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Sea urchin (*Paracentrotus lividus*) gut biomass as a co-product with antioxidant and antibacterial potential to supplement aquafeeds

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Abstract – Sea urchin processing practices by the canning industry result in a large volume of waste due to a high fraction of inedible parts, in special the gut, that can still be a valuable source of bioactive compounds. Therefore, this work aimed to thoroughly characterise gut biomass from *Paracentrotus lividus* evaluating its bioactive potential. The gut biomass was evaluated in terms of bioactive capacity, and biochemical composition in both males and females. Although no statistical differences were found between sexes in any of these parameters, this study provided a proof of concept on the potential of sea urchin's gut, obtained as a co-product in the sea urchin industry, for feed supplementation. The gut biomass exhibited a high antioxidant capacity ($IC_{50} \approx 0.5–1.0$ mg_{DW} mL^{-1} in four different assays) and bactericidal activity ($IC_{50} < 1.0$ mg_{DW} mL^{-1} against *Vibrio parahaemolyticus*, *Edwardsiella tarda*, and *Tenacibaculum maritimum*). The gut co-product can also be used as a source of phenolic compounds, carotenoids, and PUFAs, with contents of 4.6 ± 0.4, 2.4 ± 0.8, and 93.0 ± 3.1 mg g_{DW}^{-1}, respectively. Overall, the sea urchin's gut seems a valuable product with a remarkable potential for use in aquafeeds as a source of bioactive compounds.

Keywords: Waste valorisation / bioactive capacity / phenolic compounds / carotenoids / PUFAs

1 Introduction

The global market demand for sea urchins is estimated at 60 000–70 000 tonnes per year; most of them are consumed in traditional markets, with Japan accounting for ca. 90% of global demand, followed by France and Korea (Stefánsson *et al.*, 2017). The most popular sea urchin is *Loxechinus albus*, harvested in Chile, which accounts for roughly half of all global landings. The other major global harvesters are Russia, Japan, the United States, and Canada (Stefánsson et al., 2017). In Europe, the two most common and abundant species are *Paracentrotus lividus* and *Strongylocentrotus droebachiensis.* More specifically in Portugal, commercial harvesting of *P. lividus* was about 160 tonnes in 2019, mainly to be exported to Spain (FAO, 2022).

On the other hand, sea urchin harvesting has gained public interest due to concerns about species extinction, highly driven by illegal fishing and rising sea temperatures (Zila et al., 2023). *Paracentrotus lividus* plays a structural role as a herbivore in Mediterranean ecosystems, therefore, when this region suffers from overfishing it is expected losses in macroalgal communities and biodiversity (Farina et al., 2020). A potential

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solution to mitigate overgrazing in overfished regions comes with efficient management and control of the harvesting. However, unregulated direct extraction can lead to population collapses and ecological impacts (Farina et al., 2020). Integrated management strategies have been developed in various regions to address this situation, relying on knowledge of sea urchin biology and population dynamics (Farina et al., 2020, Zila et al., 2023). Sustainable sea urchin fisheries with size and timing regulations have been implemented in countries like New Zealand and Italy. Additionally, aquaculture studies have shown promise, offering opportunities for a steady fish supply and stable prices while supporting conservation efforts against illegal fishing and harmful harvesting methods (Zila et al., 2023).

Sea urchin gonads are a highly valued delicacy worldwide, thus making their trade quite profitable. As a result, several sea urchin species have been overexploited, consequently compromising wild stocks, and favouring population fragmentation (Baião et al., 2022). Furthermore, gonads are the only edible part of the animal and yet they are a mere fraction of 5–30% of the whole sea urchin, therefore, most of the biomass ends up as waste (Marzorati et al., 2021). As happens with current seafood processing, the sea urchins' industry leads to the accumulation of a considerable amount of waste products – especially shells, spines, and viscera. Nevertheless, such wastes are still rich in several high-value components that still lack proper management. In addition, incorrect waste disposal has been directly damaging the environment, and consequently human health (Yadav et al., 2019). A representative example of an interesting by-product from sea urchin processing is collagen, which is extracted from their shells without resorting to damaging procedures, so its structural integrity and associated mechanical performance are preserved. This procedure is an important factor in biomaterial design because the collagen found in human tissues is extremely similar to that found in marine collagen (Zilia et al., 2021). For instance, Ferrario et al. (2020) suggested sea urchin shells as a source of fibrillar glycosaminoglycan-rich collagen to produce bilayer collagen-based skin-like scaffolds. Marzorati et al. (2021) have further observed the presence of antioxidant compounds (spironochromes) in the supercritical CO_2 extract of *P. lividus* shells. Recent studies also have identified bioactive compounds in the sea urchin gut, such as fatty acids (Anedda et al., 2021) – which may constitute a suitable supplement in fish feed, since juvenile marine fish generally require ca. 0.5–1.0% of dry weight of their diet be accounted for by highly unsaturated fatty acids. However, for this a thorough assessment of gut biomass as feedstock urges more detailed studies.

In terms of bioactive compounds found in the gut of sea urchins, three groups stand out for their recognized bioactive potential, namely carotenoids, phenolic compounds, and polyunsaturated fatty acids (PUFAs).

