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Evaluation of Purple sea urchins Paracentrotus lividus grazing on alien species Rugulopteryx okamurae.

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Abstract

Rugulopteryx okamurae is an invasive nonindigenous marine species (NIS) of macroalgae present in the benthic communities of the Strait of Gibraltar that have colonised quickly most of the coast of this strait triggering ecological, economic and social consequences. Sea urchins (*Paracentrotus lividus)* are generalist herbivore with potential to provide resistance to this NIS. The objectives of this study were to assess if the generalist herbivore sea urchin consumes the invasive macroalgae *R. okamurae* under laboratory conditions, to known the grazing preferences when this NIS macroalgae is offered together with the native species *Ulva ohnoi*, as well as to find biochemical tissue markers related with *R. okamurae* ingestion. In this study two experiments were carried out. In the first experiment sea urchins were evaluated in groups for two months, and afterwards, in a second experiment, the same specimens were used to analyse individual preferences, feeding rates and fatty acid profiles. Daily food consumption rates showed that sea urchins accepted the NIS seaweed *R. okamurae* as food under laboratory conditions and even increase its intake over a long exposure period. In the same way, we observed that elective values for non-native species improve when *P. lividus* was exposed to *R. okamurae* for a long time, also showing differences among individuals. Regarding the fatty acids profiles of tissues, our results shown that arachidonic acid discriminates intestines of sea urchins fed with *R. okamurae*, while the gonad fatty acid composition was not directly related with diet.

1. Introduction

Non-indigenous (NIS) marine macroalgae are considered one of the greatest threats to the native biodiversity itself and to the overall value of the ocean´s resource (Schaffelke and Hewitt 2007). They constitute a considerable proportion of NIS invasions in coastal habitats and globally represent approximately 10–40% of the total NIS (Schaffelke et al. 2006). Generally, the invasion of NIS macroalgae produces drastic effects on native communities in terms of the species' structure and diversity (Robert and Patrick 2006; Piazzi 2009). Macroalgae invasion decreases native macroalgae diversity (Piazzi et al. 2001; Hoffman et al. 2008), reduces the diversity of the indigenous fauna (Piazzi and Cinelli 2003) and they can even promote the settlement and establishment of NIS grazers (Hoffman et al. 2011).

In many cases, the success of NIS macroalgae is due to they are more competitive than native species owing to characteristics such as vegetative reproduction through propagules, production of toxic metabolites, a high dispersal potential, phenotypic plasticity and the fact that most are perennial species (Blanco et al. 2020). Biological invasions are also frequently associated to ecosystem disturbances such as the removal of native competitors and predators (Gaither et al. 2013). Thus, exotic species which are scarce in their natural habitats, sometimes thrive and become very abundant in recipient communities, often because of the lack of coevolved enemies (herbivores and pathogens) in the new habitats (Elton 1958; Gillett 1962). However, in cases were generalist herbivores populations are well conserved and are able to graze on exotic algae, they can be harmful and hinder the NIS macroalgae propagation (Cebrian et al. 2011; Neilson et al. 2018; Noè et al. 2018). The effects of herbivores species on NIS macroalgae

depend of some aspects like interspecific competition between NIS and native macroalgae (Noè et al. 2018) or the invasion history in the invaded area (Santamaría et al. 2022). In this sense, Santamaría et al. (2022) reported that native herbivores learn to consume the invader progressively (within less than a decade). Thus, at recent sites of invasion the herbivores ignore the alga, shifting to distinct preferences and high consumption rates at older sites (Santamaría et al. 2022).

Rugulopteryx okamurae is a NIS macroalga that is recently colonizing southwestern European coasts. It is usually widely distributed in the north western Pacific ocean (Hwang et al. 2009), but has been regularly reported on the Strait of Gibraltar coast (both shores) since it was first identified in this area in 2015 (Altamirano-Jeschke et al. 2016; El Aamrı et al. 2018). Its presence has been reported in all Andalusian's Mediterranean coast (Villanueva Ojeda 2022), reaching the highest invasion levels in the Strait of Gibraltar where it reaches coverage rates of over 85% in depths between 10 and 30 m (García-Gómez et al. 2021). This has caused changes in the structure of benthic communities and the regression of bioindicator species (i.e., *Paramuricea clavata* and *Astroides calycularis*) as well as others macroalgae (García-Gómez et al. 2020; García-Gómez et al. 2021; Sempere-Valverde et al. 2021). Indeed, García-Gómez et al. (2021) has observed the disappearance of sea urchin *Arbacia lixula* (Linnaeus, 1758) and *Paracentrotus lividus* (Lamarck, 1816)] from different shallow rocky bottoms following the establishment of *R. okamurae*.

The purple sea urchin *Paracentrotus lividus* is one the most common native generalist herbivores in the area invaded by *R. okamurae*. Grazing by sea urchins have a remarkable effect on some macroalgae assemblages such as in intertidal pools, kelp forest and seagrass meadows (Valentine and Heck 1991; Benedetti-Cecchi and Cinelli 1995; Leinaas and Christie 1996; Cardoso et al. 2020), playing a major role in the structure of macrophyte communities (Cardoso et al. 2020). However, these effects differ as a function of macroalgal species abundance and sea urchin feeding preference (Andrew and Underwood 1989). Therefore, feeding preference studies are essential to evaluate the potential of sea urchins to control invasive macroalgae. Recently, Cardoso et al. (2020) have provided evidence relating to *P. lividus* preferences under laboratory conditions. They prove that this species did not show a specific feeding preference for any of the different seaweeds offered (native and NIS). Nevertheless, several studies on feeding behaviour have demonstrated that consumption is non-random, and that some association exists between consumers and certain types of food (Chiantore et al. 2008; Monteiro et al. 2009; Cebrian et al. 2011).

Dietary biomarkers can provide essential insight to explore dietary preferences in nature. Many fatty acids (FA) have been used as dietary biomarkers (Dalsgaard et al. 2003; Kelly and Scheibling 2012; Schram et al. 2018). It is remarkable that dietary FA are often modified by the consumer, either by elongation, desaturation, de novo synthesis (González-Durán et al. 2008; Kabeya et al. 2017), or by selective store (Castell et al. 2004). Organism FA composition can also vary seasonally and spatially, complicating the use of FA as biomarkers (Martínez-Pita et al. 2010; Dethier et al. 2013). Despite all these factors, the FA profiles can be reliably associated with a variety of primary producers and consumers thus the use of FA

biomarkers is supported by a high volume of scientific research (Kelly and Scheibling 2012; Dethier et al. 2013; Schram et al. 2018).

Current research focuses on sea urchins cultured in captivity and fed with *R. okamurae*. Our main hypotheses were firstly, that these herbivores can feed on *R. okamurae* over a long period of time. Secondly, that *R. okamurae* consumption and selectivity, when these macroalgae are offered together with native species, present interindividual variability. This may lead, over time, to a selection of specimens adapted to *R. okamurae* consumption. Finally, that the use of some sea urchin fatty acid levels as dietary biomarkers associated to *R. okamurae* consumption is feasible. The result of this prolonged exposure of sea urchins to *R. okamurae* was evaluated in two experiments. In the first experiment, *R. okamurae* consumption and algal selectivity were evaluated in groups for two months. In the second one, lasting another two months, the same specimens subjected to the same diets were used to analyse individual feeding rates, feeding preferences and tissues lipid composition.

2. Material And Methods

2.1. Algae collection and acclimatation

Both algae used in this study (*Ulva ohnoi* and *R. okamurae*) were collected in their natural habitats every 2 weeks and transport to the experimental facilities in cooled container between February and July. *U. ohnoi* were collected in sea ponds in IFAPA El Toruño facilities (El Puerto de Santa María, Spain) (36°34’48”N; 6°12’33”W), and *R. okamurae* were collected in “Punta de las Azofeas” (Algeciras, Spain) (36°04’16”N; 5°25’45”W). Until algae were used to feed sea urchins, they were maintained in tanks (400 L) with a continuous water flow in a closed flow-through system. All water tanks were changed every week. A modified f/2 (Guillard 1975) medium with 1.8 mM nitrate and 0.1 mM phosphate were used to fertilise culture mediums. Tanks were illuminated (15–30 µmol photons m^{-2} s^{-1} on surface of the tank) with fluorescent tubes in a 8:16 h (light:darkness) photoperiod. Temperature, salinity and oxygen were daily monitored, and they oscillated in the ranges of [17–19] °C, [37–41] psu and [6–8] ppm, respectively. Algae were carefully cleaned with seawater and centrifuged before being used for feeding sea urchins. A sample of each batch of algae was freeze dried for biochemical analysis.

2.2. Sea urchins collection and acclimatation

Individual sea urchins (*P. lividus*) (130) were collected three weeks before the start of the experimental period in southern Spain (La Herradura, Granada) (36°43’20”N; 3°43’39”W). They were transported wrapped in wet towels in cooled container to the IFAPA Center El Toruño and maintained in tanks (400 L) until the beginning of the experimental procedures. During this period, animals were fed with *U. ohnoi*. No mortality or disease signs were observed in this period.

Acclimatation and experimental procedures were conducted in tanks (400 L) with 200% water renewal per hour. All tanks were connected to a multiple recirculation system (RAS) equipped with a cooling, mechanical filter, skimmer, ultraviolet lights and biofilter. Tanks were illuminated (1.5–1.7 µmol photons

m^{-2} s^{-1} on surface of the tank) with fluorescent tubes in a 8:16 h (light:darkness) photoperiod. Throughout sea urchins acclimatisation and during experimental period, temperature, salinity, oxygen, ammonia, nitrite and nitrate ranged between [18–20] ºC, [37–41] psu, [6–7] ppm, [0.00-0.04] mg L^{-1}, [0.02–0.05] mg L^{-1} and [0.64–1.64] mg L^{-1}, respectively.

2.3. Experimental design

Experiment 1

Sea urchin individuals were starved for 48 h before the experiment to standardized the initial hunger conditions. The experiment lasted for 58 days, between February 22 and April 13. The mean weight of sea urchins at the beginning of the experiment was 60.8 ± 1.21 g (mean ± SE). Three treatments were established: i) sea urchins fed *R. okamurae* (RO); ii) sea urchins fed *U. ohnoi* (U); and iii) sea urchins fed both macroalgae in the same amounts (RO + U). In order to test the three feeding treatments in triplicate, sea urchins were placed in 9 oyster culture baskets adapted for sea urchins of 13 cm depth, 36 cm diameter and 2 mm plastic-mesh pore (Fig. 1A,C). Thirteen sea urchins were randomly distributed in each basket, and recipients were allocated in three tanks (one replicated per tank, Fig. 2A). All baskets were randomly distributed in the tanks and their position rotated every week to avoid possible biases due to their location in the water mass (Fig. 2A). Total biomass per litre in tanks was 5.85–6.14 g/L.

