets (Table 3). All diets resulted in a final body weight 2.5 times superior to the initial weight. Sex and diet had no significant effect on gonad weight. All diets were effective in enhancing gonad size that increased from an initial weight of 2.2±1.3 g and 2.3±0.8 g to a final weight of 6.1±1.0 g and 5.7±0.4 g in female and male, respectively; Table 4). GSI values varied from 12.2-14.0% in

female and 11.4-14.2% in male, with no significant differences between sex or diets (Table 4).

Table 3 - Growth performance and feed utilization of *Paracentrotus lividus* fed the five experimental diets over 60 days at 18 °C.

	Experimental diets					One-way ANOVA <i>p</i> -value
	CTRL	L_SYN	H_SYN	L_PAP	H_PAP	
Weight gain^a (g)	2.7 ± 1.4	2.4 ± 0.5	1.9 ± 0.6	2.0 ± 0.5	2.7 ± 0.8	0.778
SGR^b	0.1 ± 0.05	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.02	0.1 ± 0.03	0.691
VFI^c (g/100 g/day)	0.4 ± 0.03	0.4 ± 0.01	0.4 ± 0.04	0.4 ± 0.03	0.4 ± 0.06	0.699
FCR^d	4.5 ± 2.5	4.8 ± 1.0	5.6 ± 1.2	5.1 ± 0.9	4.1 ± 0.6	0.901

Values are presented as mean ± standard deviation (for each sex: n=3 in CTRL and LSYN and n=2 in HSYN, LPAP and HPAP treatments. Absence of superscript indicates no significant difference between treatments ($P \geq 0.05$).

^aWeight gain= final body weight (g) - initial body weight (g)

^bSpecific growth rate

^cVoluntary feed intake

^dFeed conversion ratio

3.3. Proximal composition of gonads

Overall, gonads were characterised by a high protein and low lipid level. Male showed higher moisture, protein and ash content and lower lipid and energy content than female (Table 4). Protein content ranged between 12.6-13.5% WW and 14.1-14.9% WW, while lipids varied from 4.0-4.8% WW and 2.1-3.3% WW, in female and male, respectively. Energy content in gonads followed a similar pattern of total lipids, being higher in female (4.4-5.0 kJ g⁻¹) than in male (4.0-4.2 kJ g⁻¹) and similar among dietary treatments. Gonad moisture was also not affected by dietary treatments but was significantly lower in the female when compared to male (78-80% vs 80-81%, respectively). A negative correlation was found between moisture and protein, and moisture and energy levels, of both female and male gonads, while protein level was positively correlated to energy in both sexes (Table 5). The dietary treatments did not affect the total carotenoid level of sea urchins' gonads, but male showed significantly higher concentration (33.6 – 49.4 µg g⁻¹ WW) than female (21.2–27.4 µg g⁻¹ WW) (Table 4). Three different carotenoid pigments were identified in sea urchins' gonads: β-carotene, echinenone and zeaxanthin; their profiles were similar among dietary treatments within each sex. However, β-carotene and echinenone levels were significantly higher in male than in female (Table 4). Gonadal echinenone displayed a positive correlation with β-carotene (Table 5), and it was the pigment detected in highest levels in both male and female gonads (36.4-41.8 and 16.6-22.8 µg g⁻¹ WW, respectively).

Table 4 - Final gonad yield, proximal composition, carotenoids content, colour and texture of female and male *Paracentrotus lividus* fed the five experimental diets for 8 weeks.

Sex	Female					Male					Two-way ANOVA		
											<i>p</i> -value		
Diet	CTRL	L_SYN	H_SYN	L_PAP	H_PAP	CTRL	L_SYN	H_SYN	L_PAP	H_PAP	Sex	Diet	SxD
Yield													
Gonad weight (g) ¹	5.8 ± 1.3	6.2 ± 2.1	5.9 ± 1.6	6.1 ± 2.3	5.4 ± 1.6	5.5 ± 1.4	6.7 ± 2.0	5.2 ± 1.8	5.7 ± 1.6	5.5 ± 1.0	0.316	0.296	0.684
GSI (%) ²	11.7 ± 2.7	12.6 ± 4.0	12.8 ± 3.3	13.4 ± 4.1	12.4 ± 3.3	11.0 ± 2.9	13.4 ± 3.0	10.2 ± 3.3	11.6 ± 2.8	11.4 ± 1.6	0.057	0.277	0.379
Gonad composition (% WW)													
Moisture	79.3 ± 0.5	80.1 ± 0.5	78.8 ± 0.4	77.7 ± 1.2	77.7 ± 1.3	80.3 ± 2	80.1 ± 0.8	80.5 ± 1.1	79.9 ± 0.7	80.0 ± 0.04	0.011	0.308	0.441
Protein	12.9 ± 0.4	12.6 ± 0.1	13.4 ± 0.4	13.5 ± 0.2	13.0 ± 0.5	14.6 ± 0.8	14.6 ± 0.5	14.7 ± 1	14.1 ± 0.6	14.9 ± 0.4	<0.001	0.680	0.318
Lipid	4.1 ± 1.0	4.0 ± 0.8	4.8 ± 0.1	4.7 ± 0.01	4.4 ± 0.2	2.7 ± 0.5	2.4 ± 0.3	2.1 ± 0.4	3.3 ± 0.5	3.3 ± 0.3	<0.001	0.259	0.325
Ash	2.1 ± 0.1	2.1 ± 0.2	2.1 ± 0.1	2.1 ± 0.2	2.1 ± 0.1	2.5 ± 0.1	2.4 ± 0.2	2.7 ± 0.2	2.3 ± 0.04	2.5 ± 0.2	<0.001	0.282	0.258
Energy (kJ g ⁻¹)	4.6 ± 0.1	4.4 ± 0.1	4.7 ± 0.3	5.0 ± 0.1	5.0 ± 0.2	4.2 ± 0.6	4.1 ± 0.2	4.0 ± 0.4	4.2 ± 0.4	4.1 ± 0.1	<0.001	0.464	0.549
Carotenoids content (µg g⁻¹ WW)													
Total carotenoids	21.2 ± 5.7	23.5 ± 1.2	27.0 ± 0.8	27.4 ± 8.8	27.4 ± 6.0	41.5 ± 8.5	36.9 ± 2.9	49.4 ± 2.3	43.9 ± 3.8	33.6 ± 0.8	<0.001	0.203	0.311
β-carotene	1.9 ± 0.4	2.1 ± 0.4	2.7 ± 0.1	3.4 ± 2.0	2.8 ± 0.5	5.1 ± 1.8	3.7 ± 1.3	6.0 ± 0.2	6.5 ± 0.4	3.5 ± 2.3	<0.001	0.109	0.388
Echinenone	18.3 ± 7.0	16.6 ± 2.7	22.2 ± 1.9	22.8 ± 3.8	21.2 ± 6.4	37.1 ± 4.3	36.4 ± 1.4	41.8 ± 1.3	40.9 ± 6.0	38.1 ± 3.0	<0.001	0.198	0.967
Zeaxanthin	0.5 ± 0.1	0.6 ± 0.3	0.5 ± 0.2	0.5 ± 0.4	0.7 ± 0.1	0.1 ± 0.1	0.04 ± 0.1	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	<0.001	0.931	0.692
Colour (CIE)													
<i>L</i> *	45.0 ± 6.2	43.6 ± 4.2	45.2 ± 8.9	45.3 ± 5.4	44.5 ± 5.8	48.5 ± 6.7	49.4 ± 5.5	48.7 ± 6.7	48.3 ± 7.3	47.4 ± 5.5	<0.001	0.492	0.533
<i>a</i> *	18.7 ± 3.8 ^b	23.5 ± 4.3 ^a	21.2 ± 7.6 ^{ab}	20.8 ± 3.7 ^{ab}	21.3 ± 4.4 ^{ab}	13.2 ± 3.9 ^b	17.6 ± 4.6 ^a	13.1 ± 5.4 ^b	14.0 ± 3.4 ^{ab}	14.2 ± 2.6 ^{ab}	<0.001	0.001	0.585
<i>b</i> *	31.5 ± 5.1 ^b	34.8 ± 4.8 ^a	30.5 ± 8.2 ^b	33.5 ± 6.1 ^{ab}	33.3 ± 5.3 ^{ab}	23.5 ± 6.0 ^b	30.8 ± 6.5 ^a	23.9 ± 7.0 ^b	23.9 ± 6.4 ^b	26.3 ± 4.8 ^{ab}	<0.001	0.029	0.286
<i>C</i> *	36.6 ± 6.0	42.1 ± 5.8	38.4 ± 9.6	40.5 ± 6.1	39.7 ± 5.9	26.6 ± 6.5 ^b	34.7 ± 7.3 ^a	28.7 ± 6.7 ^{ab}	27.8 ± 6.7 ^b	30.0 ± 5.1 ^{ab}	<0.001	0.006	0.492
<i>h</i> *	58.3 ± 4.2	54.9 ± 3.5	54.6 ± 2.3	57.7 ± 3.1	56.2 ± 3.6	58.2 ± 4.7	59.6 ± 2.5	58.3 ± 4.5	58.1 ± 4.2	60.7 ± 4.2	<0.001	0.310	0.057
ΔE76	14.3 ± 5.6 ^b	9.0 ± 6.0 ^a	7.7 ± 5.3 ^a	11.2 ± 3.4 ^{ab}	12.3 ± 5.6 ^{ab}	14.1 ± 6.6	9.9 ± 4.3	11.2 ± 6.0	12.3 ± 4.2	11.5 ± 5.7	0.336	0.005	0.633
Texture													
Firmness (g)	16.8 ± 4.3	16.6 ± 5.5	19.1 ± 6.6	17.2 ± 4.2	16.2 ± 3.5	17.7 ± 4.0	21.6 ± 5.0	17.1 ± 2.6	17.9 ± 5.1	19.5 ± 4.3	0.058	0.655	0.068
Resilience (%)	84.9 ± 6.0	84.8 ± 6.4	84.1 ± 5.5	87.5 ± 5.1	85.1 ± 7.6	83.4 ± 3.9	85.2 ± 5.5	87.6 ± 3.2	85.9 ± 5.8	84.8 ± 2.2	0.911	0.520	0.435
Acceptance	7.2 ± 1.5 ^{ab}	7.6 ± 1.3 ^a	6.9 ± 1.5 ^{ab}	6.2 ± 2.4 ^b	7.3 ± 1.3 ^{ab}	6.5 ± 1.5 ^a	6.9 ± 1.0 ^a	5.3 ± 2.2 ^b	5.9 ± 1.7 ^{ab}	6.7 ± 1.2 ^a	0.001	0.001	0.264