Carotenoids have been shown to boost antioxidative status and immune system function in farmed fish, thus resulting in disease resistance, improved growth performance, higher survival, and improved egg quality without cytotoxicity or side effects (Nakano and Wiegertjes, 2020). Therefore, such compounds are widely recognised as crucial supplements in aquaculture feeds. The antioxidant and provitamin A activities of β-carotene are particularly noteworthy (Ponce-Palafox et al., 2006). Furthermore, marine creatures cannot synthesize carotenoids on their own, so the provision of these components in their diet appears critical (Galasso et al., 2017). As a result of the rising demand for aquaculture, β-carotene (especially from natural sources) has become preeminent.

Moreover, during the past decade, polyphenols and polyphenol-rich additives have been studied as functional feed additives in aquaculture. Such polyphenolic substances as flavonoids, phenolic acids, lignans, and stilbenes have indeed been shown to improve fish's overall performance and immunity – thus enhancing the health and productivity of fish farms (Ahmadifar et al., 2021).

In terms of PUFAs, fish, like all vertebrates, require a dietary source of PUFAs because they cannot be produced *de novo*. Inadequate n-3 long-chain (C20) PUFAs compromise fish health and growth, entailing caution when selecting lipid sources for aquafeed formulations (Alhazzaa et al., 2018). These compounds play important structural roles as constituents of phospholipids, which are the major components of cellular biomembranes, and confer various functional properties by influencing both the physicochemical properties of the membrane and the functions of membrane proteins (Tocher, 2015). The majority of marine and some freshwater fish species require n-3 PUFA, particularly EPA and DHA, with requirements ranging from 0.5 to 2% of their diet (Alhazzaa et al., 2018).

Nonetheless, the use of seafood waste as a major component of feed is yet to be fully accepted by fish farmers – chiefly due to their wide variation in nutrient profile, seasonality, and geographic distribution (Uyeh et al., 2021). Another important parameter is diet regime; tailored artificial diets were shown as effective in promoting *P. lividus'* gonad size (Baião et al., 2022). A few studies have indeed tackled the effects of different diets on gonad quality and quantity, yet information on the effect on gut composition is scarce.

This work aimed to evaluate the potential of gut biomass from *P. lividus* as a possible supply of functional ingredients for feeds whilst promoting a circular bioeconomy targeting zero waste. To achieve this goal, the nutritional value, bioactive capacity (antioxidant and antibacterial), and biochemical composition (total phenols, carotenoids profile and polyunsaturated profile) of the obtained biomass were determined.

2 Material and methods

2.1 Growth trial

Adult purple sea urchins (*P. lividus*) were collected on the intertidal shores of Viana do Castelo, Portugal and transported to the Research facilities (Murtosa, Aveiro, Portugal). After an acclimation period of 2 weeks, two homogeneous groups of 14 sea urchins (initial body weight of 56.40 ± 13.47 g) were randomly distributed inside plastic mesh cages ($25 \times 30 \times 15$ cm) placed in 250 L tanks in a saltwater recirculating aquaculture system (4 L min^{-1}), under the following conditions: 15.4 ± 0.7 °C, salinity at 33.4 ± 0.3‰, and 10 h:14 h light:dark photoperiod. The sea urchins were fed until apparent satiation according to Baião et al. (2019) with a diet formulated based on

previous studies (Baião et al., 2022) supplemented with *Dunaliella salina* (Nutrally, Spain) at 1.5%. After 8 weeks, sea urchins were fasted for 48 h before sampling.

2.2 Gut recovery

After 8 weeks of the experimental trial, sea urchins were individually weighed and sampled for gut removal. The animals were split into pools of 10 animals, according to sex, in a total of two pools per sex ($n = 2$). The gut was carefully separated from the gonads, snap-frozen in liquid nitrogen, and stored at −80 °C until further processing. Samples were then duly freeze-dried and kept under darkness in a desiccator, at room temperature until further analysis.

2.3 Nutritional characterisation

Dry matter was evaluated by subtracting the weight after drying from the fresh weight. Total protein, lipid, ash, and carbohydrate were assessed, in duplicate, in dried samples using Official Methods of Analysis of AOAC international methods: Dumas' assay, based on 990.03 AOAC for proteins; chloroform-methanol extraction method, based on 983.23 AOAC for lipids; combustion of organic matter based on 942.05 AOAC method for ash and carbohydrates content estimated as the difference from the other components (i.e., Carbohydrates = 100% − Proteins % − Fat % − Ash %).

2.4 Biochemical composition

2.4.1 Total phenolic content

The total soluble phenolic content of the gut of *P. lividus* was determined (in analytical triplicates) using the Folin-Ciocalteu method as adapted by Magalhães et al. (2010), using gallic acid as a standard. In summary, using a 96-well plate, 25 µL of the sample was mixed with 125 µL of H_2O and 25 µL of Folin-Ciocalteu reagent; the mixture was incubated for 5 min in the dark. Then, 75 µL of Na_2CO_3 (0.63 M) was added, and absorbance read at 760 nm after 90 min in the dark. The results were expressed as $mg_{GAE}\ g_{DW}^{1}$ (milligram of gallic acid equivalent per gram of dry weight).