Experiment 2

Once finished the first experiment, sea urchins were maintained in oyster baskets with the same algae used during the first experiment until the beginning of the second trial (for 4 weeks). The second experiment lasted for 67 days, between May 13 and July 19, and two experimental treatments (30 replicates by treatment) were set: i) sea urchins fed *R. okamurae* (RO); and ii) sea urchins fed *U. ohnoi* and *R. okamurae* in the same amounts (RO + U) (Fig. 2B). To carry out individual monitoring of sea urchins, oyster culture baskets were divided into four compartments (Fig. 1B). In each basket we place four sea urchins (one per compartment; two fed *R. okamurae* and the other two fed *U. ohnoi* and *R. okamurae*), and five baskets with sea urchins were allocated by tank (3 tanks and ten dietary treatment replicate by tank). All baskets were randomly distributed in the tanks and their position rotated every week to avoid possible biases due to their location in the water mass (Fig. 2B). Total biomass per litre in tanks was 2.6–3.1 g/L.

Sea urchins wet weight (WW) was determined every 2 week for Experiment 1 and every 4 weeks for experiment 2. Sea urchin specimens were weighed after 5 min of drying on absorbent paper. In both experiments, offered seaweeds were pre-weighed (wet weight; excess of water was blotted off with blotting paper), placed in each dietary replicate at the same time and allowed to be graze for 7 days. A week later, the remaining algae was weighed to evaluate biomass differences due to herbivory and a new pre-weighted algae was offered. For each dietary treatment, three control baskets (C) devoid of sea urchins were placed in tanks to correct for any variation of the macroalgal biomass due to degradation in

both experiments (Fig. 1C and Fig. 2). When average macroalgae loss in controls was equal or higher than 40%, this sampling was not considered to calculate macroalgae ingestion.

The Ivlev's index was used as a measure of electivity (E) for invasive algae species: $E = (d_i - a_i)/(d_i + a_i)$ where d_i = % of invasive algae in the gut content (use prevalence in diet), and a_i = % of invasive algae available in the diet (prevalence in the environment). Values of Ivlev's index (E) range from − 1.00 (complete avoidance) to + 1.00 (exclusive selection) and $E > 0$ means that food type is eaten more than its availability in the environment (selected).

2.4. Lipid analysis

At the end of Experiment 2 all Sea urchins were weighed, sacrificed in ice and dissected. Gonads and intestine were weighed and sampled, and both were kept at -80ºC until lipid analysis. Total lipids were extracted in chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant (Christie 2003). The organic solvent was evaporated under a stream of nitrogen and the lipid content determinate gravimetrically. For fatty acids (FA) analyses, total lipid extracts were subjected to acid-catalysed transmethylation overnight (16–20 h) at 50°C, using 1 ml of toluene and 2 ml of 1% sulphuric acid (v/v) in methanol. Fatty acids methyl esters (FAME) were separated and quantified by using a Shimadzu GC 2010-Plus gas chromatograph equipped with a flame ionization detector (280°C) and a fused silica capillary column SUPRAWAX-280 (15 m × 0.1 mm I.D.) (Teknokroma, San Cugat del Valles, Spain). Hydrogen was used as carrier gas and the initial oven temperature was 100°C for 0.5 min, followed by an increase at a rate of 20°C min^{-1} to a final temperature of 250°C for 8 min. Individual FAME were identified and quantified using external standard (Sigma-Aldrich).

2.5. Statistical Analyses

All data were presented as mean ± standard error of mean (SE). All data were checked for normality, as well as for homogeneity of variances and, when necessary, log or arcsin transformation was applied. When a normal distribution and/or homogeneity of the variances were not achieved, data were analysed using a Kruskal–Wallis test. Analyses of weight gain and daily food consumption were carried out using repeated-measures ANOVA, with dietary treatment, basket and tank as fixed factors, and the initial weight of sea urchins as covariable. The differences in sea urchins gonadosomatic index (GSI) and between gonad and intestinal fatty acids were tested using a One-way ANOVA test. The possible relationships between daily food consumption and GSI, or Ivlev's Index and ARA intestinal content were analysed using Pearson's or Spearman's correlation. Principal Component Analyses (PCAs) were carried out to show the relationship among diets according to the FA matrices for intestine and gonad. ANOVA logistic regression and regression analysis were performed using SPSS statistics version 22 software (IBM, Armonk, NY, USA). The multivariate analyses were carried out using the PRIMER v.6 plus PERMANOVA package (PRIMER-e, Auckland, New Zealand).

3. Results

3.1. Fatty acids composition of macroalga

The two macroalgae that were used as food (*R. okamurae* and *U. ohnoi*) had a distinct fatty acids composition (Table 1). *R. okamurae* was characterised by a higher content of myristic acid (MA, 14:0), stearic acid (SA, 18:0), vaccenic acid (VA, 18:1n-7), gadoleic acid (GA, 20:1n-11), linoleic acid (LA, 18:2n-6), arachidonic acid (ARA, 20:4n-6), adrenic acid (AA, 22:4n-6), eicosatetraenoic acid (ETA, 20:4n-3) and eicosapentanoic acid (EPA, 20:5n-3), while *U. ohnoi* was richer in palmitic acid (PA, 16:0) and palmitoleic acid (16:1n-7).

3.2. Experiment 1

Ingestion analysis

Analysis of ingestion showed differences between dietary treatments (repeated-measures ANOVA, F = 235.668, p = 0.0005) (Fig. 3). However, no differences in the total ingestion rates with factor “time” were found (repeated-measures ANOVA, F = 1.206, p = 0.3632). In general, sea urchins fed *U. ohnoi* (U) presented the highest ingestion values (1.9% ww day^{-1}), followed by group RO + U (1.6% ww day^{-1}) and finally by sea urchins fed only RO (0.6% ww day^{-1}). *R. okamurae* ingestion remained stable along the experimental period in both dietary treatments (RO and RO + U), while the ingestion of *U. ohnoi* decreased in both cases (RO + U and U) from 22 days after the experiment started (Fig. 3). Regarding to dietary preference, the Ivlev’s Electivity Index displayed a preference of *P. lividus* for *U. ohnoi* (E = -0.47 ± 0.21), with no significant changes throughout the experimental period (repeat-measures ANOVA, F = 2.834, p = 0.187).

Sea urchins development

In this trial, sea urchins growth was monitored for 58 days by measuring weight (W) using a longitudinal approach (Fig. 4). In general, a significant change in fresh weight was detected in sea urchins throughout the experimental period (repeated-measures ANOVA, F = 36.490, p = 0.0002). The repeated-measures ANOVA analysis identified a statistically significant interaction between diet and time (repeated-measures ANOVA, F = 27.057, p = 0.0003), indicating that weight change was different between experimental treatments. Indeed, animals fed *U. ohnoi* (U) and both algae (RO + U) increased weight throughout the experimental period (6.5% and 3.2% of their wet weight, respectively); while those fed only *R. okamurae* (RO) lost 1.1% of their wet weight. Survival was not statistically different between groups, and it was higher than 98% in all cases.

3.3. Experiment 2

Ingestion analysis

Analysis of ingestion showed significant differences in the total amounts of algae ingested between both dietary treatments (repeated-measures ANOVA, F = 12.709, p = 0.0014) and throughout the experimental period (repeated-measures ANOVA, F = 4.360, p = 0.0027) (Fig. 5). Moreover, an interaction between the

factors “diet” and “time” was detected (repeated-measures ANOVA, F = 3.485, p = 0.0103). The pair-wise tests of interaction reflected that differences for the total algae ingested were only significant for periods 40–49 (ANOVA, F = 5.679, P = 0.025), 49–57 (ANOVA, F = 19.028, P = 0.0002) and 57–67 (ANOVA, F = 8.253, P = 0.0080). In general, the ingestion of *R. okamurae* remained stable for both treatments (R and R + U) throughout the all experimental period, while *U. ohnoi* ingestion (for treatment R + U) increased towards the second part of the experiment (Fig. 5).

In general, the Ivlev’s Electivity Index displayed a slight preference of *P. lividus* for *U. ohnoi* (E = -0.15 ± 0.04), although with high inter-individual variability and without differences throughout the experimental periods (repeated-measures ANOVA, F = 0.583, P = 0.676). Interestingly, the analysis of relationships between amounts of *U. ohnoi* and *R. okamurae* ingested by each sea urchin specimen showed a weak positive correlation (r = 0.374, p = 0.045) (Supplementary file 1). Analysis of differences between individuals showed significant differences among them (repeated-measures ANOVA, F = 20.117, p = 0.0000). Sea urchins with references 55, 59, 25, 9, 15, 39 and 57 consume *R. okamurae* in proportion to its availability or preferred, whilst. The Ivlev’s Electivity Index of these specimens (E = 0.17) was significantly different than those of specimens with references 53, 51, 49 and 3 (E = -0.52).

Sea urchins development

In this trial, sea urchins wet weight (W) was monitored individually for 67 days. A longitudinal approach was performed to evaluate weight changes with time and related to dietary treatments. In general, a significant change in weight was detected throughout the experimental period (repeated-measures ANOVA, F = 6.675, p = 0.0026) (Fig. 6). The repeated-measures ANOVA analysis identified a statistically significant interaction between diet and time, indicating that weight change was different between experimental treatments (repeated-measures ANOVA, F = 7.339, p = 0.0015). Moreover, significant differences were detected between both experimental groups for estimated marginal means of weight (repeated-measures ANOVA, F = 8.388, p = 0.0060) and for all sampling evaluated (ANOVA, p < 0.05). No mortality was recorded during the all experimental period.

Regarding to gonadal development, the dietary treatment had a significant effect on gonadosomatic index (GSI), and sea urchins fed diet RO presented lower GSI (1.0 *vs.* 4.9, for treatment RO and RO + U respectively) (ANOVA, F = 42.64, p = 0.0000) (Fig. 7). Interestingly, when the relationship between GSI and algae ingestion was explored, a moderate but significant correlation between the total amounts of macroalgae ingested and GSI in both treatments was observed (Fig. 8).