Values are presented as mean $\pm$ standard deviation (n=3 of pooled samples/sex/CTRL, L_SYN; n=2 of pooled samples/sex/H_SYN, L_PAP, H_PAP). Lowercase superscripts indicate differences between diets ($p < 0.050$) within each sex, according to the Least Significant Difference test. ¹Initial gonad weight: 2.2 $\pm$ 1.3 g for females and 2.3 $\pm$ 0.8 g for males. ²Initial GSI: 4.8 $\pm$ 2.1% for females and 5.8 $\pm$ 1.5% for males.

Table 5 - Values of Pearson's correlation coefficients between yield, biochemical composition, carotenoids content and colour of *P. lividus* female and male gonads fed with the five experimental diets for eight weeks.

		GSI	Moisture	Protein	Energy	a*	b*	β -carotene	Echinenone	Zeaxanthin
Females										
Yield (%) and Biochemical composition (%WW)	Moisture			-0.76**	-0.95**				-0.59*	
	Protein				0.77**					
Colour parameters (CIE)	L*					-0.72**				
	a*						0.66*			
Carotenoids content ($\mu\text{g g}^{-1}$ WW)	Total carotenoids							0.86**	0.84**	0.67*
	β-carotene								0.76**	0.58*
	Echinenone			0.76**	0.64*					
Males										
Yield (%) and Biochemical composition (%WW)	Moisture									
	Protein			-0.71**						
	Energy			-0.94**	0.74**					
Colour parameters (CIE)	b*	0.64*				0.90**				
Carotenoids content ($\mu\text{g g}^{-1}$ WW)	Total carotenoids							0.80**	0.76**	
	Echinenone							0.66*		

n=12, ** $p < 0.010$ (two-tailed) and * $p < 0.050$ (two-tailed).

3.4. Gonad colour and texture

Colour evaluation of L^* , a^* , b^* , C^* and h^* parameters of gonads is presented in Table 4. L^* of gonads was not affected by the dietary treatments; however, differences were found between sex, with male exhibiting lighter gonads than female (47.4-49.4 and 43.6-45.3, respectively). Gonad a^* , b^* , and C^* values varied significantly between diets and sexes. L_SYN improved gonad colouration, showing significantly higher a^* (23.5 and 17.6 for females and males, respectively) than CTRL; higher b^* (34.8 and 30.8 for females and males, respectively) than CTRL and H_SYN in both males and females and higher b^* than L_PAP only in males; higher C^* values were also observed in males (34.7) compared to those fed the CTRL and L_PAP diets. Female showed more reddish, yellowish, and colourfulness gonads than male. A positive correlation between a^* and b^* values of gonads was found for both sexes, being stronger in male than in female (Table 5). In male, GSI was positively correlated with b^* values, but the same was not observed in female.

Firmness values ranged between 16.2-19.1 g and 17.1-21.6 g, while resilience varied from 84.1-87.5% and 83.4-87.6% in female and male, respectively (Table 4). Gonad firmness and resilience were not affected by the dietary treatments nor the sex of sea urchins. The texture was not correlated with any of the analysed parameters.

3.5. Gonad sensory quality

3.5.1 Overall liking

Regarding overall liking (Table 4), results from the two-way ANOVA show that there is a significant effect of diet fed to sea urchins ($P = 0.001$) and sex ($P = 0.001$) on gonads acceptance, but there is no interaction between diet and sex ($P = 0.264$). L_SYN showed higher values of acceptance when compared to the other diets, whilst demonstrating significant differences only with L_PAP in females and H_SYN in males. Female were better accepted than male.

3.5.2 Check-All-That-Apply (CATA)

Figure 2 shows the configuration of the samples in the first and second dimensions of correspondence analysis, which explained 78.7 % of the data variation. The representation shows that consumers of sea urchin gonads clustered the samples in three different groups. All female (CTRL, L_SYN, H_SYN, L_PAP and H_PAP) and male fed H_PAP are characterised by attributes such as appealing appearance and orange colour, and also by a pleasant and sea odour. Female fed H_SYN or L_PAP are also associated with a soft appearance. In another group, male fed H_SYN are described as not appealing, and finally, the remaining male (CTRL, L_SYN) constituted a group characterised by a grainy appearance and yellow colour. These results show that a high percentage of the data

is explained by the CATA method, in which samples of the first group are related with positive attributes (pleasant and appealing), whereas the other samples are mainly characterised with less demanded attributes, for instance, not appealing, yellow and grainy.

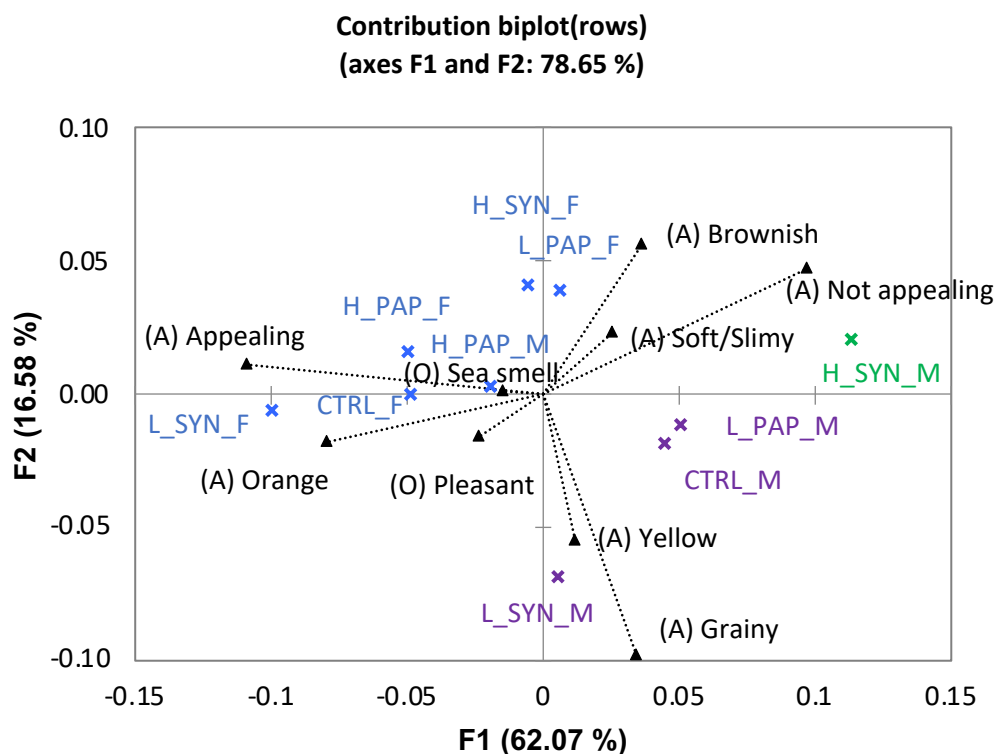


Figure 2 – Dimensions 1 and 2 of correspondence analysis of CATA (Check-All-That-Apply) questionnaire applied to sea urchins' gonads samples. Abbreviations are M=Male, F=Female, O=Odour, and A=Appearance.

