Journal of Applied Phycology
https://doi.org/10.1007/s10811-023-02925-0

RESEARCH

Ulva as potential stimulant and attractant for a valuable sea urchin species: a chemosensory study

Pierantonio Addis[1] · Viviana Pasquini[1] · Alberto Angioni[1] · Veronica Malavasi[3] · Davide Moccia[1] · Paolo Solari[2]

Received: 23 December 2022 / Revised and accepted: 7 February 2023

Abstract

The green seaweed *Ulva* is close to becoming popular due to its suitability as potential feedstock production and for food items. However, there is a general lack of studies on the aversion or acceptability of this alga by marine organisms, particularly on its role as a chemoattractant and/or phagostimulant activity. Here we tested the effect of *Ulva compressa* and other biochemicals as potential chemostimulating compounds for a valuable sea urchin species, *Paracentrotus lividus*, selected as model species for our tests. Sea urchins' chemical sensitivity was estimated by analysing movements of spines, pedicellariae, tube feet, and individual locomotion using an innovative bioassay. Our results showed that all forms of *Ulva* (fresh, defrosted, and fragmented) resulted in an effective stimulus, evoking in sea urchins strong responses with robust activation of spines and tube feet, where the defrosted one was the most stimulating. Among the amino acids tested, glycine, alanine, and glutamine produced a significant response, highlighting for the latter a concentration–response relationship. Sea urchins responded to glucose, not to fructose and sucrose. Spirulina resulted as the most effective stimulus, acting in a dose-dependent manner. Major results indicate the role of *Ulva* as a chemostimulant and strongly attractant for such herbivore species. From an applied point of view, the presence of potential *Ulva*'s feed-related compounds, acting as chemoattractants (to reduce food searching time) and/or feeding stimulants (to stimulate ingestion), would improve the several applications of *Ulva* in the formulation of the feeds for sustainable aquaculture.

Keywords *Ulva* spp. · Biochemical composition · Feed · Chemosensory · Sea urchin

Introduction

The boosting of sustainable aquaculture for aquatic plants will play a key role in the future of human and animal feeds production, agriculture biofertilizers, pharmaceuticals, cosmetics, emerging packaging, and waste treatment (Pereira, 2016; Cai et al 2021). The margin on economic growth for this industry is mainly relevant in Europe, which contributes only 0.8% of world seaweed production (Cai et al 2021).

Regarding this challenge, the green algae belonging to the genus *Ulva* (Chlorophyta) have been identified as one of the most suitable candidates for sustainable mariculture and are currently under the attention of the international scientific community generating many expectations (Cai et al 2021; COST Action 20106). The members of Ulvaceae are widely distributed in coastal marine ecosystems, where they provide nutrients and habitat for a variety of invertebrates and fish species. At the same time, they have the capacity to grow rapidly in response to eutrophication, resulting in massive nuisance blooms (Škaloud et al. 2018). The use of *Ulva* species may cover a wide range of products, from human food, animal feed and food ingredients to chemical constituents and, on a broader view, environmental benefits and ecosystem services (Campbell et al. 2019). However, citizen appreciation of *Ulva* and its usefulness in human diets, especially in western countries, must be further investigated and validated. On the other hand, its use in the feed industry and aquaculture, specifically in echinoculture, is already considered a primary ingredient or a supplement included in prepared diets (Van Alstyne et al. 2001; Akakabe and Kajiwara 2008; Cyrus et al. 2015; Etwarysing

✉ Pierantonio Addis
addisp@unica.it

[1] Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy

[2] Department of Biomedical Sciences, Section of Physiology, University of Cagliari, Cagliari, Italy

[3] Department of Botany, Charles University, Prague, Czech Republic

Published online: 17 March 2023

et al. 2017; Prato et al. 2018; Shpigel et al. 2018; Kwon et al. 2021). In the context of new feed formulation, which considers *Ulva* as a biomass source, one of the unknown aspects is whether the target farmed species appreciate this green alga. The primary concern is whether *Ulva*'s biochemical components or trace elements cause a chemical stimulus or chemoattraction to farmed species.