Fatty acids analysis

Intestine and gonad fatty acids profile of sea urchins fed both dietary treatments are shown in Tables 2 and 3. The percentage of saturated fatty acids (SAT) was significantly lower in the intestine of sea urchins fed only *R. okamurae*, mainly due to the lower content of MA (14:0) and PA (16:0). The level of total monounsaturated fatty acids (MUFA) was similar in the intestine of both treatments, although percentage of VA (18:1n-7) and paullinic acid (PA, 20:1n-7) were lower for sea urchins fed only *R.*

okamurae, while the percentage of GA (20:1 n-11) and erucic acid (EA, 22:1n-9) were higher than RO + U treatment. Finally, the total content of polyunsaturated fatty acids (PUFA) in the intestine of sea urchins fed *R. okamurae* was higher than those fed both macroalgae, mainly due to the high content of ARA (20:4n-6), but also due to differences in eicosadienoic acid (20:2n-6) and AA (22:4n-6). However, the intestine of sea urchins fed both macroalgae (RO + U) present a higher percentage of n-3 PUFA, due to the higher content in all n-3 PUFA fatty acids (18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3).

In gonads, no differences were detected in SAT although we observe higher content of SA (18:0) and lower content of MA (14:0) in gonad of treatment RO. The percentage of total MUFA was higher in treatment RO due to the higher content in GA (20:1 n-11) and EA (22:1n-9). No differences were detected in total PUFA, although gonads of sea urchins fed *R. okamurae* present a higher content of n-6 PUFA, especially due to the high content in ARA, and lower content of n-3 PUFA, due to the lower content in almost all n-3 PUFA fatty acids (except for EPA and DHA).

Changes in intestinal and gonadal FA profiles were further explored by a PCA. Regarding intestinal fatty acids, two PCA components explained 91.4% of total variance (Fig. 9). Principal component 1 (86.1% total variance) correlated positively with ARA ($r = 0.878$, $p < 0.001$) and clearly separated sea urchins fed *R. okamurae* from those fed both algae. Principal component 2 (5.32% total variance) correlated positively with 20:2 D5,11 NMID ($r = 0.427$, $p < 0.025$) and EPA ($r = 0.613$, $p < 0.0025$), but axes were not correlated with any experimental condition. In gonad, PC1 and PC2 explained 88.7% of total variance (Fig. 10). PC1 (82.9% total variance) positively correlated with ARA ($r = 0.695$, $p < 0.001$) and separated samples by GSI. Regarding PC2 (5.8% total variance), this axis significantly correlated with EPA ($r = -0.500$, $p < 0.025$) and 16:0 ($r = 0.618$, $p < 0.001$), but separation was not related to any variable analysed.

4. Discussion

Results obtained in the present study indicate that adult purple sea urchin accepted *R. okamurae* as food under laboratory conditions. Values of daily ingestion were, in general, similar to reported by Cardoso et al (2020), Casal et al. (2021), McCarron et al. (2009) and Lourenço et al. (2020), but lower to those found by Castilla-Gavilán et al. (2019) and Grosso et al. (2021). In the first experiment (Experiment 1), we observed that the ingestion rates of sea urchins fed only *R. okamurae* (RO) were low (0.6% ww day^{-1}). These values were similar to reported by Casal et al. (2021) for sea urchins fed *R. okamurae* in a 24 hours experiment, and significantly lower than the ingestion rates of sea urchins fed only *U. ohnoi* (U, 1.9% ww day^{-1}) or fed *R. okamurae* and *U. ohnoi* (RO + U, 1.3% ww day^{-1}), both observed in the present work (Experiment 1). Casal et al. (2021) attributed the low consumption of *R. okamurae* to the deterrent properties of dilkamural, a secondary metabolite isolated from this alga. Nevertheless, it is interesting to note that sea urchins increased *R. okamurae* ingestion in Experiment 2 (RO, 1.0% ww day^{-1}). In this experiment, the ingestion values were close to those of sea urchins fed both macroalgae (RO + U, 1.2% ww day^{-1}), although with statistical differences. Differences between Experiment 1 and 2 can be explain by a previous adaptation of sea urchins to feeding on *R. okamurae* during Experiment 1. The previous adaptation to feeding is a consequence of using the same sea urchin individuals subjected to the same

diets in both experiments. This experimental approach probably brings on carry-over effects in all variables analysed. But also, it allows us to carry out a long-term assessment of *R. okamurae* ingestion effects, as well as to include an individualised analysis in Experiment 2. Regarding to long-term effects, Santamaría et al. (2022) proved for other generalist herbivore (*Salpa salpa*), that with time, as native species become increasingly familiar with the invader, the strength of consumer-resource interactions increases. Unquestionably, with current information it is speculative to propose that sea urchins could increase grazing over the invader alga with time, and finally control their populations. Nevertheless, our work clearly shows that sea urchins manage feed on *R. okamurae*, and it is even able to increase its intake over a long exposure period.

Concerning sea urchins algae electively, results found in this work reinforce our conclusions about differences on *R. okamurae* ingestion between Experiments 1 and 2 discussed above. In Experiment 1, we observed that sea urchins clearly preferred to eat *U. ohnoi* when it was offered with *R. okamurae* (Ivlev's idex=-0.47). However, the electively values change in Experiment 2, in which *P. lividus* showed only a slight preference for *U. ohnoi* (E = -0.15). These differences are probably related to the increased of *R. okamurae* intake over a long-time exposure as discussed above. Cardoso et al. (2020) showed that sea urchins did not show a specific preference for non-native or native macroalgae species, and for any of the different seaweeds of each phylum offered. However, Monteiro et al. (2009) and Cebrian et al. (2011) reported that *P. lividus* sometimes avoid NIS in invaded areas and selected the native. Although in some cases sea urchins preferred the NIS (Cebrian et al. 2011). Our results show that the exposure time to NIS macroalgae determinates the electively values for sea urchins. So that, *P. lividus* will select more frequently *R. okamurae* as its preferred food after cumulative interaction with this macroalgae. In any case, all these data reinforce the importance of continued work on sea urchins electively when *R. okamurae* is present, preferably in long-term studies carried out in the natural environment.

Furthermore, in relation to algae electively results, it is interesting to highlight the individual differences found in Experiment 2, underlining that we found specimens that even preferred ingest the NIS *R. okamurae* instead the native species *U. ohnoi* (E = 0.17). As for individual variability, Boudouresque and Verlaque (2007) reported that sea urchins present a rhythmic feeding periodicity, with feeding peaks every 3–6 days. These cycles could interfere with the assessment of daily food consumption per individual if time between sampling is less than sea urchins feeding cycle. In Experiment 2, the average time for the analysis of algae ingestion was 7 days, so it is unlikely that individual feeding cycles may have interfered with the evaluation of algae ingested. The fact that we found sea urchins specimens that preferred ingest *R. okamurae* instead a native species indicate a possible future in which sea urchin are selected based on algae electively, and populations become more prone to graze on *R. okamurae*, substantially increasing the control of *R. okamurae* by *P. lividus*.

In general, growth registered for sea urchins were low but in agreement with reports by other authors in this size range (McCarron et al. 2009; Lourenço et al. 2020; Grosso et al. 2021). Statistically significant differences were found between experimental treatments in both experiments, and sea urchins fed *U. ohnoi* (U) or fed *R. okamurae* and *U. ohnoi* (RO + U) grew more than those fed exclusively *R. okamurae*

(R). Nevertheless, growth differences were minor in Experiment 2. Our main hypothesis is that the higher ingestion values in Experiment 2 for sea urchins fed exclusively *R. okamurae* could explain a growth improvement for this experimental treatment. This is in line with results reported by McCarron et al. (2009), who found that *P. lividus* body weight was significantly affected by feeding regimens (intermittent *vs*. continuous). Regarding the effect of *R. okamurae* consumption on sea urchins physiology, we observed a reduction of GSI in animals fed only *R. okamurae*. It is possible that, like occurs with growth, GSI differences were related with the ingestion of *R. okamurae*. Our data confirm this hypothesis, so a significant positive correlation between total daily algae ingested and GSI was found for both treatments. Frantzis and Grémare (1993) also found a clear relationship between ingested organic matter and gonadal growth. Finally, we observed high survival values throughout the experimental period (above 98%), without differences between the experimental groups. In this sense, Cornell and Hawkins (2003) suggests that, when a secondary metabolite is already present in the new range, generalist herbivores are likely to possess the ability to detoxify algae defences and feed on it, showing a more responsive growth to toxicity differences than survival. Gonad growth probably follow the same pattern, being a response variable more sensible to the experimental factors than survival was.

Arachidonic acid (ARA, 20:4n-6) is one of the most abundant fatty acids in macroalgae *R. okamurae* (7.38 *vs*. 0% of TFA, in *R. okamurae* and *U. ohnoi* respectively). Our results show that this fatty acid is accumulate in the intestine of *P. lividus* and clearly discriminates intestines of sea urchins fed *R. okamurae* (R) from those of treatments R + U (see PCA analysis). Furthermore, a strong positive correlation between Ivlev's electively index and the level of intestinal ARA ($r = 0.654$, $p = 0.040$) was found (data not shown), which reinforce the idea that intestinal ARA could be a reliable marker of *R. okamurae* ingestion. These results are in line with what is reported by Cook et al. (2000), which found high levels of 20:4n-6 in the gonads of *Psammechinus miliaris* fed with *Laminaria saccharina*, a macroalgae enriched in this fatty acid. Moreover, Schram et al. (2018) observed that sea urchin *Strongylocentrotus droebachiensis* and *S. purpuratus* selectively retained ARA; suggesting that it is feasible to develop FA resource libraries, for future use in Bayesian mixing models for further evaluating in situ urchin trophic linkages in situ. Conversely, other fatty acids also quantitively important in *R. okamurae* were not univocal markers of *R. okamurae* consumption. For example, eicosanoid acid (EPA, 20:5n-3) or eicosatetraenoic acid (ETA, 20:4n-3), both with relevant differences in diets (higher in *R. okamurae*), did not reflect these differences in sea urchin intestines. This is probably due to the intensive biosynthetic and selective dietary sparing capacity of sea urchins (Kabeya et al. 2017; White et al. 2017). Synthesis processes were especially significant for EPA in RO + U treatment. In these intestines, in addition to detecting a greater amount of EPA, we found higher levels of all EPA precursors 18:4n3, 20:3n3 and 20:4n3 than in sea urchins fed *R. okamuae*.