3.5.3 Multiple dimensional analysis (MDA)

Based on the outcomes of the CA, we determined the multidimensional relationship between products and attributes using MDA. Figure 3 shows that female fed L_SYN, CTRL or H_PAP are highly associated with an orange colour and appealing appearance. Moreover, female fed H_PAP is also positively related to a sea smell. Female fed L_SYN did not differ from CTRL or H_PAP but differed from L_PAP and H_SYN. These data are consistent with the interpretation of Figure 2. Female fed the L_SYN and CTRL diets were inversely associated with a not appealing and soft appearance, whereas female fed the H_PAP diet was only inversely associated with the not appealing attribute. Female fed L_PAP and, to a slightly lesser extent, H_SYN are not very strongly associated with any of the attributes. Figure 4 demonstrates that male fed L_PAP and H_SYN diets are intrinsic related to not desirable attributes, such as brownish colour and soft and not appealing appearance. Male fed L_SYN or CTRL diet, as indicated from the two-dimensional display of the CA results in Figure 2, are associated with a grainy appearance. However, male fed H_PAP diets were strongly related to a pleasant odour.

FEMALES

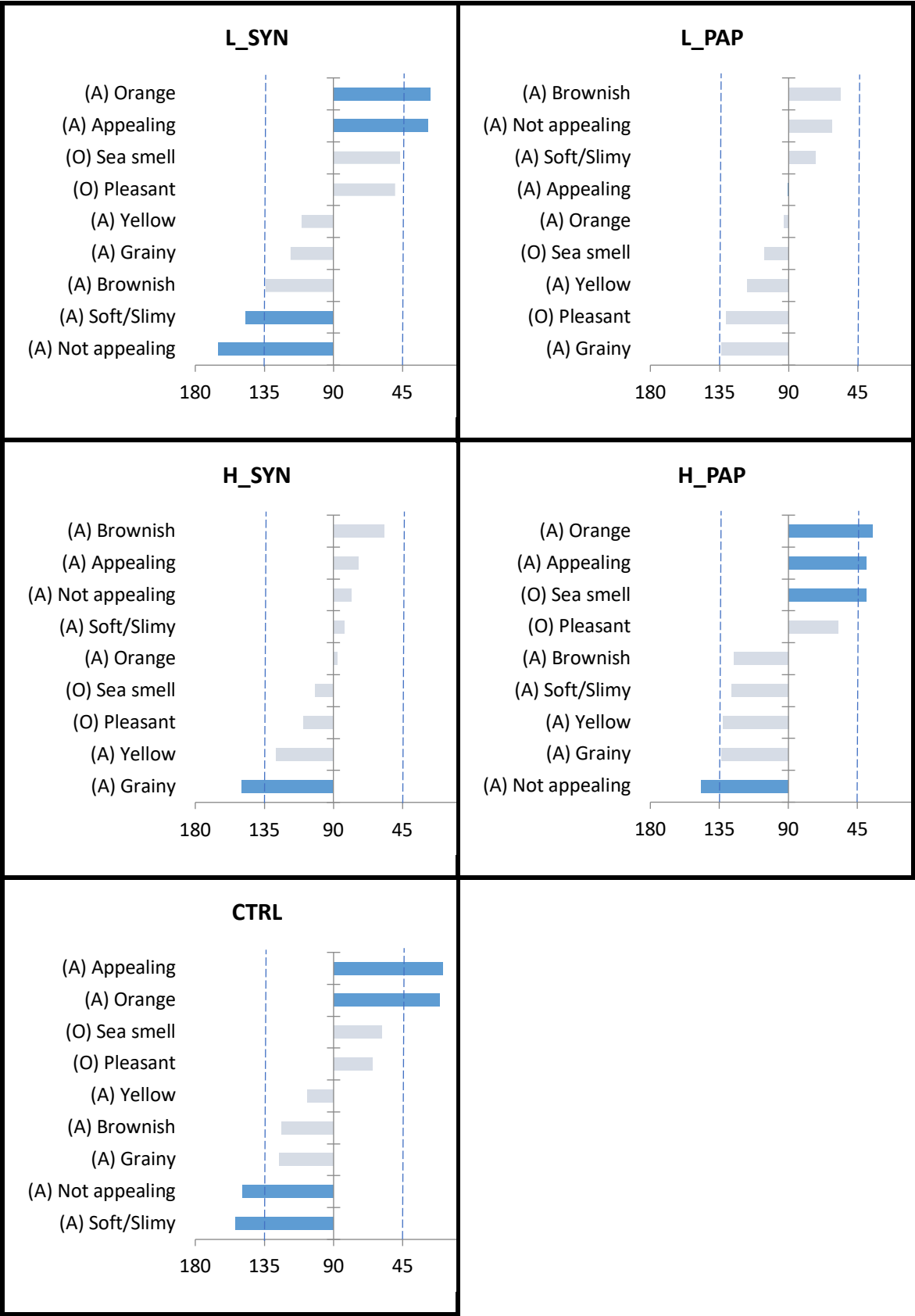


Figure 3 - Associations between attributes and products based on the vectorial angle (0–180°) between samples and attributes, based on the MDA (Females). Abbreviations are O=Odour, and A=Appearance.

MALES

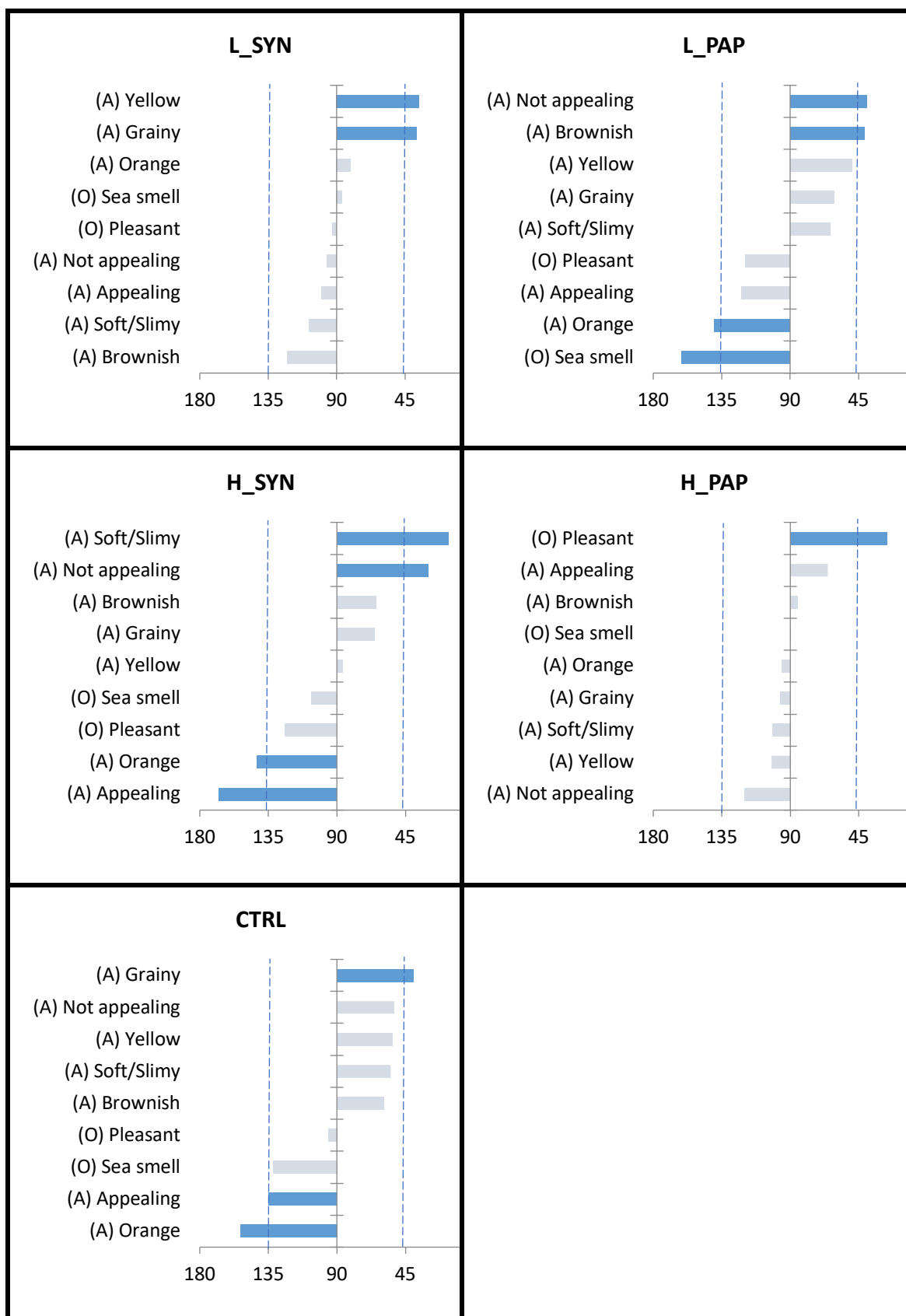


Figure 4 - Associations between attributes and products based on the vectorial angle (0–180°) between samples and attributes, based on the MDA (Males). Abbreviations are O=Odour, and A=Appearance.