In general, animals can perceive and respond to chemical cues in all environments, including aquatic habitats, where changes in behaviour after the detection of waterborne molecules have been extensively documented for many species and in many behavioural contexts (Bargmann 2006; Kamio and Derby 2017; Mollo et al. 2017).

Sea urchins are generalist herbivores that can have a key role in either structuring or altering benthic macroalgal communities (Lawrence 2013). They are slow-moving, broadcast-spawning marine invertebrates that rely on chemical signals to produce appropriate behavioural responses, such as avoidance of predators, spatial orientation, identification of suitable habitats, localization of potential food sources, and conspecific mates (Lawrence 2013). Pioneering studies have shown that sea urchins may trigger the activation of spines, tube feet, and pedicellariae in conspecifics (Snyder and Snyder 1970; Campbell 1983). For instance, it is known that *Strongylocentrotus* sp. is attracted by algae recognized as food (Vadas 1977), while *Lytechinus variegatus* can orient to chemicals emanating from potential food resources over distance, even under turbulent water flow conditions (Pisut 2004). Sea urchins have been reported to respond to distant feeding stimuli with upstream orientation, using odour-guided rheotaxis for chemtrail navigation and odour source localization (Atema 2012). In this respect, their slow speed may facilitate temporal sampling of chemical cues, and the widely distributed array of chemosensory organs may enhance spatial resolution (Weissburg 2000).

Sea urchins, like other echinoderms, also use chemicals in fine-tuning breeding aggregations and spawning synchrony strategies to increase the probability of gamete encounter in animals with external fertilization (Mercier and Hamel 2009; Reuter and Levitan 2010). Despite the broad chemical sensitivity of both larvae and adults of sea urchins, chemoreceptive organs have yet to be identified with certainty. Based on behavioural and histological studies, three main systems have been reported to respond to chemical cues: the "spine system", the tube feet, and the pedicellariae, with responses ranging from simple, local reflex reactions of these systems, to fully coordinated chemotaxis, in which the whole animal moves toward or away from a stimulus source (Sloan and Campbell 1982; Campbell et al. 2001). Sea urchin chemoreceptors belong to the family of the G-protein-coupled receptors (GPCRs), and their high number—up to several hundred—is comparable with that identified in many other animals, thus suggesting that sea urchins possess a sophisticated chemosensory system (Burke et al. 2006; Raible et al. 2006).

In this study, we considered the Mediterranean sea urchin *Paracentrotus lividus* as a model species to investigate chemosensory responses to a set of *Ulva*-related stimuli. Moreover, since chemical sensitivity does not necessarily imply positive (attractive) or negative (repulsive) chemotaxis, we also aimed to ascertain if sea urchins are attracted by *Ulva*.

The main questions regard the identification of the proper form in which these macroalgae should be used as the primary feed, and then the identification of its biochemical compound that can cause chemical stimulation or chemoattraction towards the model species.

To address these questions we carried out a series of trials that considered the following stimuli: fresh, defrosted, and freeze-dried *Ulva* (*Ulva compressa*), seawater conditioned with urchins fed with fresh *Ulva*, and seawater conditioned with faeces from urchins fed with fresh *Ulva*. Among biochemical compounds, we considered three sugars and six amino acids. Finally, the blue-green alga Spirulina (*Arthrospira platensis*) was considered as a reference stimulus as it was tested in a previous investigation on the attractant response of sea urchins (Solari et al. 2021a). Under the assumption that any of these substances could be stimulant and, therefore, could subsequently be considered as a potential food source, we analysed by means of an animal bioassay the behavioural responses of sea urchins in terms of their locomotion and movement of spines, pedicellariae, and tube feet.