2.4.2 Carotenoid profile

Identification and quantification of carotenoids in the gut of *P. lividus* were performed via high-performance liquid chromatography (HPLC), using a Waters Alliance liquid chromatograph consisting of a Model e2695XC Separation Module (gradient pump and autosampler) and a Model 2998 photodiode-array (PDA) detector (Waters, USA). The HPLC instrument was operated by Empower 3 software (Waters). Samples were prepared using the acetonic extract from the bioactive potential, with the addition of an internal standard (*trans*-β-8-Apo-8′-carotenal; 170 µg mL^{-1}). Samples were dried and resuspended in 200 µL of acetone:ethyl acetate (9:1) (Guedes et al., 2011). The stationary phase was constituted by a 4 × 250 mm Purospher Star RP-18e (5 µm) column (Merck), and the mobile phase by ethyl acetate and acetonitrile:water (9:1). The sample was eluted over 55 min, under a flow rate of 1 mL min^{-1}, at 25 °C ± 2 °C (column heater (Waters, USA)). Spectral data from all peaks were collected in the range of 250 to 750 nm. The compounds were identified by comparing retention times and UV-visible spectrum with chromatographic standards (HPLC grade); these were purchased from Extrasynthese (lutein, zeaxanthin, and β-carotene) and Sigma (echinenone). The results were expressed in milligrams per gram of dry weight (mg g_{DW}^{-1}).

2.4.3 Polyunsaturated fatty acids (PUFAs) profile

Dried biomass (150 mg) was transmethylated *in situ* with methanol:dichloromethane:hydrochloric acid (10:1:1, v/v/v) and *n*-hexane: dichloromethane (4:1, v/v) with BHT (5 mg mL^{-1}), as described elsewhere (Parrish et al., 2015) with modifications. Tricosanoic acid (C23:0) was used as an internal standard and added before transmethylation. Fatty acid methyl esters were resuspended in 1.5 mL of hexane before gas chromatography (GC-FID) analysis. Gas chromatographic analysis was performed in a Shimadzu Nexis GC-2030 gas chromatograph (Kyoto, Japan), equipped with an FID and a Shimadzu AOC-20i auto-injector. The separation was carried out on an OmegaWax 250 capillary column (L × I.D. 30 m × 0.25 mm, df 0.25 µm). Operating conditions were as follows: split mode ratio of 1:50; injection volume of 1 µL; and temperature of injector and detector of 250 and 280 °C, respectively. A flow rate of 25 mL min^{-1} of helium was used as a carrier gas and 40 mL min^{-1} of hydrogen and 400 mL min^{-1} of air for the detector. The column thermal gradient was as follows: initial temperature of 50 °C for 2 min, which was increased at 50 °C min^{-1} to 174 °C, held for 14 min, then increased at 2 °C min^{-1} to 210 °C and held for 50 min. Fatty acid methyl esters were identified by comparison with a known standard mixture (Sigma 47,885-U Supelco 37 Component FAME Mix, USA), and quantified using the software GC solution for GC systems (Shimadzu).

2.5 Bioactive potential

For screening of bioactivity, 100 mg of dry gut biomass from *P. lividus* was homogenised with 5 mL acetone in a Precellys Homogenizer (Bertin, France) (6 series of 8000 rpm, for 30 s with 45 s of pause). The solution was centrifuged at 2000 × g for 10 min, and the supernatant was dried in a rotavapor. For the antioxidant capacity evaluation, the supernatant was resuspended in 10% dimethyl sulfoxide (DMSO) at 10 mg mL^{-1}, and further diluted for each assay. For antioxidant scavenging assays and antibacterial assays, the concentration able to inhibit 50% of activity (IC_{50}) was calculated with GraphPad Prism software (version 8.0), through curve spline interpolation. The FRAP value is calculated from the ferrous ion (Fe^{2+}) equivalent and expressed as mmol Fe^{2+} g^{-1}. All experiments were run in triplicate. Solvent and sample absorption were subtracted from the calculation to avoid wavelength overlap.

2.5.1 Antioxidant capacity

The antioxidant capacity of the gut was accessed by $ABTS^{\bullet+}$, $DPPH^{\bullet}$, nitric oxide ($^{\bullet}NO$) and superoxide ($O_2^{\bullet-}$) scavenging assays, and the ferric-reducing ability of plasma (FRAP).

$ABTS^{\bullet+}$

The $ABTS^{\bullet+}$ scavenging capacity was calculated in a 96-well plate. In summary, 60 µL of the sample was mixed with 180 µL of ABTS radical solution (7.46 mM ABTS + 2.44 mM $K_2S_2O_8$; further adjusted to absorbance within 0.680–0.720 at 734 nm), the mixture was left in the dark for 6 min, and absorbance read at 734 nm (Granados-Guzman et al., 2017).

$DPPH^{\bullet}$

The $DPPH^{\bullet}$ scavenging capacity was calculated in a 96-well plate. In summary, 60 µL of the sample was mixed with 180 µL of DPPH (0.15 mM in methanol; further adjusted to absorbance within 0.800–0.900 at 515 nm), the mixture was left in the dark for 30 min, and absorbance read at 515 nm (Bobo-García et al., 2015).