3.5.4 Multiple factor analysis

Multiple factor analysis (MFA) was performed to compare sea urchin gonads' carotenoid composition, gonad instrumental colour, CATA, and acceptance evaluations. Table 6 shows the RV coefficients (squared Pearson correlation coefficient) resulting from MFA. For CATA, colour and carotenoids, the RV values in the MFA configuration were very high and close to the unit, 0.821, 0.796, 0.898, respectively. The correlation between CATA and carotenoids and also colour and carotenoids was significant ($P < 0.01$), being the RV value between colour and carotenoids high (0.754).

Table 6 - RV coefficients resulting from multiple factor analysis applied to sea urchin gonads carotenoids composition, gonad instrumental colour values, CATA and acceptance evaluations (p -value is shown in brackets).

	CATA	Colour	Carotenoids	Acceptance
Colour	0.326 (0.131)	-	-	-
Carotenoids	0.542 (0.007)	0.754 (0.004)	-	-
Acceptance	0.622	0.586	0.646	-
MFA	0.821	0.796	0.898	0.633

Figure 5 shows the two first dimensions of the MFA consensus, which explains 69.7% of the variability of the experimental data. The representation shows that naïve consumers of sea urchin gonads clustered the samples in four different groups. These data are consistent with the interpretation of Figure 2, where males, except the ones fed H_PAP, are characterised by a soft, grainy and not appealing appearance and yellow or brownish colour and, also, with total carotenoids, echinenone, β -carotene, brightness and hue angle. A quite interesting finding is the relationship between male and female fed H_PAP, which tended to be projected in opposite quadrants. Males fed H_PAP were more associated with the remaining females, whereas the female fed H_PAP were associated with positive attributes, for instance, the sea smell, orange colour and appealing appearance. The remaining females were grouped in another cluster. Females were negatively loaded along F1 and were associated with an appealing appearance, orange colour and sea and pleasant odour, and, also, with zeaxanthin, yellowness, redness and chroma. Sea urchins fed the L_SYN diet were the most accepted product among consumers.

3.6. Comparison between reared and wild gonads

At the end of the trial, the predominant maturation stage was the premature gonads (stage III). Both the L_SYN, H_SYN and H_PAP presented similar colour, while the L_SYN proved to be more accepted among the consumers (correlation with the acceptance in Figure 5). For this reason, sea urchins fed L_SYN diet, supplemented with a lower amount of β -carotene, were further compared to marketable wild specimens at a similar maturation stage. This comparison was performed separately for female and male sea urchin (Table 7). GSI of reared and wild sea urchins were similar independently of the sex (12.6 vs 14.8%

in female and 13.4% in all males). Wild individuals showed higher levels of protein, lipids and energy than those fed L_SYN (14-17 vs 13-15 % WW, 3-5 vs 2-4 % WW, 5 vs 4 kJ g⁻¹, respectively). Female fed the L_SYN diet showed a significantly lower content of total carotenoids, β -carotene and echinenone (23.5 vs 72.2 $\mu\text{g g}^{-1}$ WW, 2.1 vs 14.6 $\mu\text{g g}^{-1}$ WW and 16.6 vs 59.1 $\mu\text{g g}^{-1}$ WW, respectively), and also less reddish, yellowish and colourfulness gonads (23.9 vs 29.0, 34.2 vs 39.6 and 41.8 vs 49.1, respectively) when compared to wild female. The gonads from female fed formulated diets also had lower firmness and higher resilience than wild individuals (26.1 vs 16.6 g and 79.8 vs 84.8 %, respectively). On the contrary, carotenoid concentration, pigment levels and colour (L^* , a^* and b^*) values of male fed the L_SYN were similar to those of wild specimens. Male fed L_SYN had a similar firmness and higher resilience when compared with wild male (21.6 vs 24.1 g and 85.2 vs 77.6%, respectively). L_SYN females and males did not demonstrated significant differences of acceptance in relation to wild individuals (Table 7).

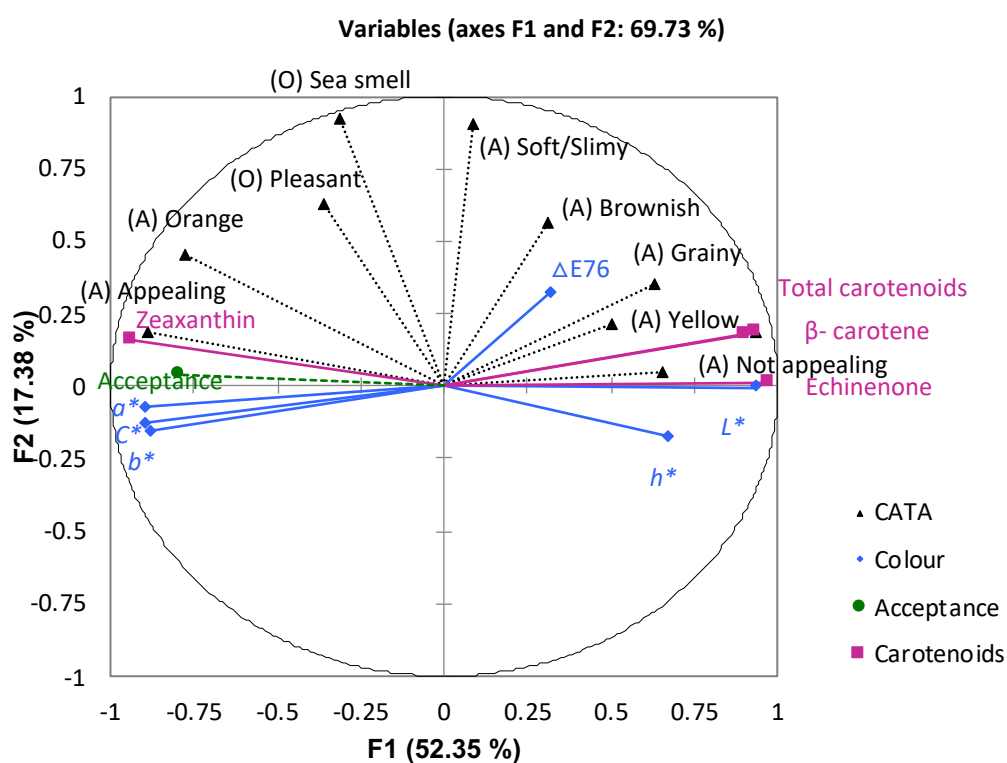
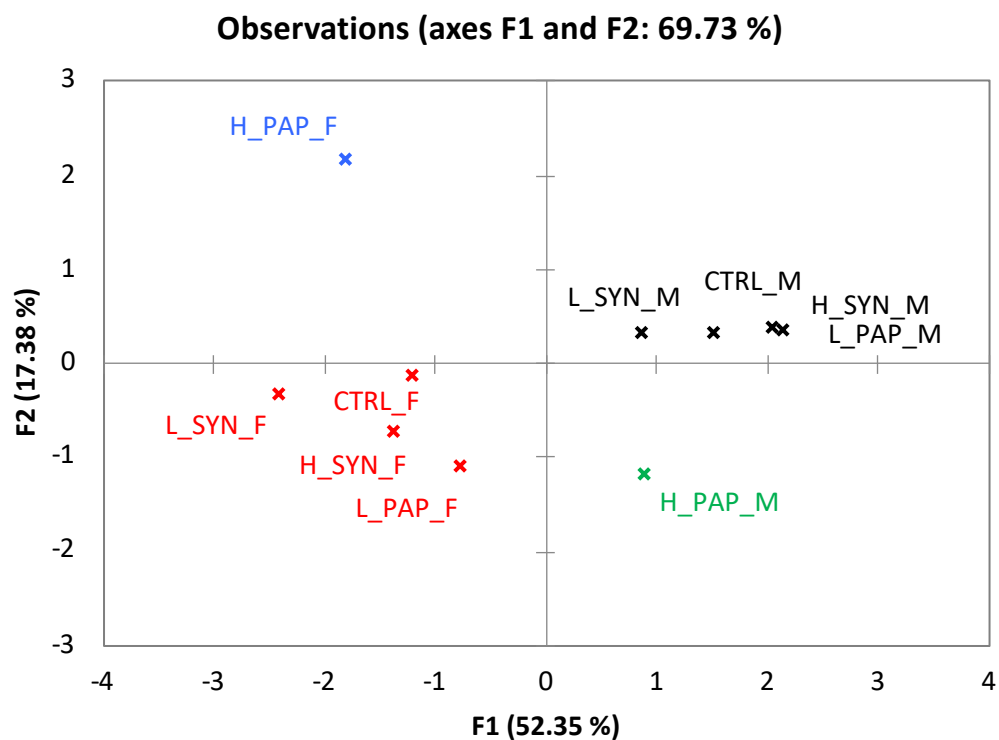


Figure 5 - Dimension 1 and 2 of multiple factor analysis applied to sea urchin gonads carotenoids (purple lines), instrumental colour features (blue lines), CATA (red lines) and acceptance (green lines).