Materials & Methods

Animal collection and rearing conditions

Wild specimens of *Paracentrotus lividus* ($n = 100$) measuring 30 mm in test diameter (corresponding to the third age class) were collected at a depth of about 5 m from the south coast of Sardinia (Italy). Prior to the experiments, the sea urchins were acclimated for two weeks in Plexiglas tanks containing about 60 L of natural, aerated seawater (SW) at 20 ± 0.5 °C, 34‰ salinity, and a 12 h light/12 h dark photoperiodic regime. According to a previous protocol adopted by Pusceddu et al. (2021), animals were fed with *Ulva* three times a week; uneaten food and/or faecal material were removed every 2 days along a partial (less than 10%) water exchange. The sea urchins were not fed for 48 h preceding the experiments to prevent any potential adaptation of their chemoreceptor neurons (CRNs) to chemical stimulation. All experiments were carried out in full accordance with the EU Directive 2010/63/EU.

Stimuli and supply protocol

Experiments were performed considering the following sets of stimuli: *Ulva*-, amino acids-, sugars-, and spirulina-based experiments. The *Ulva* biomass was collected during the late spring season (May to June 2022) from a coastal lagoon located in the Gulf of Cagliari on the southern part of Sardinia, Italy (Santa Gilla 39° 13.760'N; 9° 4.761'E). The biomass collection was carried out using manual harvesting, and it was washed with seawater to remove unwanted debris and stored in sterile plastic bags. Morphological characterization was carried out in the laboratory for species identification (*Ulva compressa,* hereafter referred to as *Ulva*) using an inverted light microscope (Olympus CKX41, Japan) (Malavasi et al. 2020).

Fresh, defrosted, and fragmented *Ulva* cultured waters were considered as stimuli at 10 mg mL^{-1}. Cultured waters were obtained leaving *Ulva* for 24 h in an aerated photobioreactor containing static seawater (hereafter SW). Freeze-dried *Ulva* was prepared by suspending the relative amount of 10 mg mL^{-1} in SW and filtered (Whatman Filter Paper, Sigma-Aldrich, Italy). Moreover, faeces from *Ulva*-fed sea urchins (1 mg mL^{-1} of faeces obtained from specimens fed with 10 mg mL^{-1} of fresh biomass) were also considered as stimulants.

Among the amino acids, we considered the essential ones (methionine, valine) and non-essential ones (alanine, glycine, glutamine, serine) chosen as the most abundant (weight ratio) from the biochemical composition of *Ulva* (Prato et al. 2018), in order to ascertain if the stimulating effects of *Ulva* could be attributable, at least in part, to its single components of the amino acid fraction. Among sugars, glucose, fructose, and sucrose were tested as stimuli. All amino acids and sugars were first dissolved in SW at 10^{-1} mol L^{-1} and were then stored frozen as stock solutions.

On the day of the experiments, stock solutions were thawed and serially diluted in SW to 10^{-3}, 10^{-5} mol L^{-1} so that each compound was supplied at the three increasing concentrations 10^{-5}, 10^{-3}, and 10^{-1} mol L^{-1} to obtain a dose-response relationship. These concentrations were chosen according to those previously adopted to build dose-response curves following stimulation with the same compounds in other aquatic invertebrates (Solari et al. 2015, 2017, 2021b).

Spirulina (*Arthrospira platensis*) was prepared by suspending the finely hashed power in SW at a final concentration of 5 mg mL^{-1}. This preparation was then filtered (Whatman Filter Paper, Sigma-Aldrich, Italy) to remove any particulate which could mechanically stimulate the sea urchins. Then, the Spirulina-containing solution was serially diluted in SW to be used at 1, 0.1, and 0.01 mg mL^{-1}.

Chemicals were obtained from Sigma-Aldrich (Italy), while freeze-dried spirulina was purchased from Livegreen Società Agricola (Italy).

During this stimulation sequence, the water was not replaced (i.e., stepwise stimulations were used, according to Solari et al. 2015, 2021a).

Sea urchin bioassay

Sea urchins were independently exposed to stimuli according to the procedure adopted by Solari et al. (2021a, b). The experimental rack consisted of a small rectangular Plexiglas tank containing about 350 mL seawater, which was connected to two different channels of a peristaltic pump (Gilson, Minipuls Evolution) operating at a flow rate of 10 mL min^{-1} and thus ensuring a constant flow within the tank. The inflow and outflow terminals allow the SW and chemical stimuli to be delivered into and removed from the tank. The outflow terminal was connected to a secondary tank for waste collection. At the beginning of each test, animals were immersed in the experimental tank and allowed to acclimatize until becoming motionless, typically within 15 min. Before the stimulus supply, the response of each animal to the same aliquot of seawater (blank Control) was monitored for 5 min.