Regarding the fatty acid content of the gonads of sea urchins, we found that fatty acid profile were not directly related with diet. Principal components analysis clearly showed a separation of samples by GSI levels, independently of diet. All specimens with GSI < 1 were sea urchins of treatment RO (discussed above). Nevertheless, in the group of sea urchins with GSI > 2 we found 4 of 13 specimens analysed from RO treatment. The gonads of these three specimens displayed values of EPA, DHA and others n-3 PUFA,

like 20:3n-3 or 20:4n-3, similar to those found in gonads of treatment RO + U (data not shown). This leads us to propose that lipids incorporated in developing gonads have specific features in terms of n-3 HUFA, and probably are biosynthesised or incorporated from diets and selectively sparing. So, gonad fatty acid composition is more related with gonadal cycle, like previously indicate other authors (Martínez-Pita et al. 2010).

In conclusion, our results indicate that *P. lividus* accepted the NIS *R. okamurae* as food under laboratory conditions, increasing its intake over a long exposure time. These results open the door to new studies directed at proving if *P. lividus* is feeding on NIS in invaded coastal areas, and to check if ingestion rate in these areas is dependent on the invasion history. The use of ARA as dietary biomarkers can be a suitable tool to apply in studies in a natural environment, in combination with others such as the analysis of sea urchins intestinal content. On the other hand, the present study indicates that there exists a high variability between the specimens of *P. lividus* in *R. okamurae* electivity values. It would be very interesting to study if this variability is connected with sea urchins genotypes, and if it can become a character trait that selects (in natural habitat) families more adapted to consume this NIS. In summary, this work aims to start new studies related to the use of sea urchin as a biocontrol species in areas invaded by *R. okamurae*.

Declarations

Acknowledgements

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Teresa Jiménez, Miriam Muñoz, Eugenia Zuasti and Ismael Hachero-Cruzado. Photographs were taken by Teresa Jiménez. The first draft of the manuscript was written by Ismael Hachero-Cruzado and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

References

1. Altamirano-Jeschke M, De la Rosa Álamos J, Martínez Medina FJ (2016) Arribazones de la especia exótica rugulopteryx okamurae (EY Dawson) en el Estrecho de Gibraltar
2. Andrew NL, Underwood AJ (1989) Patterns of abundance of the sea urchin Centrostephanus rodgersii (Agassiz) on the central coast of New South Wales, Australia. J Exp Mar Biol Ecol 131: 61-80 doi https://doi.org/10.1016/0022-0981(89)90011-7
3. Benedetti-Cecchi L, Cinelli F (1995) Habitat heterogeneity, sea urchin grazing and the distribution of algae in littoral rock pools on the west coast of Italy (western Mediterranean). Mar Ecol Prog Ser 126: 203-212
4. Blanco A, Lemos MF, Pereira L, Gaspar R, Mouga T, Neto JM, Troncoso J, Olabarria C (2020) Mapping invasive macroalgae in the western Iberian Peninsula: a methodological guide. Universidade de Vigo. Politécnico de Leiria. Universidade de Coimbra
5. Boudouresque C, Verlaque M (2007) Chapter 13 Ecology of Paracentrotus lividus. In: Lawrence JM (ed) Developments in Aquaculture and Fisheries Science. Elsevier, pp 243-285
6. Cardoso AC, Arenas F, Sousa-Pinto I, Barreiro A, Franco JN (2020) Sea urchin grazing preferences on native and non-native macroalgae. Ecol Indic 111: 106046 doi https://doi.org/10.1016/j.ecolind.2019.106046
7. Casal I, Zubía E, Brun F (2021) Dilkamural: A novel chemical weapon involved in the invasive capacity of the alga Rugulopteryx okamurae in the Strait of Gibraltar. Estuar Coast Shelf Sci 257: 107398 doi 10.1016/j.ecss.2021.107398
8. Castell JD, Kennedy EJ, Robinson SMC, Parsons GJ, Blair TJ, Gonzalez-Duran E (2004) Effect of dietary lipids on fatty acid composition and metabolism in juvenile green sea urchins (Strongylocentrotus droebachiensis). Aqua 242: 417-435 doi https://doi.org/10.1016/j.aquaculture.2003.11.003
9. Castilla-Gavilán M, Cognie B, Ragueneau E, Turpin V, Decottignies P (2019) Evaluation of dried macrophytes as an alternative diet for the rearing of the sea urchin Paracentrotus lividus (Lamarck, 1816). Aquac Res 50: 1762-1769 doi 10.1111/are.14045
10. Cebrian E, Ballesteros E, Linares C, Tomas F (2011) Do native herbivores provide resistance to Mediterranean marine bioinvasions? A seaweed example. Biol Invasions 13: 1397-1408 doi 10.1007/s10530-010-9898-1
11. Chiantore M, Vielmini I, Privitera D, Mangialajo L, Cattaneo-Vietti R (2008) Habitat effects on the population structure of Paracentrotus lividus and Arbacia lixula. Chem Ecol 24: 145-157 doi 10.1080/02757540801965423
12. Christie WW (2003) Lipid Analysis: Isolation, Separation, Identification, and Structural Analysis of Lipids. Amer Oil Chemists Society

13. Cook EJ, Bell MV, Black KD, Kelly MS (2000) Fatty acid compositions of gonadal material and diets of the sea urchin, Psammechinus miliaris: trophic and nutritional implications. J Exp Mar Biol Ecol 255: 261-274 doi https://doi.org/10.1016/S0022-0981(00)00301-4
14. Cornell Howard V, Hawkins Bradford A (2003) Herbivore Responses to Plant Secondary Compounds: A Test of Phytochemical Coevolution Theory. Am Nat 161: 507-522 doi 10.1086/368346
15. Dalsgaard J, St. John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment Advances in Marine Biology. Academic Press, pp 225-340
16. Dethier M, Sosik E, Galloway A, Duggins D, Simenstad C (2013) Addressing assumptions: Variation in stable isotopes and fatty acids of marine macrophytes can confound conclusions of food web studies. Mar Ecol Prog Ser 478: 1-14 doi 10.3354/meps10310
17. El Aamrı F, Idhalla M, Naoufal Tamsourı M (2018) Occurrence of the invasive brown seaweed Rugulopteryx okamurae (E.Y.Dawson) I.K.Hwang, W.J.Lee & H.S.Kim (Dictyotales, Phaeophyta) in Morocco (Mediterranean Sea). Mediterranean Fisheries and Aquaculture Research 1
18. Elton CSECS (1958) The ecology of invasions by animals and plants
19. Frantzis A, Grémare A (1993) Ingestion, absorption, and growth rate of Paracentrotus lividus (Echinodermata: Echinoidea) fed different macrophytes. Marine Ecology-progress Series - MAR ECOL-PROGR SER 95: 169-183 doi 10.3354/meps095169
20. Gaither MR, Bowen BW, Toonen RJ (2013) Population structure in the native range predicts the spread of introduced marine species. Proceedings of the Royal Society B: Biological Sciences 280: 20130409 doi 10.1098/rspb.2013.0409
21. García-Gómez JC, Florido M, Olaya-Ponzone L, Rey Díaz de Rada J, Donázar-Aramendía I, Chacón M, Quintero JJ, Magariño S, Megina C (2021) Monitoring Extreme Impacts of Rugulopteryx okamurae (Dictyotales, Ochrophyta) in El Estrecho Natural Park (Biosphere Reserve). Showing Radical Changes in the Underwater Seascape. Front Ecol Evol 9 doi 10.3389/fevo.2021.639161
22. García-Gómez JC, Sempere-Valverde J, González AR, Martínez-Chacón M, Olaya-Ponzone L, Sánchez-Moyano E, Ostalé-Valriberas E, Megina C (2020) From exotic to invasive in record time: The extreme impact of Rugulopteryx okamurae (Dictyotales, Ochrophyta) in the strait of Gibraltar. Sci Total Environ 704: 135408 doi https://doi.org/10.1016/j.scitotenv.2019.135408
23. Gillett J (1962) Pest pressure, an underestimated factor in evolution. Systematics Association Publication 4: r46
24. González-Durán E, Castell JD, Robinson SMC, Blair TJ (2008) Effects of dietary lipids on the fatty acid composition and lipid metabolism of the green sea urchin Strongylocentrotus droebachiensis. Aqua 276: 120-129 doi https://doi.org/10.1016/j.aquaculture.2008.01.010
25. Grosso L, Rakaj A, Fianchini A, Morroni L, Cataudella S, Scardi M (2021) Integrated Multi-Trophic Aquaculture (IMTA) system combining the sea urchin Paracentrotus lividus, as primary species, and the sea cucumber Holothuria tubulosa as extractive species. Aqua 534: 736268 doi https://doi.org/10.1016/j.aquaculture.2020.736268