Table 7 - Comparison of gonad yield, proximal composition, carotenoids content, colour and texture of *Paracentrotus lividus* fed the L_SYN diet for eight weeks and animals harvested from the wild at similar maturation stage (Stage III).

Sex	Female		T-test	Male		T-test
Diet	L_SYN	Wild	<i>p-value</i>	L_SYN	Wild	<i>p-value</i>
Yield						
GSI (%)	12.6 ± 4.0	14.8 ± 2.9	0.156	13.4 ± 3.0	13.4 ± 0.7	0.949
Proximal composition (% of WW)						
Moisture	80.1 ± 0.5	76.4 ± 0.9	<0.001	80.1 ± 0.8	76.5 ± 0.7	<0.001
Protein	12.6 ± 0.1	13.6 ± 0.5	0.029	14.6 ± 0.5	16.5 ± 0.4	<0.001
Lipid	4.0 ± 0.8	4.9 ± 0.3	0.161	2.4 ± 0.3	3.3 ± 0.4	<0.001
Ash	2.1 ± 0.2	1.8 ± 0.1	0.079	2.4 ± 0.2	2.8 ± 0.2	0.056
Gross energy (kJ g ⁻¹)	4.4 ± 0.1	5.0 ± 0.2	<0.001	4.1 ± 0.2	4.8 ± 0.2	0.013
Carotenoids content (µg g ⁻¹ WW)						
Total carotenoids	23.5 ± 1.2	72.2 ± 11.2	<0.001	36.9 ± 2.9	60.7 ± 17.3	0.072
β-carotene	2.1 ± 0.4	14.6 ± 5.0	0.012	3.7 ± 1.3	7.5 ± 3.3	0.134
Echinenone	16.6 ± 2.7	59.1 ± 7.3	<0.001	36.4 ± 1.4	37.7 ± 7.5	0.778
Colour (CIE)						
<i>L</i> *	43.6 ± 4.2	42.3 ± 2.5	0.341	49.4 ± 5.5	54.1 ± 4.5	0.075
<i>a</i> *	23.5 ± 4.3	29.0 ± 2.5	0.005	17.6 ± 4.6	19.4 ± 3.9	0.400
<i>b</i> *	34.8 ± 4.8	39.6 ± 2.9	0.004	30.8 ± 6.5a	32.7 ± 5.9	0.329
<i>C</i> *	42.1 ± 5.8	49.1 ± 2.6	0.002	34.7 ± 7.3a	38.3 ± 5.3	0.279
<i>h</i> *	54.9 ± 3.5	53.7 ± 3.2	0.249	59.6 ± 2.5	58.9 ± 7.4	0.782
Texture						
Firmness (g)	16.6 ± 5.5	26.1 ± 4.5	<0.001	21.6 ± 5.0	24.1 ± 7.8	0.408
Resilience (%)	84.8 ± 6.4	79.8 ± 3.4	0.040	85.2 ± 5.5	77.6 ± 2.9	0.004
Acceptance	7.6 ± 1.3	7.4 ± 1.4	0.405	6.9 ± 1.0	6.9 ± 1.5	0.638

Values are presented as mean ± standard deviation (n=3 of pooled samples/sex/L_SYN, Wild). Significant differences are considered at *P*<0.05. Wild acceptance data was retrieved from published data by Baião *et al* (2021).

4. Discussion

Micronutrients, such as carotenoids, are prone to be damaged by the feed manufacturing process (Rickman *et al.*, 2007). Carotenoids are sensitive organic compounds that can be impaired by water, oxygen, heat, among others (Jintasataporn and

Yuangsoi, 2012). Moreover, the feed manufacturing process involves water, high temperature, and mechanical stresses, all of which can affect the carotenoid stability in the feed, consequently altering the carotenoid levels of feeds (Camire et al., 1990; Rodriguez-Amaya, 1999). In the present study, the inclusion of either a synthetic or a natural source of β -carotene to a mix of ingredients prior extrusion resulted in a high β -carotene degradation rate (69.5-94.3%) in the final extruded diet. Nevertheless, the synthetic β -carotene was better preserved than that from paprika, during the pellets processing and extrusion, probably due to its prior microencapsulation. Suckling et al. (2011) used freshly made diets for sea urchin supplemented with a natural β -carotene source (Algro Natural[®]) and still noticed a 19-51% pigment degradation. However, the production of fresh diets is a strong limitation to equinoculture, evidencing the importance of pigment protection prior feed processing and/or the need to select softer extrusion techniques to produce commercial extruded diets for sea urchin.

The quality of sea urchin gonads mainly depends on their size, colour, taste and firmness (Pearce et al., 2002; Prato et al., 2018a). Our study clearly shows that all diets were well accepted by the sea urchins and were effective in promoting gonadal growth. The dietary inclusion of carotenoids did not affect *P. lividus* growth performance. These results agree with previous studies that demonstrated that dietary carotenoids did not affect gonad growth of *Lytechinus variegatus* (Plank et al., 2002) and *P. lividus* (Shpigel et al., 2006). Moreover, GSI values (10-13%) were significantly higher than those observed at the beginning of the trial (4.8-5.8%) or those reported in previous studies with adult *P. lividus* reared in captivity (3-9%) (Prato et al., 2018b; Volpe et al., 2018). Baião et al. (2019), during a 15-week trial, achieved even higher gonad weight and GSI (10-11 g and 20-23%, respectively) using a diet with 30% CP and 6% CF, which is mainly explained by higher initial gonad size and GSI values (3.7 g and 10%). The proximal composition of gonads was not affected by dietary treatments, and still differed significantly between female and male. Likewise, Baião et al. (2019) reported that ovaries had a higher lipid and DM content, but lower protein content than testis, suggesting that males and females differ in their ability to incorporate dietary nutrients, which might explain differences in metabolic processes.

Carotenoid pigments are responsible for the red, orange and yellow colouration and may modulate sea urchin gonads' colour after dietary inclusion (Robinson et al., 2002; Shpigel et al., 2005). Echinenone, metabolised from dietary β -carotene (Symonds et al., 2007), was positively correlated with gonad β -carotene (Table 5), and was the most abundant pigment both in males and females' gonads, as described in previous studies (Griffiths and Perrott, 1976; Sartori and Gaion, 2016; Shpigel et al., 2005; Shpigel et al., 2006; Tsushima, 2007). However, in most nutritional studies sea urchin gonadal colour and carotenoid profile was evaluated without sexual differentiation (Pearce et al., 2004;

Robinson et al., 2002; Shpigel et al., 2006; Symonds et al., 2007), so comparisons have to be done with caution. In our study, *P. lividus* colour and carotenoid profile were determined separately in males and females. Although no significant correlations could be established between colour parameters and pigment concentration in the gonads of both sexes using the Pearson correlation analysis (Table 5), the MFA evidenced a strong positive correlation between gonad colour and carotenoids content (Table 6; $RV = 0.754$; $p = 0.004$). Multiple factor analysis (MFA) (Pagès and Husson, 2014) is a multivariate data analysis method for summarizing and visualizing a complex data table in which individuals are described by several sets of variables (quantitative and/or qualitative) structured into groups. It takes into account the contribution of all active groups of variables to define the distance between individuals. In this analysis, males had significantly higher β -carotene and echinenone content than females, whilst zeaxanthin content was highest in females. Since both sexes were fed identical diets, sex-specific pathways for carotenoid metabolism may be suggested (Robinson et al., 2002). A previous study also reported that during the maturation stage, deposition of carotenoids into the gonads of *L. variegatus* is outpaced by the growth rate of the organ itself, resulting in a lower gonadal concentration of carotenoids (Plank et al., 2002). However, *P. lividus* gonads by the end of the present study were of similar size and predominantly in the premature stage, irrespectively of diet or sex, so maturity and size do not seem to have played a major role in sea urchin gonads colour and pigment concentration.