Stimuli were added to the tank for 1 min by switching the inflow terminal from seawater to a different reservoir, and each sea urchin was allowed 4 min to respond, starting from the time the stimulus entered the experimental tank (typically 45 s after switching). During this time, the SW flow rate was maintained at 10 mL min^{-1}. This time frame was selected because of previous observations on dye diffusion in the experimental tank. Dye tests were also performed to verify the effectiveness of the perfusion/stimulation device. Trials were video-recorded for later analysis, using a colour digital camera (Samsung SMX-F34, Korea) mounted above the experimental tank. Video recordings were analysed by an independent observer blind to the experimental treatment.

The behavioural response was determined by measuring the movement rate of spines, tube feet, and the fully coordinated locomotion, if any, by which the whole animal moves toward or away from the outlet of the stimulus supply. A single sea urchin was tested for only one compound to satisfy the assumption of independence of data for statistical analysis, while at the end of each experiment, every sea urchin was returned to the holding tank.

Detection of sea urchin movements

All visible movements of the sea urchin spines and tube feet, as well as the fully coordinated locomotory activity, when present, of the whole animal within the experimental tank, were captured by means of video recordings followed

by a frame-to-frame computer analysis of the movements, according to the procedure adopted by Middleton et al. (2006) and Solari et al. (2021a, b). Briefly, this approach produces an "urchinogram" in which the movements at several sites and levels on the same animal can be recorded and compared. The video recordings were converted to a resolution of 640 × 480 pixels at 5 frames s^{-1} (300 frames min^{-1}) so that each frame could account for the instantaneous "movement state" of the sea urchin at 200 ms intervals. Each video was analysed using a custom program (AviLine, http://biolpc22.york.ac.uk/drosophila/ovary/) while the computer mouse was used to overlay lines on the video frames so that each line crossed the light/dark boundary between the animal (dark) and the background (clear). We adopted a grid with a total of 22 (13 vertical + 9 horizontal) evenly spaced lines to cover the entire area of the experimental tank everywhere the animal moved. The mean square difference in light intensity (MSD) at each point of the lines in the grid between successive pairs of frames was plotted during the whole experiment. Therefore, the movements of the dark animal on the clear background generated changes in pixel intensity along the lines, which was used as an index of the movement rate of spines, tube feet, and locomotion of each sea urchin. Recording the MSD provides great sensitivity and good discrimination of movement, as it considers the change in every pixel along the line.

This analysis protocol recorded the displacement in the focus plane, but any movement in the vertical direction was not measured. Data were saved in a Microsoft Excel format and mean peak height and intervals between peaks were calculated. For each frame, the sum of values for all lines was calculated, to pick all movements of the spine, tube feet, and whole animal anywhere within the experimental tank. In this way, the amplitude of the sea urchin movements could be evaluated before and after the supply of the different stimuli. Data were normalized to be compared to SW condition (SW = 100% of response).

Detection of *Ulva* attractiveness

The experimental system consisted of ten circular plastic tanks (30 cm in diameter, 8 cm high), each with an area of 0.07 m^2, containing 4 L of SW at 20 ± 0.5 °C and 34‰ salinity. In accordance with a procedure already used by Pusceddu et al. (2021), sea urchins ($n = 10$) were individually exposed to fresh *Ulva* (one individual at a time in each experimental tank). Animals were starved for 48 h preceding the experiments. Fresh *Ulva* (about 500 mg each) was inserted into two ceramic filter rings for each tank to prevent algal floating. The two *Ulva*-containing rings were positioned along the tank's outer edge, alternating with the other two empty rings (acting as a Control), following a radial arrangement (Supplementary Fig. 1). The sea urchin was then placed in the center of the tank allowing 1 h to respond. Individual movement in the experimental tank was recorded by a colour digital camera (Samsung SMX-F34, Samsung, Korea) supported by an aluminum framework and positioned 60 cm above the tank, with an aerial view of the experimental arena. The detection system has the following measurable parameters for the *Ulva* attractiveness: a) the percentage of tested animals that visit *Ulva* within 1 h and remained in contact with it for at least 10 min; b) distance and time (min) travelled (mm) to find *Ulva* substrate; c) mean speed (mm min^{-1}), determined as the ratio between the time to the target and the actual distance moved to reach it and; d) tortuosity of the sea urchin's route to the *Ulva* substrate, determined as the ratio between the distance (mm) to the item and the shorter distance (mm) from the center of the tank and the targeted item.