$O_2^{\bullet-}$

The $O_2^{\bullet-}$ scavenging capacity was calculated in a 96-well plate. In summary, 50 µL of the sample was mixed with 50 µL of NADH (166 µM), 150 µL of NBT (43 µM), and 50 µL of PMS (2.7µM). The mixture was left in the dark for 5 min, and absorbance was read at 560 nm (Pinho et al., 2011).

$^{\bullet}NO$

The $^{\bullet}NO$ scavenging capacity was calculated in a 96-well plate. In summary, 100 µL of sample was added to 100 µL of SNP (20 mM) and incubated for 60 min under 20 $\mu mol_{photons}\, m^{-2}\, s^{-1}$ of fluorescent light. Then, 100 µL of Griess reagent (1.0% sulphanilamide and 0.1% N-(1)-naphthylethylenediamine in 2% phosphoric acid) was added to the solution, followed by incubation for 10 min in the dark; absorbance was finally read at 562 nm (Pinho et al., 2011).

FRAP

The FRAP value was calculated in a 96-well plate reaction. In summary, 20 µL of the sample was added to 200 µL of FRAP solution (composed of acetate buffer (300 mM, pH 3.6), TPTZ (10 mM) and $FeCl_3$ (20 mM) at a ratio of 10:1:1). The mixture was incubated at 37 °C for 10 min, and absorbance was read at 593 nm (Benzie and Strain, 1996). A standard curve was calculated using $FeSO_4$ (within the range of 0.1–2.0 mM).

2.6 Antibacterial potential

The *in vitro* bactericidal capacity of *P. lividus* gut was tested against a selection of the most relevant pathogenic bacteria for aquacultures species (i.e., *Vibrio anguillarum*, *V. harveyi*, *V. parahaemolyticus*, *Aeromonas hydrophila*, *Yersinia ruckeri*, *Edwardsiella tarda*, *Photobacterium damselae* subsp. *piscicida* – Phdp and *Tenacibaculum maritimum*).

All bacteria were cultured for 24 or 48 h at 25 °C, with tryptic soy agar (TSA; Condalab, Spain) – or, in the case of *T. maritimum*, with marine agar (MA; Condalab, Spain); and then inoculated in the corresponding broth prior to assay.

The assay was performed on 96-well plates. In summary, 20 µL of the sample was incubated with shaking for 150 min at 25 °C, with 20 µL of each bacterial suspension (with an optical density within 0.200–0.300 at 600 nm). Then, 25 µL of Thiazolyl Blue Tetrazolium Bromide (Sigma-Aldrich) at 1 $mg\, mL^{-1}$ was added, and the mixture was incubated for 10 min at 25 °C with shaking. Finally, 200 µL of DMSO (Sigma-Aldrich) was added, and absorbance was read at 560 nm (Stevens et al., 1991). The bactericidal activity was calculated as the percentage of non-viable bacteria and concentration able to inhibit 50% bacterial viability. For positive control, 10% DMSO was used instead of gut samples, whereas 10% DMSO was used instead of bacterial suspension for negative control.

2.7 Statistical analysis

All extractions and analyses were performed in analytical triplicates (pseudoreplicates). Data were analysed by comparing sexes individually, considering the two cultivated pools ($n = 2$). Mann-Whitney tests were performed to check differences between groups in all evaluated parameters, using GraphPad Prism v.8 software (GraphPad, USA).

3 Results

The potential of gut biomass from *P. lividus* as a possible source of nutritional or bioactive compounds for feed was evaluated. Results regarding Mann-Whitney tests indicate that no statistical differences were found between sexes ($p > 0.05$) for the evaluated parameters, so, the four pools were considered as a single group, to evaluate the potential of sea urchin gut biomass ($n = 4$).

3.3.1 Nutritional content and biochemical composition

The dry matter, total protein, lipid, carbohydrate, and ash content of freeze-dried gut biomass was evaluated. Protein is the main component of gut biomass, with an average of $42.9 \pm 3.0\%_{DW}$. Carbohydrates and lipids come second, with an average of $24.6 \pm 8.2\%_{DW}$ and $22.2 \pm 6.0\%_{DW}$, respectively. Finally, the ash content averaged $10.3 \pm 0.9\%_{DW}$. Dry matter represented $21.5 \pm 3.0\%$ of wet weight.

When it comes to the bioactive compounds – PUFAs, carotenoids and phenolic compounds – the sea urchin gut biomass showed a content of 93.0 ± 3.1, 2.4 ± 0.8, and, 4.6 ± 0.4 mg g_{DW}^{-1}, respectively.

In terms of profile, a huge amount of total PUFAs was observed (Tab. 1), representing about 40% of total lipids. The main PUFA was linoleic acid (C18:2 n-6), representing ca. 46% of the total PUFAs. Other high-content PUFAs were

Table 1. Polyunsaturated fatty acid profile and quantification in gut from *P. lividus* (average ± standard deviation, $n = 4$).