To the best of our knowledge, there are no sensory studies neither in *P. lividus* nor other sea urchin's species that correlate sensory profiling with pigment deposition and instrumental colour evaluation. In our study, differences in the sensory quality of gonads are mainly correlated with sex, as previously reported either in *P. lividus* (Baião et al., 2021) and *E. chloroticus* (Phillips et al., 2009; Phillips et al., 2010b). Baião et al. (2020), using exploratory sensory profiling techniques based on projective mapping, have demonstrated that male gonads tend to have a pale-yellow colour and a sweet taste and soft texture, while female gonads reveal a bright-orange colour and an intense sweet tropical taste. Baião et al. (2021), using a full sensory profiling with the same CATA methodology, showed that wild males had a brownish and milky white fluid appearance, while wild females were strongly associated with a fresh odour and an orange and appealing appearance. This is in general accordance to our study, which showed that females were more orange, appealing and pleasant, and presented higher values of a^* , b^* and C^* , and lower values of L^* compared with the yellow, grainy and not appealing males.

Gonadal colour differences emerged between diets, besides the significant differences between ovaries and testis. L_SYN improved gonad colouration, showing significantly higher a^* and b^* values than those fed the CTRL and L_PAP diets. Females

fed with the L_SYN diet were the most accepted product among consumers. While for males, those fed with the L_SYN diet presented the lowest total colour difference and most similar pigment levels and colour (L^* , a^* , b^* , C^* and h^*) values compared to wild individuals. Furthermore, acceptance of L_SYN females and males among consumers did not differ from their wild counterparts, suggesting that the incorporation of low levels of synthetic β -carotene (L_SYN) could improve sea urchins' gonad colour and carotenoid content resulting in better acceptance. Moreover, there was a positive correlation between yellowness (b^*), redness (a^*), chroma (C^*) and zeaxanthin content with 'appealing appearance' and consumer's acceptance, while the 'not appealing appearance' attribute and other carotenoids were primarily associated with the brightness (L^*) and hue angle (h^*) parameters. Lower values of redness and yellowness (pale-yellow or brownish coloured gonads, Figure 5) were associated with higher echinenone and β -carotene values, irrespectively of the diet. This is consistent with Shpigel et al. (2005) that reported that higher concentrations of echinenone are thought to originate more intense and darker gonads, but it is not consistent with previous works that have positively correlated high proportions of these carotenoids with the orange colouration (Barker et al., 1998; Griffiths and Perrott, 1976; McBride et al., 2004; Pearce et al., 2004; Shpigel et al., 2006). Moreover, Robinson et al. (2002) and Shpigel et al. (2005) have demonstrated that the dietary inclusion of carotenoid pigments is crucial to enhance the desirable bright yellow/orange colouration of gonads sought by the market. In our study, wild female specimens presented a much higher echinenone and β -carotene content that were associated with better colour parameters, suggesting that sea urchin fed carotenoid-rich diets were unable to incorporate the dietary pigments in the gonads, or that natural foods contain higher pigments' bioavailability than the artificial diets. Moreover, the biochemical nature of the pigments content measured in gonads failed to be correlated with enhanced redness (a^*) and yellowness (b^*), suggesting that visual perception of colour may be influenced by other sex-dependent nutritional or metabolic factors.

According to Shpigel et al. (2006) and Suckling et al. (2011) freshly made diets with the incorporation of a rich natural β -carotene source, *Dunaliella* spp. concentrate could improve gonad colour in *P. lividus* and *P. miliaris*, respectively. Shpigel et al. (2006) also demonstrated that the addition of 66 mg kg⁻¹ DM natural β -carotene to agar-based diets was more effective at inducing good colouration in gonads than 25 mg kg⁻¹ DM synthetic β -carotene, although doses were not comparable. Moreover, natural β -carotene enhanced the bioavailability of 9'-cis echinenone isomer which was shown to be the major carotenoid contributing to the pigmentation/colour of the gonad (Symonds et al., 2007). Different results were reported by Robinson et al. (2002) that showed that paprika, known to have high concentrations of β -carotene, could not improve *S. droebachiensis* colouration when

incorporated up to 10% in the diet. Likewise, in the present study, although paprika was included as an oleoresin concentrated form in the diet, it was highly susceptible to degradation during the extrusion process, and was unable to improve *P. lividus* gonad colour. Furthermore, besides echinenone, zeaxanthin is also metabolised from carotenes and is mainly associated with orange colour, primarily deposited in females (orange colour, Figure 5). In a parallel perspective, O'Hare et al. (2015) showed that high-zeaxanthin concentration improved the orange colour of sweet-corn relatively to yellow carotenoids such as lutein. Female presented higher values of a^* , b^* and zeaxanthin was strongly associated with higher product acceptance rates. It is still not very well-defined in the literature why some natural β -carotene sources seem to outperform synthetic β -carotene, neither the relationship between gonad colouration and the bioavailability, bioabsorption or bioconversion of the supplemented pigments in the gonad, so further studies are still warranted.

Gonad firmness and resilience were not affected by the dietary treatments nor the sex of sea urchins, which agrees with several studies (Pearce et al., 2002; Phillips et al., 2009; Senaratna et al., 2005). Higher values of firmness were observed in wild females with access to natural food sources compared to those fed formulated diets, confirming previous studies demonstrating that kelp-fed and wild individuals had slightly better gonad firmness than those fed prepared diets (Pearce et al., 2004; Phillips et al., 2010a; Siikavuopio et al., 2007). However, male fed L_SYN had a similar firmness and even higher resilience than wild male, suggesting that male sea urchins fed this experimental diet had similar characteristics from their wild counterparts.

Despite the findings of this work, it is noteworthy that colour and texture perception can be distinct among consumers of different regions. For instance, in Japan, high-priced sea urchins are represented by a yellowish colour as well as a sweet taste and a firm texture (Barker, 2015; Stefánsson et al., 2017), whereas in the Mediterranean countries, the preference is represented by a bittersweet flavour, firm texture, reddish-orange colour (Sartori and Gaion, 2016). Therefore, selecting the most appropriate diet for sea urchins should consider the reference market. Moreover, incorporating a concentrated natural β -carotene in sea urchin diets, more resistant to the extrusion processes, may introduce higher quantities of this pigment into the carotenoid metabolism pathways, improving the β -carotene retention and, consequently, gonad colour. Nonetheless, it is also quite clear that there is a set of unknown parameters, besides carotenoids, which have the ability to modulate colour.

5. Conclusions

This study clearly shows that the incorporation of synthetic or natural pigments does not affect the growth or pigments content in the sea urchin gonads. Gonads' sensory profiling and pigments content did not differ among diets. L_SYN diet was effective in promoting gonad colour enhancement: sea urchin fed with L_SYN were the most accepted among consumers, both in males and females, and the males fed this diet displayed a carotenoid content and gonad colour similar to wild specimens. However, female' wild urchins had higher echinenone and β -carotene content, accompanied by higher redness, yellowness and firmness values than female' urchins fed the L_SYN diet. Appearance properties, mainly colour, are crucial for the acceptance and choice of consumers concerning seafood. Indeed, these visual impressions are usually the first visual sensory stimuli presented to a consumer, being key purchase determinants. Thus, additional nutritional strategies are required for improving the colour and texture of gonads.

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Chapter X.

General discussion, conclusions and final considerations

General discussion

The present chapter aims to integrate the main findings of Chapters II to IX, as well as to build a bridge to the general and specific objectives previously delineated for this thesis. In general, the current work focused on assessing and developing the sensory profile of sea urchin and the value of its intrinsic characteristics, as well as to enhance a diet capable of improving sea urchin gonad size and quality. To this end, four specific objectives were established. The first one focused on the characterization of the seasonal and sex variation of the sea urchin gonad quality and further determination of the best harvest period (Chapter II). The second aimed to define the consumer's perception, acceptance and consumption habits of this product category (Chapters III and IV). The third one intended to identify the sensory profile of *P. lividus* (Chapter V, VI and VII). The last objective aimed at the modulation of sea urchin gonad growth and quality (Chapter VIII and IX) through dietary formulation.

P. lividus gonads are considered a prized and gourmet seafood delicacy due to their unique flavour, making their commercialization highly profitable. The depletion of wild stocks of echinoids due to over-fishing, and the constantly growing market demand for good quality sea urchin products highlights the importance of echinoculture in bridging the gap between demand and supply of this gourmet niche with valuable commodity. Nonetheless, several technical bottlenecks need to be overcome before aquaculture can significantly contribute to sea urchin production. This thesis strictly focused on commercial sized sea urchins, hence, the identification of the main organoleptic properties that determine gonads' marketability, consumer's acceptance and purchase decision, are crucial for the valorisation of this caviar-like product, as well as the formulation of an inert diet capable of inducing high-quality gonads, meeting consumers' expectations.