Data analysis

A paired t-test was used to evaluate the effect of each form of *Ulva* on the behavioural response of sea urchins, while the effects of different concentrations of the other tested compounds were evaluated by one-way repeated measures ANOVA. Post-hoc comparisons were performed using Dunnett's test to assess significant differences between each stimulus concentration and the relative blank (Control) mean. When data did not conform to a normal distribution (Kolmogorov–Smirnov test for goodness of fit), Friedman's test was used for comparisons of repeated measures, followed by Dunn's post hoc test.

All statistical analyses were carried out by using the Prism program (GraphPad Software, USA). Differences were considered significant for $p \leq 0.05$.

Results

After being acclimatized in the experimental tank, the sea urchins became motionless and displayed only negligible basal activity, consisting of slow oscillations of a few spines and limited movements of tube feet. Conversely, when exposed to a stimulating compound, sea urchins started a typical response characterized at first by an increase in spine movements, coupled with a marked enhancement in tube feet projectivity. This behaviour often culminated in a coordinated locomotory activity of the whole animal within the experimental tank. All these responses were considered together as an index of the chemical sensitivity of sea urchins toward a stimulus, according to what was previously reported by Campbell et al. (2001).

All forms of *Ulva* resulted in an effective stimulus, evoking a robust activation of spines and tube feet in the sea urchins (Fig. 1). In detail, compared to the Control, the

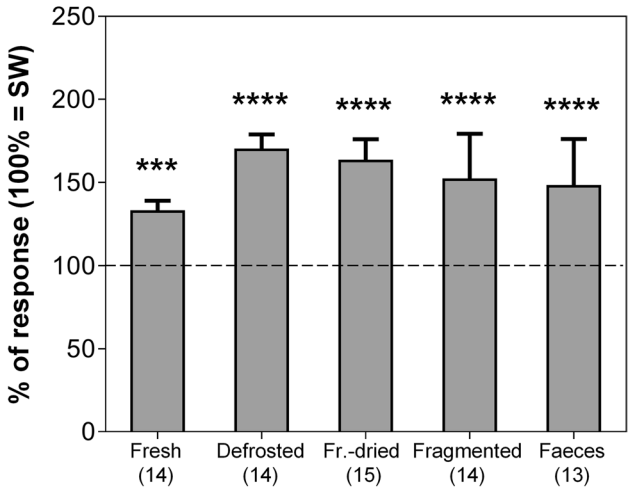

Fig. 1 Normalized movement rate of sea urchins, recorded as mean square difference in light intensity (summed peak heights for each preparation during a 2-min stimulation) ± se (vertical bars), following supply of seawater (SW) conditioned with fresh, defrosted, freeze-dried (Dry) and fragmented *Ulva* and with faeces from *Ulva*-fed sea urchins, compared to SW (100% of response, dashed line). *** and **** indicate significant differences for $p < 0.001$ and $p < 0.0001$, respectively (paired t-test) with respect to SW. Values are presented as means ± standard errors (vertical bars). The number of sea urchins tested for each stimulus is indicated in brackets

increase in animal response ranged from 132.4 ± 6.4%, following stimulation with fresh *Ulva,* up to 169.7 ± 9.3% after the supply of defrosted one, which therefore resulted in the most stimulating form of the algae (MSD for the Control was 95201 ± 1894 in the case of fresh and 96978 ± 2339 for the defrosted *Ulva*, 100% of the response). As shown in the same figure, the sea urchins were also sensitive to the faeces from *Ulva*-fed animals, showing an increase in the movement rate of 147 ± 7.9%, with respect to the Control (MSD was 93231 ± 2487).