Fatty acid	Content (mg g_{DW}^{-1})
C16:2 n-4	0.68 ± 0.03
C16.3 n-4	0.19 ± 0.01
C16:4 n-1	0.60 ± 0.04
cis C18:2 n-6	42.34 ± 2.08
trans C18:2 n-6	0.11 ± 0.01
C18:3 n-6	0.46 ± 0.03
C18:3 n-3	21.76 ± 1.22
C18:4 n-3	0.88 ± 0.05
C20:2 n-6	4.98 ± 0.61
C20:3 n-6	1.92 ± 0.31
C20:4 n-6	8.99 ± 0.67
C20:3 n-3	1.76 ± 0.17
C20:4 n-3	0.64 ± 0.02
C20:5 n-3	6.14 ± 0.35
C22:2 n-6	0.34 ± 0.04
C22:5 n-3	0.17 ± 0.02
C22:6 n-3	1.66 ± 0.14
Σ ω3	32.55 ± 0.89
Σ ω6	58.99 ± 2.22
Σ EPA+DHA	7.64 ± 0.53
Σ PUFA	93.01 ± 3.06

Table 2. Carotenoids' profile and quantification of gut from *P. lividus* (average ± standard deviation, $n = 3$).

Carotenoid (mg g_{DW}^{-1})	Content (mg g_{DW}^{-1})
Lutein	0.38 ± 0.03
Zeaxanthin	0.18 ± 0.01
Echinenone	0.12 ± 0.01
α-carotene	0.25 ± 0.02
Trans-β-carotene	0.51 ± 0.04
Cis-β-carotene	0.15 ± 0.01
β-carotene derivatives	0.82 ± 0.05
Σ β-carotene	0.66 ± 0.03
Σ Identified carotenoids	2.42 ± 0.14

α-linolenic acid (C18:3 n-3) and arachidonic acid (C20:4 n-6), with averages of 21.76 ± 1.21 and 8.99 ± 0.67 mg g_{DW}^{-1}, respectively. Interestingly, major concentrations of eicosapentaenoic acid (EPA; C20:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3) were observed (6.19 ± 0.36 and 1.66 ± 0.14 mg g_{DW}^{-1}, respectively).

In terms of the carotenoid profile (Tab. 2), results showed that the gut biomass is mainly composed of β-carotene and its derivatives (ca. 60%). The average ratio between *trans*- and *cis*-β-carotene was 3.4. Moreover, a significant amount of known antioxidant carotenoids, such as lutein and α-carotene, and β-carotene was observed (0.38 ± 0.03, 0.25 ± 0.02 and 0.66 ± 0.03 mg g_{DW}^{-1}, respectively).

3.2 Bioactive potential

3.2.1 Antioxidant capacity

The gut biomass is a complex matrix constituted by multiple compounds. As the dominant antioxidant mechanism can vary, such evaluation should consider the various types of compounds. The antioxidant capacity of sea urchin gut biomass was accordingly determined via five alternative methods, thus allowing for a better understanding of the potential bioactivity of the biomass under scrutiny (Fig. 1). The results showed that gut biomass exhibits an antioxidant capacity for all assays, both non-specific ($ABTS^{\bullet+}$ and $DPPH^{\bullet}$ assays) and specific ($^{\bullet}NO$, and $O_2^{\bullet-}$ assays, radicals present in animals), with low IC_{50} values (within 0.5–1.0 mg_{DW} mL^{-1}), and an average FRAP value of 0.23 ± 0.01 μmol Fe^{2+} mg^{-1}.

3.2.2 Antibacterial capacity

To ascertain the antibacterial capacity of the sea urchin gut, eight common bacterial pathogens in farmed aquatic animals were used: *V. anguillarum*, *V. harveyi*, *V. parahaemolyticus*, *A. hydrophila*, *Y. ruckeri*, *E. tarda*, Phdp, and *T. maritimum*. The results are expressed in IC_{50}, in Figure 2.

The results indicate that sea urchin gut possesses antibacterial capacity against seven of the eight pathogens assessed; in particular, against *V. parahaemolyticus*, *E. tarda* and *T. maritimumares* (IC_{50} of ca. 0.5-1.0 mg_{DW} mL^{-1}); followed by *V. harveyi*, with sea urchin gut had an average IC_{50} of 2.9 ± 1.11 mg_{DW} mL^{-1}. A less evident antibacterial capacity was found against *A. hydrophila* and Phdp; since in these cases, the concentration of sea urchin gut necessary to inhibit 50% bacteria would be superior to 10 mg mL^{-1}.

4 Discussion

An important indication of the feasibility of the real application of gut biomass from gonad processing is the evaluation of its productivity. Toward that goal, both gut:gonad and gut:body weight ratios were determined for each sea urchin ($n = 28$); on average, 173 mg of gut for each gram of gonad produced will be obtained as "waste" and further processed to a new product; on a more global perspective, 28.2 mg of the gut can be obtained for each gram of sea urchin produced. Considering the demand for 60 000 tonnes of sea urchins worldwide (Stefánsson et al., 2017), it would be feasible to reuse ca. 1700 tonnes of sea urchin gut every year. In Portugal, particularly, this would mean re-using ca. 500 kg of sea urchin gut annually.