26. Guillard RRL (1975) Culture of Phytoplankton for Feeding Marine Invertebrates. In: Smith WL, Chanley MH (eds) Culture of Marine Invertebrate Animals: Proceedings — 1st Conference on Culture of Marine Invertebrate Animals Greenport. Springer US, Boston, MA, pp 29-60
27. Hoffman R, Dubinsky Z, Israel A, Iluz D (2008) The mysterious disappearance of Halimeda tuna from the intertidal zone along the Israeli Mediterranean. Isr J Ecol Evol 54: 267-268
28. Hoffman R, Shemesh E, Ramot M, Dubinsky Z, Pinchasov-Grinblat Y, Iluz D (2011) First record of the Indo-Pacific seaweed Codium arabicum Kü tz. (Bryopsidales, Chlorophyta) in the Mediterranean Sea. Bot Mar 54: 487-495 doi 10.1515/BOT.2011.056
29. Hwang I-K, Lee WJ, Kim H-S, De Clerck O (2009) Taxonomic reappraisal of Dilophus okamurae (Dictyotales, Phaeophyta) from the western Pacific Ocean. Phycologia 48: 1-12 doi 10.2216/07-68.1
30. Kabeya N, Sanz-Jorquera A, Carboni S, Davie A, Oboh A, Monroig O (2017) Biosynthesis of Polyunsaturated Fatty Acids in Sea Urchins: Molecular and Functional Characterisation of Three Fatty Acyl Desaturases from Paracentrotus lividus (Lamark 1816). PLOS ONE 12: e0169374 doi 10.1371/journal.pone.0169374
31. Kelly JR, Scheibling R (2012) Fatty acids as dietary traces in benthic food webs. Mar Ecol Prog Ser 446: 1-22 doi 10.3354/meps09559
32. Leinaas HP, Christie H (1996) Effects of removing sea urchins (Strongylocentrotus droebachiensis): Stability of the barren state and succession of kelp forest recovery in the east Atlantic. Oecologia 105: 524-536 doi 10.1007/BF00330016
33. Lourenço S, José R, Andrade C, Valente LMP (2020) Growth performance and gonad yield of sea urchin Paracentrotus lividus (Lamarck, 1816) fed with diets of increasing protein: energy ratios. Anim Feed Sci Technol 270: 114690 doi https://doi.org/10.1016/j.anifeedsci.2020.114690
34. Martínez-Pita I, Garcia FJ, Pita ML (2010) 2010 Effect seasonality gonad fatty acids P lividus A lixula J Shell Res 29 517 525. J Shellfish Res 29: 517-525
35. McCarron E, Burnell G, Mouzakitis G (2009) Growth assessment on three size classes of the purple sea urchin Paracentrotus lividus using continuous and intermittent feeding regimes. Aqua 288: 83-91 doi https://doi.org/10.1016/j.aquaculture.2008.11.025
36. Monteiro C, Engelen A, Santos R (2009) Macro- and mesoherbivores prefer native seaweeds over the invasive brown seaweed Sargassum muticum: A potential regulating role on invasions. Mar Biol 156: 2505-2515 doi 10.1007/s00227-009-1275-1
37. Neilson BJ, Wall CB, Mancini FT, Gewecke CA (2018) Herbivore biocontrol and manual removal successfully reduce invasive macroalgae on coral reefs. PeerJ 6: e5332 doi 10.7717/peerj.5332
38. Noè S, Badalamenti F, Bonaviri C, Musco L, Fernández TV, Vizzini S, Gianguzza P (2018) Food selection of a generalist herbivore exposed to native and alien seaweeds. Mar Pollut Bull 129: 469-473 doi https://doi.org/10.1016/j.marpolbul.2017.10.015
39. Piazzi L (2009) Invasion of alien macroalgae in different Mediterranean habitats. Biol Invasions v. 11: pp. 193-204-2009 v.2011 no.2002 doi 10.1007/s10530-008-9224-3
40. Piazzi L, Ceccherelli G, Cinelli F (2001) Threat to macroalgal diversity

41. effects of the introduced green alga Caulerpa racemosa in the Mediterranean. Mar Ecol Prog Ser 210: 149-159
42. Piazzi L, Cinelli F (2003) Evaluation of benthic macroalgal invasion in a harbour area of the western Mediterranean Sea. Eur J Phycol 38: 223-231
43. Robert ES, Patrick G (2006) Competitive interactions between the invasive green alga Codium fragile ssp. tomentosoides and native canopy-forming seaweeds in Nova Scotia (Canada). Mar Ecol Prog Ser 325: 1-14
44. Santamaría J, Golo R, Verdura J, Tomas F, Ballesteros E, Alcoverro T, Arthur R, Cebrian E (2022) Learning takes time: Biotic resistance by native herbivores increases through the invasion process doi 10.22541/au.164796958.87011150/v1
45. Schaffelke B, Hewitt CL (2007) Impacts of introduced seaweeds50: 397-417 doi doi:10.1515/BOT.2007.044
46. Schaffelke B, Smith JE, Hewitt CL (2006) Introduced Macroalgae – a Growing Concern. Journal of Applied Phycology 18: 529-541 doi 10.1007/s10811-006-9074-2
47. Schram JB, Kobelt JN, Dethier MN, Galloway AWE (2018) Trophic Transfer of Macroalgal Fatty Acids in Two Urchin Species: Digestion, Egestion, and Tissue Building. Front Ecol Evol 6 doi 10.3389/fevo.2018.00083
48. Sempere-Valverde J, Ostalé-Valriberas E, Maestre M, González Aranda R, Bazairi H, Espinosa F (2021) Impacts of the non-indigenous seaweed Rugulopteryx okamurae on a Mediterranean coralligenous community (Strait of Gibraltar): The role of long-term monitoring. Ecol Indic 121: 107135 doi https://doi.org/10.1016/j.ecolind.2020.107135
49. Valentine JF, Heck KL (1991) The role of sea urchin grazing in regulating subtropical seagrass meadows: evidence from field manipulations in the northern Gulf of Mexico. J Exp Mar Biol Ecol 154: 215-230 doi https://doi.org/10.1016/0022-0981(91)90165-S
50. Villanueva Ojeda E (2022) Portalrediam. Consejería de Agricultura, Ganadería, Pesca y Desarrollo Sostenible. Junta de Andalucía, Sevilla, Spain
51. White CA, Bannister RJ, Dworjanyn SA, Husa V, Nichols PD, Kutti T, Dempster T (2017) Consumption of aquaculture waste affects the fatty acid metabolism of a benthic invertebrate. Sci Total Environ 586: 1170-1181

Tables

Tables 1-3 is available in the Supplementary Files section.

Figures

Figure 1

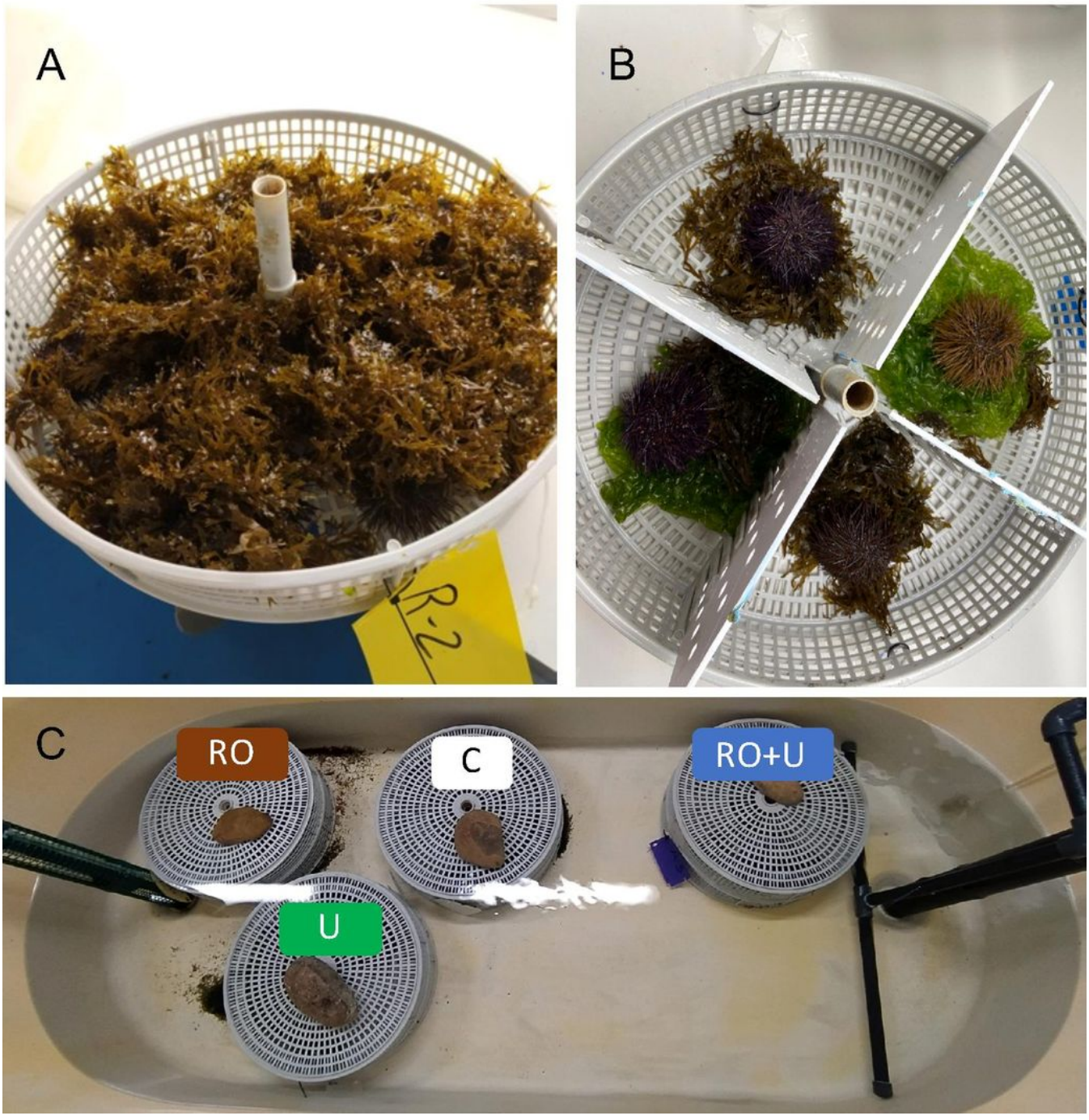


Figure 1

Experimental design. A) Open oyster culture baskets adapted for sea urchin culture in Experiment 1; B) Open oyster culture baskets adapted for individual sea urchin culture in Experiment 2; C) Layout of baskets for sea urchin culture in one tank. The amount of fresh *R. okamurae* that was placed at the beginning of each feeding period can be observed in picture A and B. RO, Sea urchins fed *R. okamurae*; C,

control basket with algae but without sea urchins; RO+U, Sea urchins fed *R. okamurae* and *Ulva ohnoi*; U, Sea urchins fed *U. ohnoi*