Results of the attractiveness test show that fresh *Ulva* evokes positive rheotaxis in *P. lividus*. In fact, all tested animals found the algal substrate and remained in contact with it for at least 10 min, covering a mean distance of 255.96 ± 45.01 mm in a mean time of 18.51 ± 4.46 min (Table 1). The tested amino acids elicited different responses (Fig. 2). Non-essential amino acids, methionine, and valine failed to stimulate the sea urchins' response, regardless of the tested concentration. Conversely, among the essential amino acids, alanine and glycine were both stimulating with respect to the Control, but only at the highest tested concentration (10^{-1} mol L^{-1}; Fig. 2). In fact, they increased the sea urchin response to 130.6 ± 8.3% and 160.7 ± 14.8%, respectively (MSD for the Control was 97679 ± 2301 and 95684 ± 1994, respectively for alanine and glycine). In the same way, glutamine turned out to be a stimulating amino acid, by

Table 1 Results of the tests conducted to assess the degree of attractiveness exerted by *Ulva* on the sea urchin *P. lividus*

Index	Values
Attraction % ($n = 10$ individuals)	100
Distance travelled (mm)	255.96 ± 45.01
Time (min)	18.51 ± 4.46
Speed (mm min^{-1})	21.97 ± 5.14
Tortuosity	2.10 ± 0.32

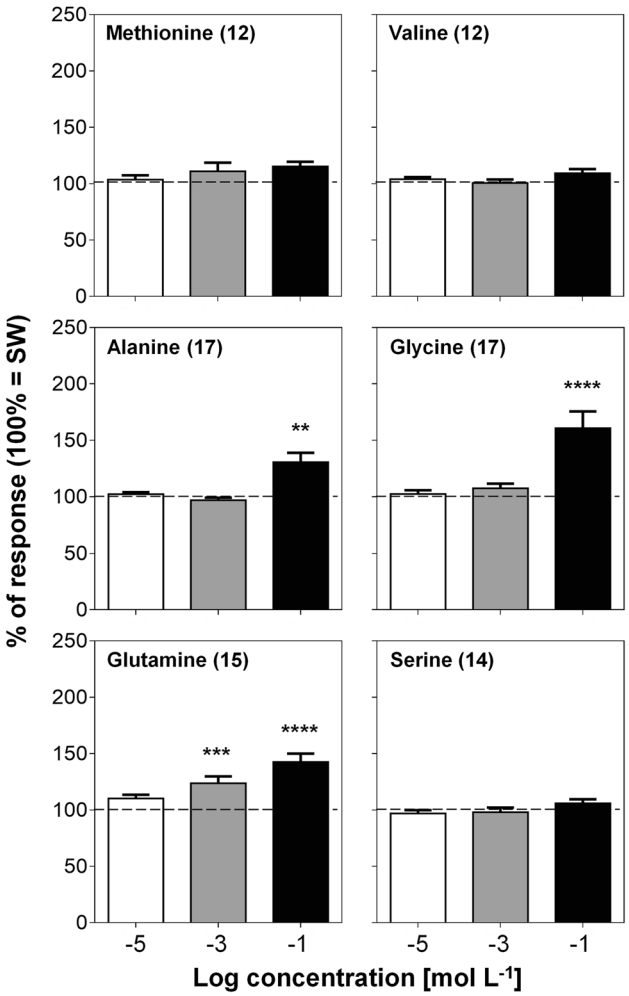

Fig. 2 Normalized movement rate of sea urchins, recorded as mean square difference in light intensity (summed peak heights for each preparation during a 2-min stimulation) ± se (vertical bars), following supply of the essential amino acids methionine and valine and the non-essential amino acids alanine, glycine, glutamine and serine, compared to seawater (SW = 100% of response, dashed line). **, *** and **** indicate significant differences for $p < 0.01$, $p < 0.001$ and $p < 0.0001$, respectively (Dunnett's multiple comparison test subsequent to One-way ANOVA for valine and alanine; Dunn's multiple comparison test subsequent to the Friedman test for methionine, glycine, glutamine and serine) with respect to SW. Values are presented as means ± standard errors (vertical bars). The number of sea urchins tested for each amino acid is indicated in brackets

increasing the sea urchin movement rate to 123.7 ± 6.1% and 142.5 ± 7.5%, compared to Control (MSD = 94002 ± 1826), when used at a concentration of 10^{-3} and 10^{-1} mol L^{-1}, respectively. Conversely, no significant changes in the movement rate were detected when the animals were presented with the essential amino acid serine, regardless the tested concentration.