The results here presented show no differences between sexes in all the evaluated parameters, which is divergent from previous studies that show that some metabolic responses have a major difference, like carotenoid accumulation. Baião et al. (2022) evaluated the growth of *P. lividus* fed with two sources of β-carotene, synthetic and from paprika (at 100 and 250 mg β −carotene kg feed $^{-1}$). Even though diets had no impact on growth or pigment accumulation, both gonad acceptance and colour showed differences between the sexes. Female gonads showed higher acceptance and were redder, yellower, and less luminous than their male counterparts – thus implying that gonad colour is not solely modulated by dietary carotenoid

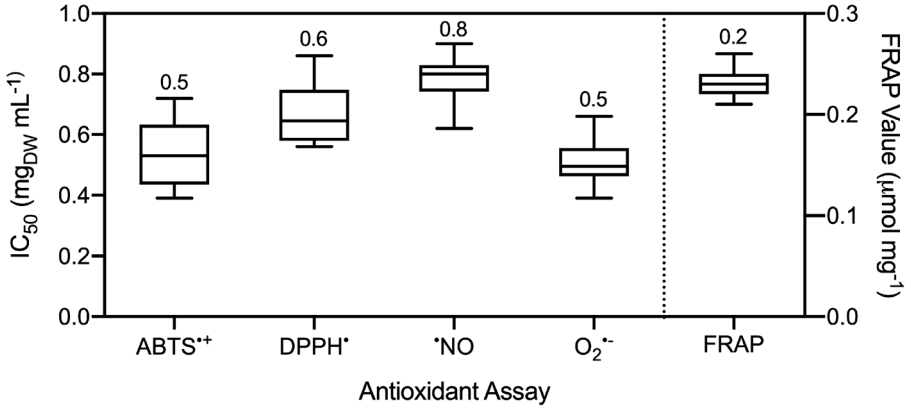

Fig. 1. Overall antioxidant capacity of gut from *P. lividus* ($n = 4$) as per different antioxidant assays. The left axis shows IC_{50} ($mg_{DW}\ mL^{-1}$) for $ABTS^{\bullet+}$, $DPPH^{\bullet}$, $^{\bullet}NO$, and $O_2^{\bullet-}$ assays, and the right axis shows FRAP value (μmol $Fe^{2+}\ mg^{-1}$) for FRAP assay. The horizontal line within the box and the number above the bar indicate the median, boundaries of the box indicate the 75th- and 25th-quartile, and whiskers indicate the highest and lowest results.

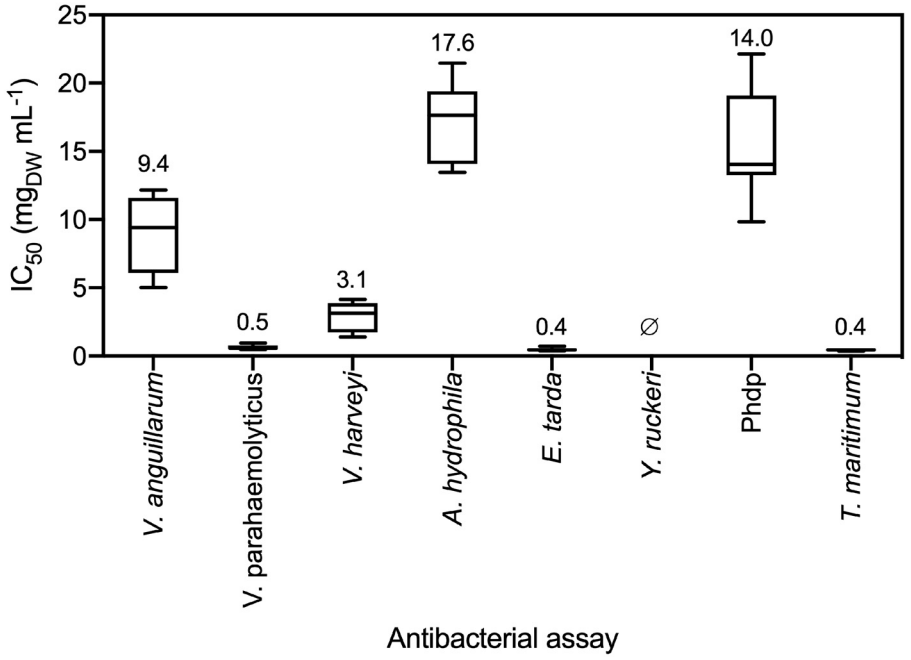

Fig. 2. Overall antibacterial capacity (IC_{50}) of gut from *P. lividus* ($n = 4$) against *Vibrio anguillarum*, *V. harveyi*, *V. parahaemolyticus*, *Aeromonas hydrophila*, *Yersinia ruckeri*, *Edwardsiella tarda*, *Photobacterium damselae* subsp. *piscicida* – Phdp, and *Tenacibaculum maritimum*. The horizontal line within the box and the number above the bar indicate the median, boundaries of the box indicate the 75th- and 25th-quartile, and whiskers indicate the highest and lowest results.

supplementation and may be influenced by other sex-dependent nutritional or metabolic factors.

Furthermore, the absence of statistical differences in the evaluated parameters regarding sex justifies an interesting utilisation of the consistent nutritional content of sea urchin gut as a waste by-product – for overcoming the concern of the feed industry regarding batch variation. However, the group variability here is still small, and a larger group should be evaluated, e.g., an annual assessment of wild specimens.