Figure 2

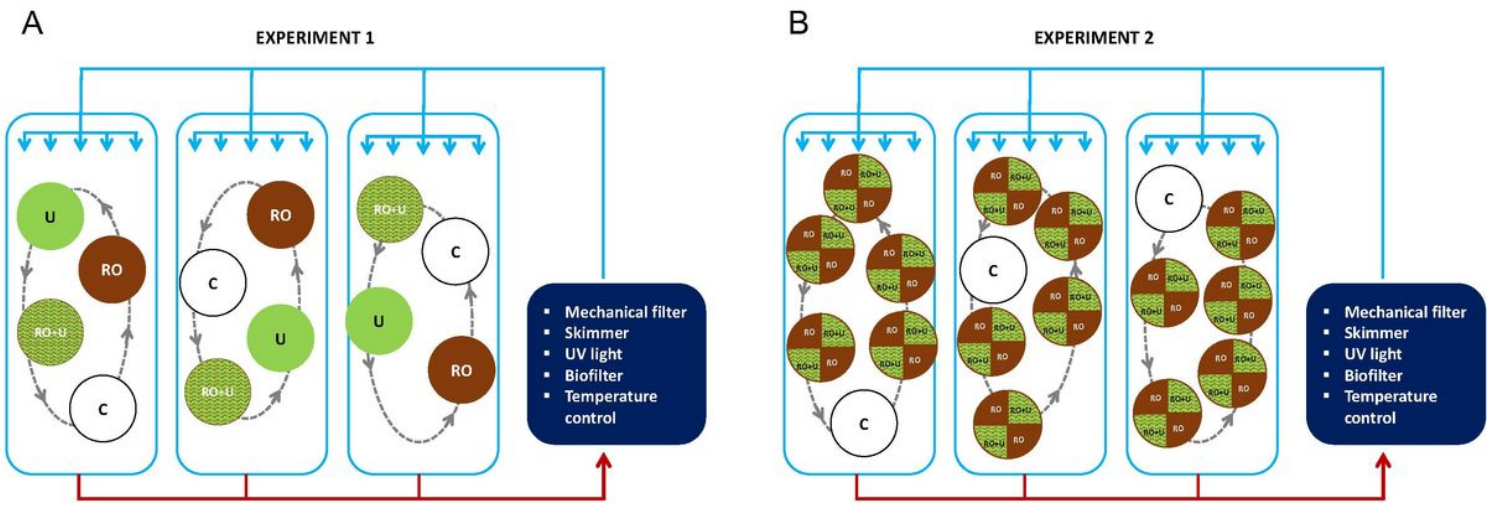
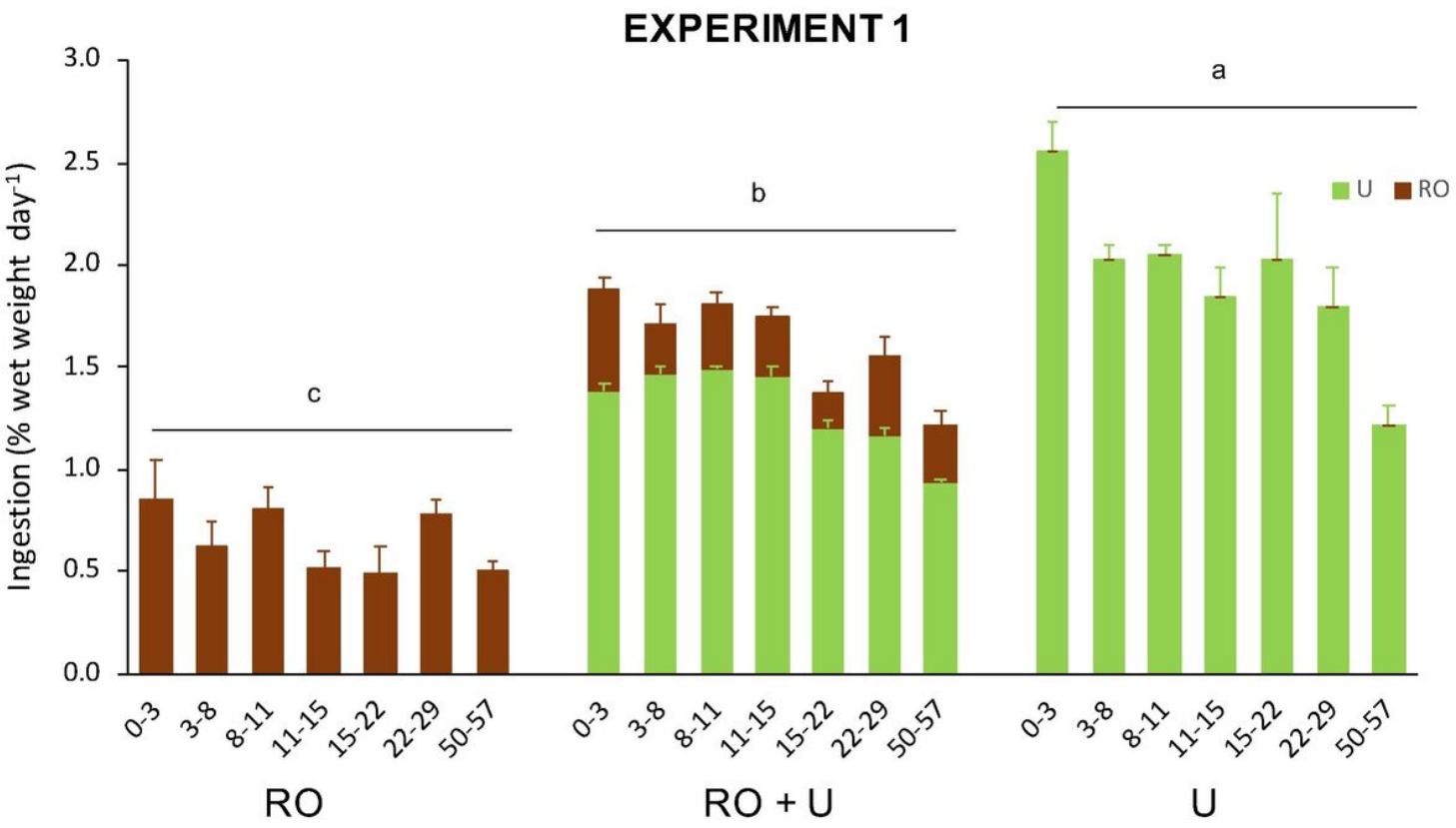


Figure 2

Experimental design. A) Tanks and baskets distribution during the Experiment 1; B) tanks and baskets distribution during the Experiment 2. Arrows indicate baskets position rotation every week. Experimental treatments: C, control basket with algae but without sea urchins; RO+U, Sea urchins fed *R. okamurae* and *U. ohnoi*; U, Sea urchins fed *U. ohnoi*

Figure 3

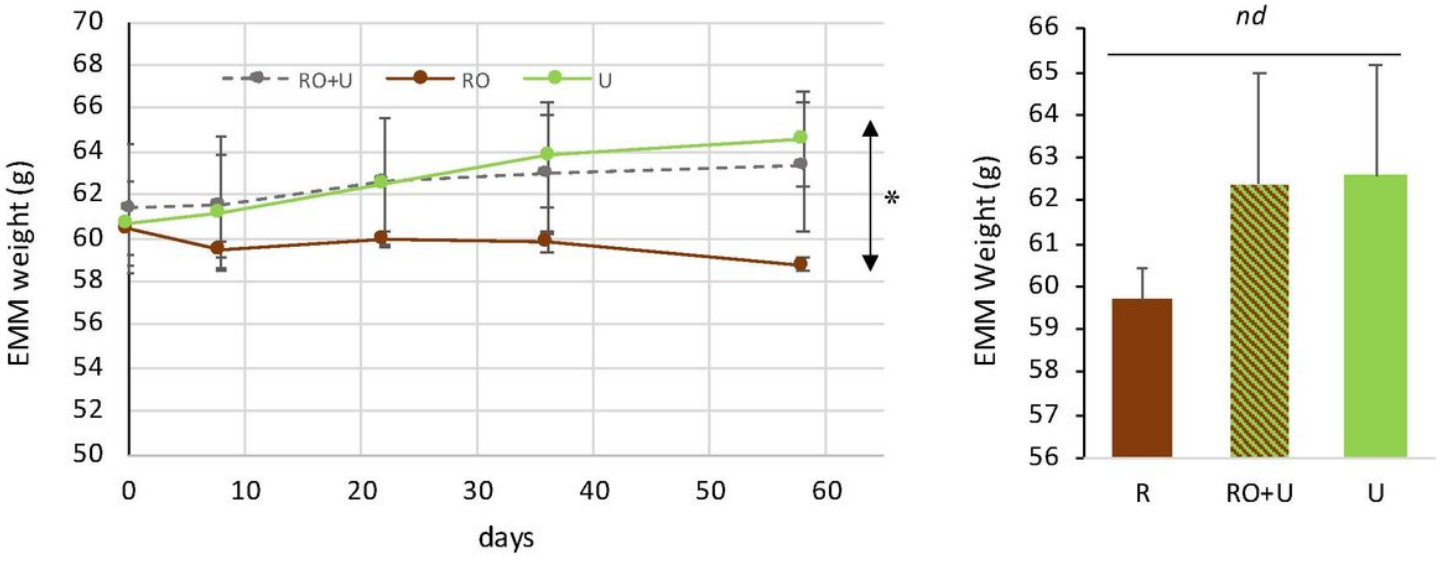


	Time (t)	TK	D	t x D	t x TK
Within	0.3632	-	-	0.1383	0.4235
Between	-	0.5583	0.0005	-	-

Figure 3

Ingestion rate of sea urchins *Paracentrotus lividus* fed with macroalgaes *R. okamurae* (RO), *R. okamurae* and *U. ohnoi*(RO+U); or *U. ohnoi* (U). The different letters above the bars denote significant differences between dietary groups (Tukey Post Hoc test). P values resulting of repeated measures ANOVA with factors diet (D) and tank (TK) are presented in table