As for the sugars (Fig. 3), the sea urchins were completely insensitive to fructose, while they responded to its isomer glucose, which significantly enhanced the movement rate of the animals to 113.3 ± 4.3, 120.6 ± 6.3 and 136.1 ± 10.4% with respect to the Control (MSD = 95738 ± 3598) at the three concentration 10^{-5}, 10^{-3} and 10^{-1} mol L^{-1}, respectively. As shown in the same figure, the disaccharide sucrose never affected the movement rate of the sea urchins at any tested concentration. Finally, Spirulina showed high stimulating effectiveness, affecting the sea urchins' motility in a dose-dependent manner (Fig. 4). In fact, even if the lowest dose (0.01 mg mL^{-1}) was ineffective compared to the Control (MSD = 102,959 ± 3423), at 0.1 mg mL^{-1} the blue-green algae evoked an increase in movement rate of the animals to 133.5 ± 5.5%, and further increased their response to 202.6 5 ± 9.1% when tested at 1 mg mL-1. At the highest dose (5 mg mL^{-1}), the algae enhanced the animal movement rate up to 184.8 ± 7.6% with respect to Control, but this response did not statistically differ from that detected at 1 mg mL^{-1}.

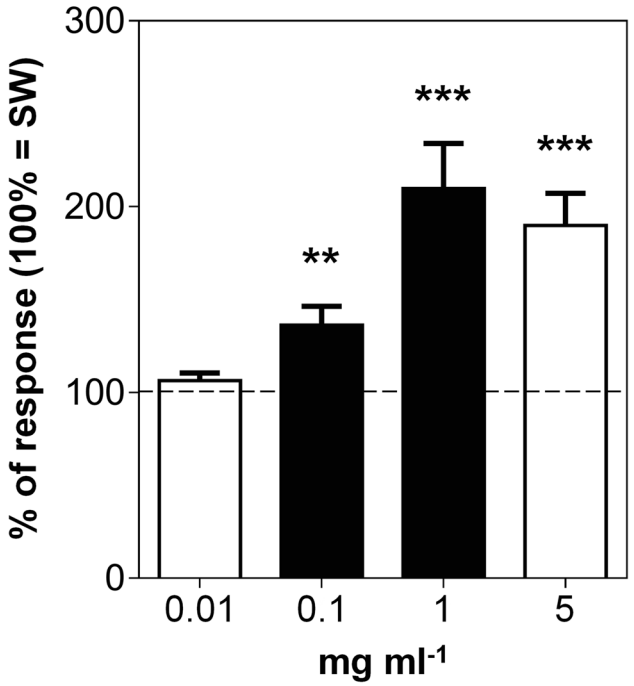

Fig. 4 Normalized movement rate of sea urchins, recorded as mean square difference in light intensity (summed peak heights for each preparation during a 2-min stimulation) ± se (vertical bars), following supply of Spirulina, compared to seawater (SW = 100% of response, dashed line). ** and *** indicate significant differences for $p < 0.01$ and $p < 0.001$, respectively Dunnett's multiple comparison test subsequent to One-way ANOVA) with respect to SW. Filled boxes indicate significant differences between a concentration and the next lower ($p < 0.05$; Tukey's multiple comparison test subsequent to One-way ANOVA). Data were obtained from 15 sea urchins

Discussion

Based on the assumption that the overall movements of spines, pedicellariae, and tube feet could be classified as a behavioural indicator of chemical detection for a sea urchin