Regarding the content of bioactive compounds, the total phenolic compounds, as well as the carotenoid and PUFA profiles were determined; for said three groups of compounds, gut from *P. lividus* showed a high content (i.e., 4.6 ± 0.4, 2.4 ± 0.8, and $93.0 \pm 3.1\ mg\ g^{-1}$, respectively). As already mentioned, shells are also a major by-product of sea urchin processing – not only as a collagen source but also as a source of arachidonic acid (Salvatore et al., 2019) and carotenoids (Kuwahara et al., 2009). However, arachidonic acid and carotenoids are found at higher levels in such tissues as gonads, or even the gut.

Phenolic compounds in animal products are derived from polyphenol-rich feeds and pastures; for instance, milk itself

contains ca. 130 mg L^{-1}. Animal-derived phenolic compounds are essential because they are more bioavailable, yet their production hinges upon the animal gut microbiota (Hashem et al., 2020).

In the case of carotenoids, the content in *P. lividus* gut biomass can be 100-fold that found in its gonads – using the data by Lourenço et al. (2020) as a reference. However, when compared to such microalgae as *Dunaliella* spp., which carotenoid contents of about 100 mg g^{-1}, *P. lividus* gut appears as a poorer source thereof. In any case, the presence of a reasonable amount of carotenoids, in addition to the potential related to the other compounds evaluated and the bioactivities described in this work, indicates that, while not a primary supplement, gut biomass is a potential source of these compounds.

Finally, the gut from *P. lividus* showed a high content (ca. 10%$_{DW}$) of PUFAs; these compounds in general, and DHA, in particular, are critical nutrients for the growth of marine fishes, especially during their larval stages (Tocher, 2015). Because these species have a limited ability to biosynthesise PUFAs *de novo*, in special during the larvae stage, these organisms when fed diets lacking these lipids have poor growth and a high death rate, which can be mitigated by supplementing PUFAs in feed. Rocha et al. (2019a) have claimed dominance of PUFAs in the lipidic content of *P. lividus* gonads, mainly EPA and arachidonic acid, which is consistent with data obtained from the gut. Moreover, those authors observed a seasonal change in fatty acid content of the wild population, which is still a concern about feed feasibility. Moreover, Rocha et al. (2019b) evaluated the nutritional composition of sea urchin gonads throughout the year and concluded that sex has a major influence. The opposite was observed in the gut of *P. lividus*, where no statistical differences were found between sexes. On the other hand, Anedda et al. (2021) reported a small content of lipids in wild *P. lividus* gut (ca. 3%$_{DW}$), and a low variability during the year in their fatty acid profile. However, α-linolenic acid and arachidonic acid content followed a similar pattern to that found in this study, being two of the most abundant PUFAs.

In terms of bioactive capacity, the gut biomass showed effective antioxidant and antibacterial capacities, with effective doses between 0.5 and 1 mg_{DW} mL^{-1}. In recent years, novel antioxidants have provided an important topic in fish protection research. The evaluation of antioxidant capacity is critical toward valorisation of any type of biomass – since oxidations underly deleterious diseases, including inflammation. Moreover, antioxidant supplements have attracted the attention of the market in recent years, because of their ability to provide a better health state for farmed fishes. Moreover, as food demand rises, aquaculture production intensifies – thus exposing fish to higher oxidative stress conditions, such as high densities, frequent fish handling and sampling, and preventive or disease treatments that expose fish to different habitat conditions (Félix et al., 2021). Hence, a poor balance between formation of reactive oxygen species (ROS) and antioxidant defence system (so-called oxidative stress) in fish may cause several deleterious reactions, such as DNA hydroxylation, protein denaturation, lipid peroxidation, apoptosis, and eventually cell damage (as happens in other organisms) (Hoseinifar et al., 2020). Various approaches, such as administration of antioxidants supplements, were used to boost antioxidant defence capability.

The evaluation of antioxidant capacity should consider different mechanisms of action and groups of compounds. Oxidative metabolism leads to several radicals, namely $O_2^{\bullet-}$, and $^{\bullet}NO$. Therefore, the effect of the complexity of such a matrix as gut biomass, composed of several groups of molecules, has a great impact upon final antioxidant potential. While $ABTS^{\bullet+}$, $DPPH^{\bullet}$, $O_2^{\bullet-}$, and $^{\bullet}NO$ assays are based on scavenging capacity, FRAP assay is based on reduction of ferric to ferrous iron; the latter is widely used to measure antioxidant capacity of foods, beverages, and nutritional supplements. In this study, the gut biomass showed a positive antioxidant capacity in all assays; for comparison, the FRAP value showed an equivalence of ca. 200 mg of ascorbic acid per gram of gut biomass – while the scavenging assays led to ca. 100 mg of ascorbic acid per gram of gut biomass. Remember that the ascorbic acid evaluated is purified and used in the food industry at ca. 10 mg kg^{-1} (Benzie and Choi, 2014; Vidya et al., 2016), considering that, using gut biomass as a natural antioxidant would allow not only a more efficient antioxidant effect, but also a higher sustainable process.