The *P. lividus* maturation stage, which varies along the annual reproductive cycle, affects gonad growth and its quality features, and consequently its market acceptance and commercial value (Walker *et al.*, 2020). In Chapter II the physiological, chemical and quality fluctuations of *P. lividus* collected along the Portuguese coast were described and related with the seasonal reproductive cycle. It was clearly demonstrated that most of the evaluated quality attributes had a seasonal variation strongly dependent on sea urchin sex. This variation along the annual reproductive cycle appears to be mainly related to water temperature, photoperiod (Boudouresque *et al.*, 2020), food availability (Livore *et al.*, 2012) and food quality (Gago *et al.*, 2003). According to the results of Chapter II, and considering the gonads' quality traits, it is suggested that the most suitable period for harvesting high-quality sea urchins in the Atlantic coast of Portugal is between November and February.

During this period, gonads are mainly at the growing and premature stages of gametogenesis, meaning that the nutritive phagocytes are full, as demonstrated by the highest levels of protein, lipid, n-3 and n-6 PUFA, carotenoids and energy content. Additionally, gonads showed lower moisture content and simultaneously higher firmness, which is a valued sensory attribute. The consumption of 100 g of sea urchin gonads in this stage corresponds to similar levels of protein and lipids of a 100 g fillet of gilthead seabream (*Sparus aurata*), which provides around 19–20 g of protein and 5–9 g of lipids (Valente *et al.*, 2011). Furthermore, the recommended daily consumption of EPA + DHA to decrease the risk of cardiovascular diseases in humans is between 0.25–0.5 g (EFSA, 2009). If sea urchins are consumed during this aforementioned period, an intake of 50 g of *P. lividus* (corresponding to 4–5 specimens) can provide nearly 0.25 g of EPA + DHA, which covers the daily recommended dose to promote human health. More important than the nutritional value, during the growing and premature stages of gametogenesis, gonads were found to be more reddish, yellowish and brighter, irrespective of sex, the values within those being considered acceptable and desirable in the European market, where a bright and orange gonad is rated as premium (Cook *et al.*, 2007; Symonds *et al.*, 2007). Therefore, gonads' commercial value is highest during the growing and premature season, when the product also presents the highest nutritional and quality features. Afterwards, when gonads mature and their GSI peaks (March), their nutritive content starts decreasing, and consequently the gonad quality deteriorates. Such biochemical changes impair gonads' flavour and texture, leading to a loss of economic value (Unuma *et al.*, 2003). Similarly to what happens in international markets, a high level of importance is placed on gonad quality since their sensory traits influence market price and consumer acceptability (Sun *et al.*, 2015).

Although seafood is often considered by consumers as a healthy product, in Southern Europe *P. lividus* is still under-consumed, mainly because it is not part of the Mediterranean gastronomy culture. However, the population from this geographic area is mostly concentrated in coastal regions and there is a close relationship between human activities and the exploitation of marine coastal ecosystems, increasing the economic potential to explore new coastal resources, especially if directed to exotic and prized species like sea urchins. Besides, in the culinary world, an increased number of rewarded Michelin Star restaurants has emerged in the past years, which boosted the development of innovative products and search for novel flavours. This has contributed to an increasing demand for seafood products, including sea urchin, not only in the *Haute* cuisine but also by consumers in general. In fact, the Chefs' and consumers' interests tend to be aligned and both end up guiding each other, setting trends. Moreover, during the last years there has also been a consumer shift towards marine products as key sources of protein, PUFA and natural antioxidants like carotenoids. There is also an increasing concern regarding

consumers' health and wellbeing, which may represent an opportunity to explore *P. lividus* as a high-valued product. In this scope, it is crucial to measure the impact of the stimulus within a natural consumer environment. Indeed, the consumption of sea urchin could be affected by many factors, namely those associated with culture, health and sensory properties. Chapter III aimed to explore and understand Portuguese consumers' preferences and consumption habits regarding sea urchin and, also, consumers' motives and barriers to their consumption. To better disclose consumer beliefs and attitudes toward this product category, a free word association task was performed, by projecting their unconscious feelings into their answers. Indeed, according to our results, the consumers who have had consumed this product more frequently associated it to emotional, sensory and health aspects, which are related to the perceived wellbeing, while consumers with a low shellfish consumption profile made almost no relevant associations with sea urchin. This suggests that participants with high consumption patterns had a high level of familiarity with this gourmet product, while consumers with a low consumption habit had poor knowledge of the product. Moreover, the participants with low levels of food neophobia were significantly more willing to taste shellfish products than participants with high levels of food neophobia. Actually, the consumers' willingness degree increased with decreasing levels of neophobia, being more likely to display adventurous behaviour in the purchase and consumption of unfamiliar shellfish products compared to those with low exposure and involvement with shellfish products, as found in several other food studies (Hayes *et al.*, 2012; Ristic *et al.*, 2016). Taking into account these results, the communication strategies regarding this product should emphasize the pleasantness obtained through sensory and emotional aspects, aligned with the aspect of health and wellbeing, while reinforcing the importance of its consumption. These pertinent aspects were further reinforced in Chapter IV that provided significant information able to increase the market value of this appraised product, contributing to the improvement of consumers' product experience. In this chapter, the need for communication and marketing strategies through locals, Chefs and gastronomic events, focused on the promotion of the consumer awareness about the exclusivity of this product and the importance of aquaculture for the future of the seafood market was highlighted. The instability in supplying high-quality sea urchin was identified as a strong barrier against including sea urchins in the main dishes of the Chefs' menus. Aquaculture could help mitigate the seasonality and lack of supply of the product, although it is still not highly considered among Chefs and consumers. In spite of all of aquaculture's positive features, consumers consider that wild seafood presents better organoleptic features and greater health benefits (Claret *et al.*, 2014), and it is their demand that defines the use of wild or aquaculture products in the Chefs' restaurants. As they seem to have the notion of the benefits surrounding aquaculture and a biased opinion concerning

aquaculture, they need to be re-educated and encouraged to follow a healthy but more conscious consumption of natural resources, and to embrace the benefits of aquaculture practices regarding their sustainability, nutrition and health (Mazur *et al.*, 2006; Rickard *et al.*, 2018). Additionally, since consumers demand a wild-like product, aquaculture's success, sustainability and market acceptability depends on the development of suitable and nutritionally adequate diets to produce high-quality gonads, building consumers' trust, reducing perceived risk and improving product valorisation. To address this further, the initial sensory organoleptic ballot obtained from high-quality sea urchin gonads was defined in two studies (Chapter III and IV), designed to explore relevant information regarding this product and its consumption among consumers and renowned cuisine Chefs. These results allowed us to further choose the best methodological approach to assess consumer perception and acceptance of this product.

Measuring the *real world* using the natural consumer environment does not mean that we ought to forsake the measurements with trained panels. Meiselman (2013) states that future scientific discussion will not be on the replacement of the trained panels by consumer panels, but how to combine harmoniously the two approaches. In a more recent study, Jaeger *et al.* (2017) implied that the amount of studies in product development, incorporating mixed methods with consumer panels and trained panels, will rise in the next few years. In Chapter V a trained panel was used to characterize the sensory features of sea urchin gonads coupled with HS-SPME/GC-MS techniques to determine the gonads' active volatile organic compounds profile. The combination of these recent methodologies provided information on the several volatile compounds and sensory descriptors, and were able to discriminate sea urchin gonads samples in terms of their sex. These differences between sea urchin sex may explain the somehow inconsistent sensory properties previously observed within wild sea urchin populations (Boudouresque *et al.*, 2020). Although without data on preference or acceptability scores, the information gathered throughout this study allowed for the development of a final ballot to be used in consumer studies to characterize the sea urchin profile, and to further cross all the information to obtain maximum liking. For that reason, we believe that a trained panel is vital for product development, being the best approach to get trustworthy data and cost-effective results, as previously suggested by Jaeger *et al.* (2017). Furthermore, a trained panel can continuously assess the quality features of what characterizes a *premium* product. Nevertheless, as suggested by Dijksterhuis (2016) and Kemp (2013) if the main objective is to increase the novel product's success, it is crucial to pay attention to the consumers' opinion and try to integrate them in the product development process. Indeed, from a sensory point of view, it is vital to understand the consumers' sensory perception of a product, because it is the closest interpretation of the final consumers' insights that we will be able to obtain (Carr *et*

al., 2001). To accomplish this, we were faced with some scientific challenges related to the disparity of the product characteristics between sex, and the initial choice of the methodological approach, which lead to the use of fast and user-friendly descriptive consumer techniques.