Figure 4

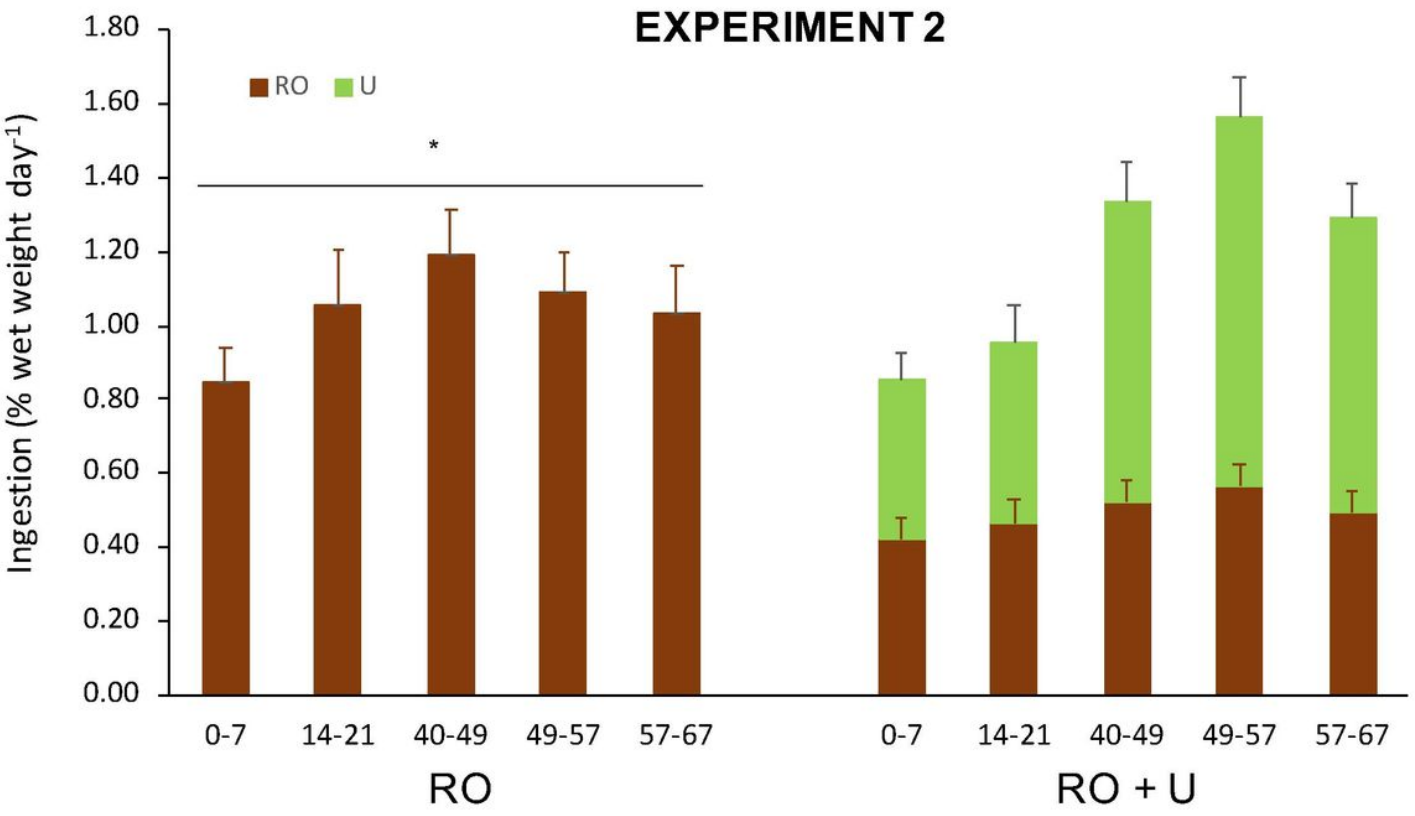


	Time (t)	TK	D	t x D	t x TK
Within	0.0002	-	-	0.0003	0.1173
Between	-	0.5911	0.3278	-	-

Figure 4

Weight of animals fed with fresh macroalgae *R. okamurae* (RO), *R. okamurae* and *U. ohnoi*(RO+U); or *U. ohnoi* (U). Lines represent the estimated marginal means (EMM) after a repeated measures ANOVA using the weight at different samplings in the evaluation periods. Asterisks on the horizontal denote if within-subjects for interaction diet × time are statistically significant. Bars on the right represent the EMM for weight in the whole period. Asterisks denote if between-subjects are statistically significant. Nd, no differences. P values resulting of repeated measures ANOVA with factors diet (D) and tank (TK) are presented in table

Figure 5

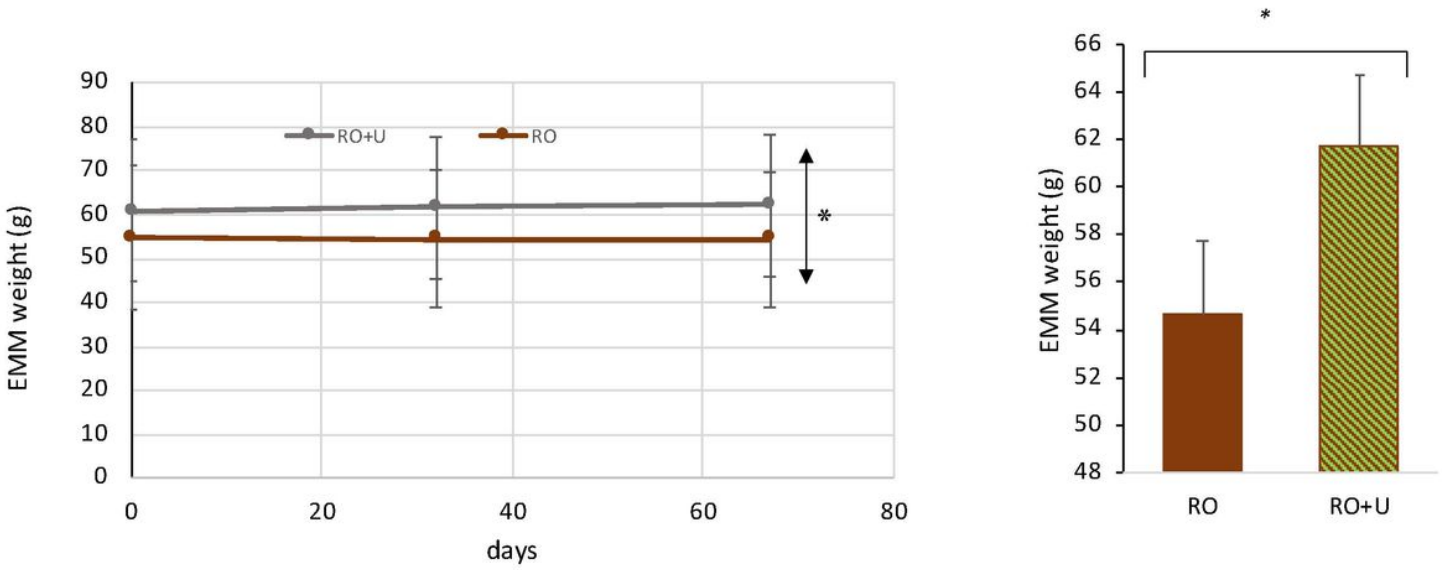


	Time (t)	Diet (D)	Tank (TK)	Basket (BK)	D x TK	D x BK	t x D
Within	0.0027	-	-	-	-	-	0.0103
Between	-	0.0014	0.1802	0.0009	0.3623	0.3134	-

Figure 5

Daily food consumption of sea urchin *Paracentrotus lividus* fed with fresh macroalgae *R. okamurae* (RO) or *R. okamurae* and *U. ohnoi* (RO+U). Asterisk above bars indicates significant differences between dietary treatments. P values resulting of repeated measures ANOVA with factors diet (D), tank (TK) and baskets (BK) are presented in table

Figure 6



	Time (t)	Diet (D)	Tank (TK)	Basket (BK)	D x TK	D x BK	t x D
Within	0.0026		-	-	-	-	0.0015
Between	-	0.0060	0.0062	0.0002	0.0417	0.2846	-

Figure 6

Weight of animals fed with the *R. okamurae* (RO) or *R. okamurae*and *U. ohnoi* (RO+U). Lines represent the estimated marginal means (EMM) after a repeated measures ANOVA using the weight at different samplings in the evaluation periods. Asterisks on the horizontal denote if within-subjects for interaction diet × time are statistically significant. Bars on the right represent the EMM for weight in the whole period. Asterisks denote if between-subjects are statistically significant. nd, no differences

Figure 7

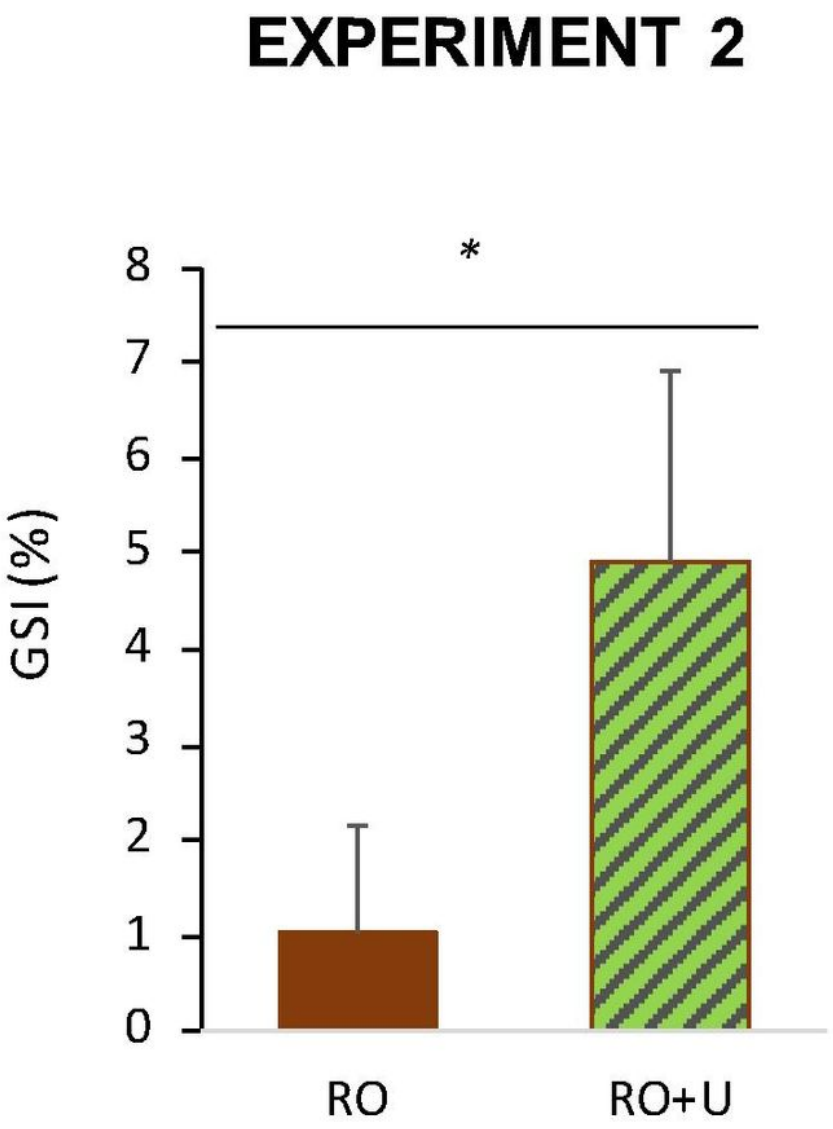


Figure 7

Gonado-somatic index (GSI) of animals fed with the *R. okamurae* (RO) or *R. okamurae* and *U. ohnoi* (RO+U). Asterisks denote significant differences between treatments