Regarding antibacterial capacity, gut from *P. lividus* showed activity against *V. anguillarum*, *V. harveyi*, *A. hydrophila*, Phdp, but in special against *V. parahaemolyticus*, *E. tarda*, and *T. maritimum* – with effective doses below 1 mg_{DW} mL^{-1}.

Vibriosis is a systemic bacterial infection caused by bacteria from the Vibrionaceae family that affects numerous species of economically significant farmed marine shrimp and fishes worldwide. Antibiotics, to which most *Vibrio* spp. are usually susceptible, are the most used method to treat bacterial infections in aquaculture, apart from vaccination. As alternative, recent practices include the use of plant extracts rich in bioactive compounds, such as carotenoids, as immunostimulants host's defence mechanisms (Mohamad et al., 2019). Vibriosis also affects sea urchins (Sweet, 2020), so presence of such compounds may prevent *Vibrio* spp. infections. This further justifies use of gut biomass as supplement for fish feeds.

As proposed here, *P. lividus* gut can be employed as a natural antiseptic and antimicrobial agent for indirect protection of humans (IC_{50} of ca. 0.5–1.0 mg_{DW} mL^{-1}). A similar amount (0.5 g kg^{-1}) was suggested by Abdel-Tawwab et al. (2010) regarding supplementation with green tea (GT), *Camellia sinensis* L., for Nile Tilapia (*Oreochromis niloticus*) feeds. There was a beneficial effect, materialized as a large reduction of *A. hydrophila* infection and mortality rates when compared to control (20% versus 80% of control).

The antibacterial capacity of *P. lividus* was previously evaluated in terms of the perivisceral celomic fluid as ecological parameter. The effect of seasonality was also observed in this immune parameter of wild *P. lividus*. The perivisceral coelomic fluid showed a bactericidal capacity of 38–80% against Phdp inhibition, and 32–72% against *V. anguillarum*, the latter being dependent on maturation status of the gonad (Fernández-Boo et al., 2018).

Antibacterial capacity is one of the main bioactivities claimed in feed additives. As bacterial infections are a major cause of production losses, and the use of antibiotics has raised a greater concern by society, supplements bearing antibacterial

activity, or able to induce higher immunologic responses are valuable, the results discussed herein, suggest a great potential for the use of gut from *P. lividus* as antibacterial agents.

Overall, *P. lividus* gut showed a remarkable potential for use as a bioactive agent and source of bioactive compounds; for being a by-product from gonad processing, its valorisation represents a big step towards a more sustainable process. However, when targeting a specific product in bioprocesses, such as gut extract, downstream processing plays a crucial role in feasibility, as there are only a few sustainable and cost-efficient alternatives for biomass extraction, which still require process-specific optimization. Green chemistry-based bio-refining offers environmentally friendly alternatives to hazardous chemical processes for food waste treatment (Venugopal, 2022). Innovative green technologies, such as fermentation and novel extraction procedures and environmental-friendly solvents, provide advantages in preserving the quality and extraction efficiency of bioactive compounds from marine by-products (Venugopal, 2022). On the other hand, process optimization is predominantly conducted in controlled laboratory settings using small volumes (as in the present study), and scaling up the processes to larger demo and industrial scales remains a challenging task. Valuable large-scale processes are often protected by patents and trade secrets, making them scarce in published literature. To overcome this challenge, process modelling and simulation using software tools offer a practical approach to assessing the feasibility of processes beyond the laboratory scale. Several authors utilize process modelling as a valuable tool to conduct environmental assessments of production schemes through the Life Cycle Assessment (LCA) methodology. Although still rare, a few examples can be found for sea urchins, such as Zilia et al. (2020, 2023).

Moreover, other strategies for the sustainability of sea urchin processing have been proposed, namely the use of integrated multitrophic aquaculture (IMTA). Shpigel et al. (2018) studied a long term IMTA (460 days) with *Ulva lactuca* (seaweed), *P. lividus* and a fish pound, in which fishes attained marketable size by the end of the first year, while sea urchins showed 80% of gonads with a bright orange colour.

Conclusion

The potential of gut biomass from *P. lividus*, which is produced as a by-product during the processing of its gonads, has been successfully demonstrated. In the present study, the productivity of this resource was shown to be on average, equivalent to 17% of the gonad produced, which would otherwise be considered as "waste.", in addition, the absence of statistical differences between sexes in the evaluated parameters regarding the gut justifies the utilization of sea urchin gut as a waste by-product with consistent nutritional content, thereby addressing concerns in the feed industry regarding batch variation. This valuable resource contains significant amounts of antioxidants, antibacterial agents, phenolic compounds, carotenoids, and polyunsaturated fatty acids (PUFAs), with effective doses for bioactive potential ranging from 0.5 to 1 mg of dry weight per millilitre. Therefore, the gut of sea urchins is a valuable product that contributes to a more sustainable future for the aquaculture industry, and its valorisation as a by-product of gonad processing represents a meaningful step towards a more sustainable process.

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Authors contributions

FP and **ACG** conceived and designed research. **FP**, **IG**, **CT** and **TGT** conducted experimental trials. **BC**, **FXM**, **LMPV**, and **ACG** were responsible for supervision and funding acquirement. **FP** wrote the original version of the manuscript. All authors read, revised, and approved the final version of the manuscript.

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