Chapter VI and VII aimed to describe a premium sea urchin gonad, based on the sensory profile and maximization of liking. Chapter VI highlighted the importance of presentation in the consumer's perception and attitudes towards the product. The mode of presentation is a key factor in food choice criteria that can improve or deteriorate the appearance leading to a better or worse acceptance of the product (Spence, 2015). Moreover, this study revealed that despite the lack of differences in overall liking and acceptance in relation to sex, consumers identified differences that go beyond liking, more specifically in the sensory profile of male and female gonads. However, the CATA format does not allow to directly measure the intensity of the perceived sensory attributes, which could mask a potential discrimination between products that have similar sensory profiles but vary in the intensity of those features (Ares *et al.*, 2014; Meyners *et al.*, 2016; Vidal *et al.*, 2018). To overcome this drawback, in Chapter VII we have used an attribute intensity measurement (RATA) as a variant to CATA questions (Ng *et al.*, 2013) to enhance sample description and discrimination. These two approaches allowed the identification of a gonad with a premium attribute set: orange and appealing gonad with a sweet, fresh and tropical flavour. These attributes were further used to help select the best nutritional approach that could also result in high quality gonads able to meet consumers wild-caught quality demands.

Likewise to other marine species, the nutritional composition of gonads seems to be highly dependent on the sea urchin diet (Lourenço *et al.*, 2019). The increase of gonad yield before gametogenesis is related to the accumulation and storage of nutrients (proteins, lipids and carbohydrates) provided through the diet, in nutritive phagocytes (Walker *et al.*, 2015). At the start of this PhD project, very little was known about the nutritional requirements of *P. lividus* and the experiment described in Chapter VIII aimed to assess the effects of different dietary protein and lipid ratios on sea urchin's growth, under a RAS. This study clearly showed that it is possible to successfully enhance sea urchin gonad yield in relation to wild animals in a RAS at 18 °C and that the diet with the lowest protein (30% DM) and lowest lipid (6% DM) was effective in promoting gonad growth level, resulting in the highest gonad yield in both sexes. However, data showed that, although such diets were successful in promoting gonad growth, they had very limited or no effect on the quality traits important for maximising consumer acceptability, such as colour. In fact, colour is the first quality attribute used by consumers, impacting the visual assessment and freshness perception of sea urchin gonads, which are key purchase determinants. The significantly

lower carotenoid content, namely β -carotene, or even its absence in the formulated feeds was potentially the main aspect responsible for the observed reduced colour quality when compared to the wild highly-acceptable gonads. The desirable orange colour seemed to be determined by carotenoids deposited in the gonads, mainly the accumulation of echinenone (Kelly *et al.*, 2013), whose content is limited by the availability, uptake and bioconversion of β -carotene from dietary sources (Shpigel *et al.*, 2005; Shpigel *et al.*, 2006). The urge for the development of new nutritional strategies to improve the colour of gonads have taken us to the last study of this PhD project.

Chapter IX aimed to produce gonads with acceptable colour with the incorporation of different sources of β -carotene (a synthetic and a natural – paprika – source) at low and high incorporation levels: 100 and 250 mg β -carotene kg⁻¹ feed, respectively (Chapter VIII). The inclusion of either a synthetic or a natural source of β -carotene to a mix of ingredients prior to extrusion resulted in a high β -carotene degradation rate (69.5-94.3%) in the final extruded diet. Carotenoids are sensitive organic compounds (Jintasataporn *et al.*, 2012) that can be prone to damage during feed manufacturing process involving water, high temperature, and mechanical stresses, all of which can affect the carotenoid stability in the feed (Camire *et al.*, 1990; Rodriguez-Amaya, 1999). The present results showed that the synthetic β -carotene was better preserved than that from paprika, during the processing and extrusion of the pellets, probably due to its prior microencapsulation. The production of fresh diets is a strong limitation to equinoculture development, highlighting the need for selecting strategies like pigment protection, prior feed processing, and/or the selection of softer extrusion techniques to produce commercially extruded diets for sea urchin. Nonetheless, in the last chapter of this thesis, synthetic or natural pigments did not affect sea urchin's growth or pigment content in the gonads. Indeed, the gonads' sensory profiling and pigment content did not differ among diets, however low levels of synthetic β -carotene were effective in promoting gonad colour enhancement. Sea urchin fed this diet showed significantly higher a^* and b^* than the control diet (without pigments incorporation), higher b^* than the diet with high levels of synthetic β -carotene in both males and females, and higher b^* than the diet with low levels of natural β -carotene only in males. Moreover, sea urchins fed the diet with low levels of synthetic β -carotene were the most accepted among consumers, both in males and females, and the males fed this diet also displayed a carotenoid content and gonad colour similar to the wild specimens. Female wild urchins had higher echinenone and β -carotene content, accompanied by higher redness, yellowness and firmness values than female urchins fed the diet with low levels of synthetic β -carotene. Nevertheless, both males and females fed this diet had similar acceptance rates among consumers comparing with premium wild sea urchin gonads, suggesting that the

incorporation of low levels of synthetic β -carotene could improve sea urchins' gonad colour and acceptance.

Appearance properties, mainly colour, are crucial for the acceptance and choice of consumers concerning seafood. Indeed, these visual impressions are usually the first visual sensory stimuli presented to a consumer, being key purchase determinants. This thesis clearly evidenced in the various studies (Chapter VI and VII) a great effect of sex in gonads colour and other sensory features: females were always more orange, appealing and pleasant, and with higher values of a^* , b^* and C^* , and lower values of L^* compared with the yellow, grainy and unappealing males. The fact that the diet with lower β -carotene content was the only one able to enhance the sea urchin colour, even without affecting the pigments incorporation in gonads, demonstrates that it is not just the pigments that influence sea urchins colour. The relationship between gonad coloration and the bioavailability, bioabsorption or bioconversion of the supplemented pigments in the gonad is not yet well-defined. In our study, the biochemical nature of the pigment content measured in the gonads failed to be correlated with enhanced redness (a^*) and yellowness (b^*), suggesting that visual perception of colour may be influenced by other sex-dependent nutritional or metabolic factors. In addition, differences in pigment metabolism and sensory quality between sexes are notorious, even when being fed with identical diets. This suggests that there are sex-specific pathways for carotenoid metabolism (Robinson *et al.*, 2002) and other metabolic and genetic factors affecting the sensory quality of these animals. In the future, further studies should be carried out to clarify the mechanisms involved in the modulation of gonad colour through dietary supplementation in order to assure the production of desirable gonads in both sexes.

Conclusions and final considerations

From a sensory point of view, sea urchin is still an under-exploited gourmet product. This thesis provides a significant contribution to the study of this product category, providing new insights into consumers' associations, perceptions and habits. It was found that consumers appreciate sea urchins for their emotional and sensory value and that there is an opportunity to promote the valorisation of sensory and emotional properties of this product, while keeping their recognised health and wellbeing value. In this context, we covered important topics such as the harvesting period, consumers' conceptualisations - useful for the construction of marketing strategies - product preparation, or even emotional and sensory drivers of liking. This work does not seek to impose a holistic "one-size-fits-all" idea and there is flexibility to use different methodologies, always depending on the response speed level required by the market and by researchers and also by the product and panel typology. In fact, a trained panel should be used for sensory quality control and naïve consumers to determine the real consumers' demands regarding a specific product. Nowadays, in the latter case, it is desirable to use a natural environment study in order to obtain a genuine answer. It is also important to mention that this opportunity to develop and apply a variety of relevant sensory analysis methodologies was fundamental for the growth of the student as well as for the company Sense Test, emphasising the relevance of a PhD in an industrial setting.

This thesis also provides a significant contribution to the ongoing development of a *P. lividus* suitable diet. It clearly proposes a diet able to significantly increase gonad size and, more widely, it will contribute to overcome the supply bottlenecks currently preventing a more extensive utilisation of these echinoids. Moreover, it provides an insight into the use of dietary pigments to enhance gonadal colour in *P. lividus*, highlighting the need for a specific dietary supplement able to enhance the production of desirable gonads colour in both sexes. It also evidences the importance of pigment protection prior to feed processing to produce commercially extruded diets for sea urchin. This work will act as a benchmark, providing crucial information for the future selection of the best nutritional add-ons, able to have a positive impact on the final quality of farmed sea urchin, and ultimately resulting in the production of premium sea urchin gonads highly accepted by consumers.

This work also opens new interesting research lines into the development of tailor-made diets that were not further explored during this PhD thesis due to time restrictions. For instance, it would have also been interesting to investigate the effects of different environmental cues on sea urchin growth and quality and their complicated interactions with

nutrition, or even deeply explore the impact of aminoacids and fatty acids in the nutritional and organoleptic quality of this gourmet product.

Many questions still remain unanswered, but by raising new scientific questions we can foster and open new research lines, promoting continuous growth of sensory analysis, consumer science and animal nutrition. Moreover, the continued effort put into studying new aquaculture species, especially those occupying lower trophic levels, will help to significantly improve the overall economic and environmental sustainability of the food industry.

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