Figure 8

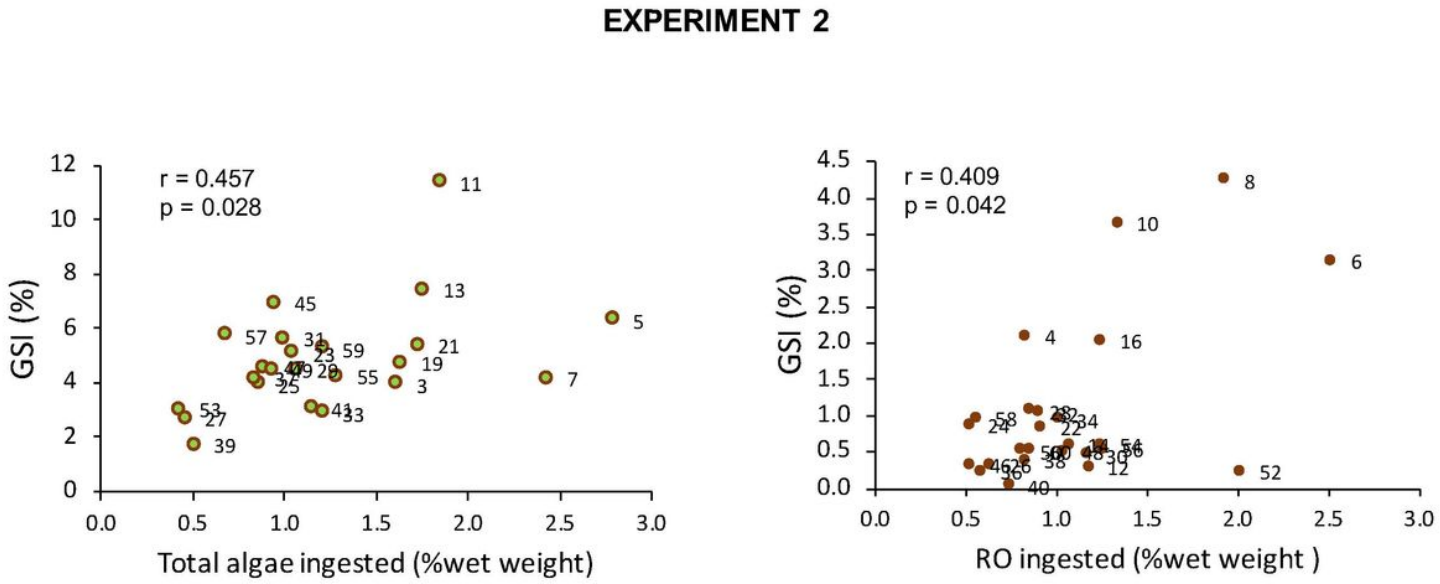


Figure 8

Correlations between gonado-somatic index (GSI) and the average daily food consumption for sea urchins fed *R. okamurae* and *U. ohnoi* (RO+U) (left); or fed only *R. okamurae* (R) (right)

Figure 9

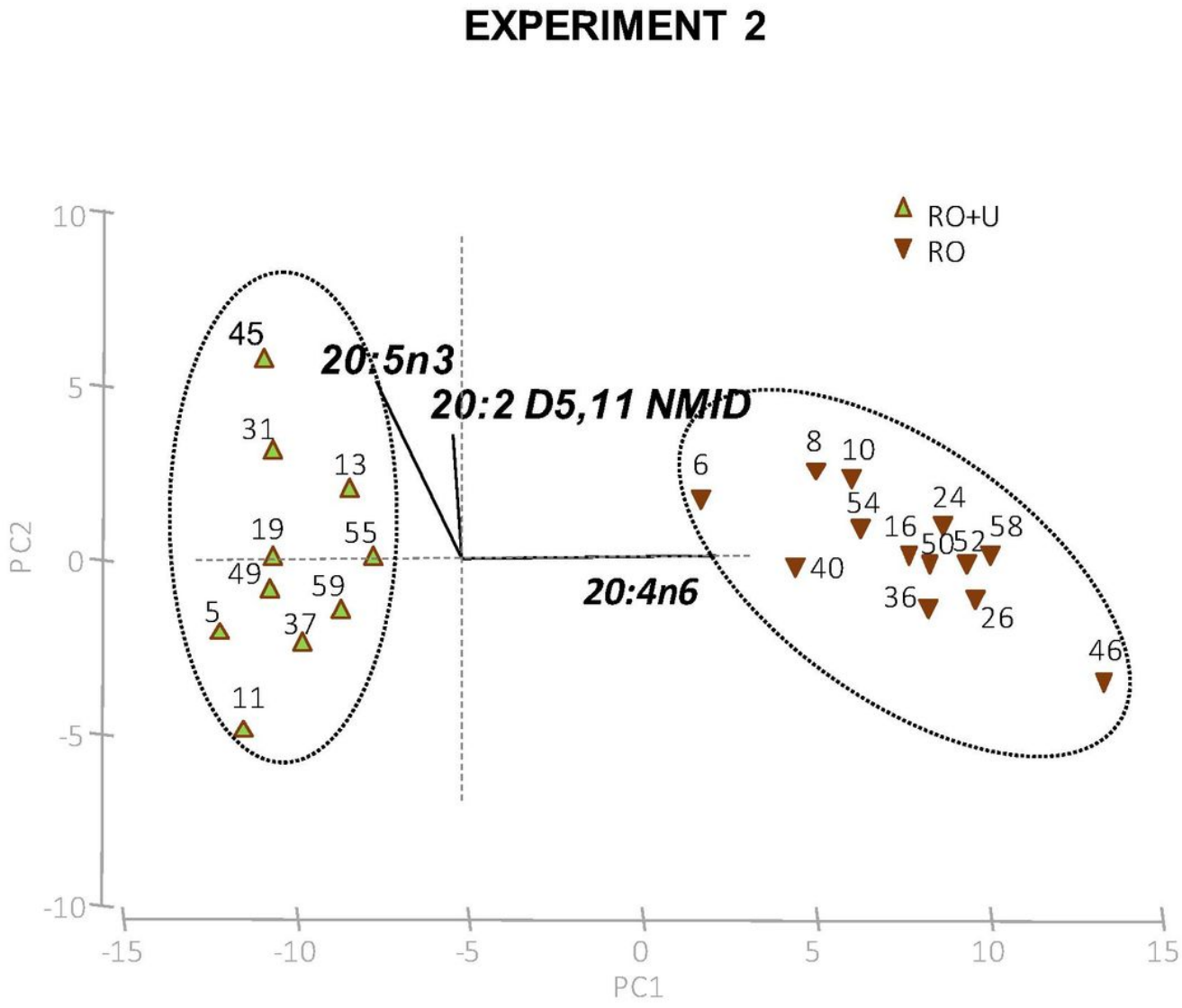


Figure 9

Principal component analysis (PCA) plot based on intestine FA composition (in % Total FA)

Figure 10

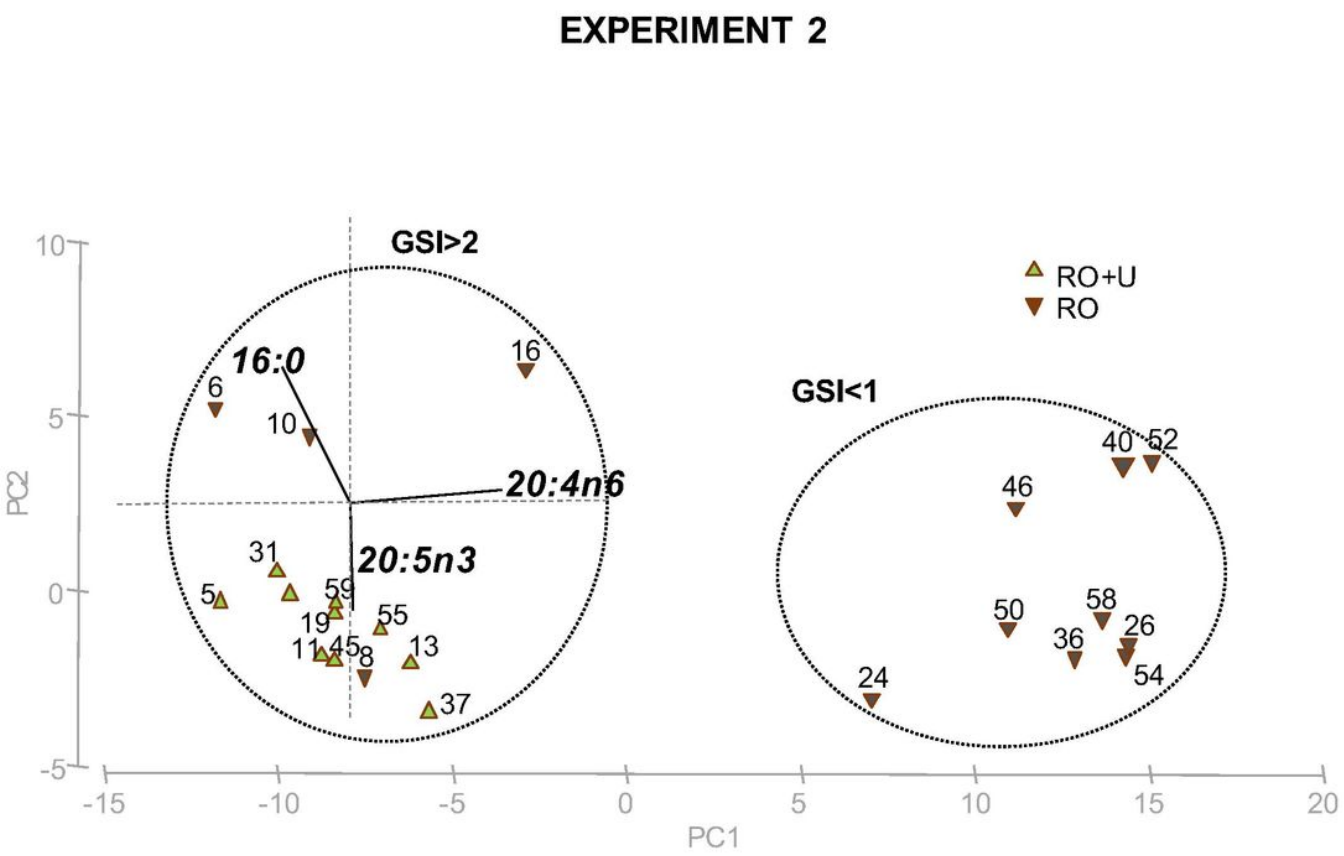


Figure 10

Principal component analysis (PCA) plot based on gonad FA composition (in % Total FA)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table13.docx
- Supplementaryfile1.pdf