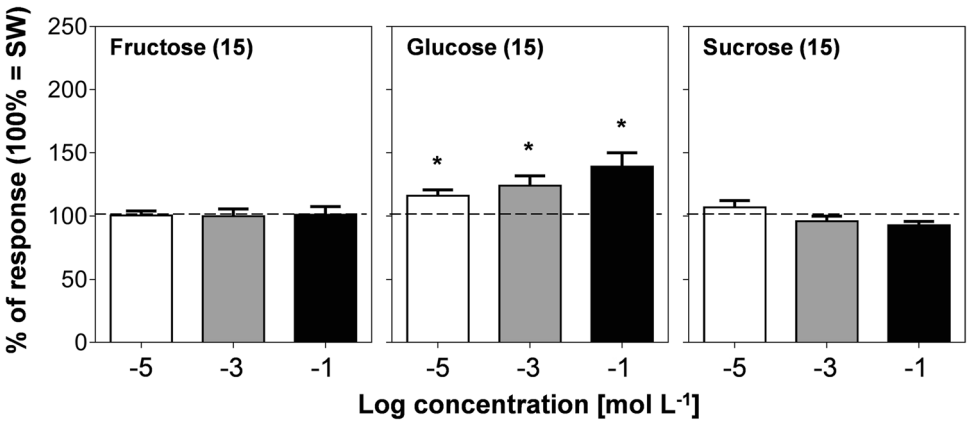

Fig. 3 Normalized movement rate of sea urchins, recorded as mean square difference in light intensity (summed peak heights for each preparation during a 2-min stimulation) ± se (vertical bars), following supply of the monosaccharides fructose and glucose and the disaccharide sucrose, compared to seawater (SW = 100% of response, dashed line). * indicates significant differences for $p < 0.05$ (Dunnett's multiple comparison test subsequent to One-way ANOVA) with respect to SW. The number of sea urchins tested for each amino acid is indicated in brackets

species, we investigated such responses under the stimuli of *Ulva* and *Ulva*-related compounds. We used a bioassay, already validated for sea urchins, in response to chemical stimulation (Solari et al. 2021a, b). On the basis of our experiments, this green alga was found to be a stimulant and attractant for the Mediterranean sea urchin *P. lividus*. This behaviour has already been demonstrated by previous research on other invertebrates and other sea urchins species (Sakata et al. 1985; Akakabe and Kajiwara 2008; Cyrus et al. 2015; Etwarysing et al. 2017; Kwon et al. 2021).

Our results have highlighted that all forms of *Ulva*, fresh, defrosted, and fragmented *Ulva* cultured water, resulting in an effective stimulus, evoking strong responses in sea urchins with robust activation of spines and tube feet. Among the different *Ulva* tested, the defrosted one was the most stimulating. This result is particularly interesting because it highlights how the post-harvested storage method of this macroalgae can affect the chemostimulatory properties and, almost certainly, its ingestion in our model species. The benefit of using defrosted *Ulva* under rearing conditions is that such a process does not alter the shape and consistency of this algae, as it occurs instead when processing the biomass using freeze-drying and fragmentation. Moreover, such biomass shows similar stability in the water as the fresh biomass, unlike observed with commonly "pelleted" prepared diets (Secci et al. 2020). In addition, a problem facing the use of wild *Ulva* in feed applications at the Mediterranean latitudes is that its harvesting is generally seasonal. Thus, seaweed must therefore be preserved and stored to supply year-round production processes. Nevertheless, we acknowledge that the cost of this kind of storage must be considered and evaluated on a case-by-case basis.

Speculatively, we can affirm that from the observations in our farming facility during the routinary feeding processes of reared sea urchins fed with *Ulva* biomass, the amount of uneaten defrosted *Ulva* is lower or absent in respect of the fresh ones (Secci et al. 2020), thus confirming its role not only as an attractant substratum but also as phagostimulant.

Freezing is one of the most popular means of long-term food storage, including macroalgae, allowing better preservation of the taste, texture, and nutritional value of foods than any other preservation technology (Choi et al. 2012). Indeed, by transforming most of the liquid water into ice, freezing greatly slows the physical and biochemical changes involved in food deterioration, together with the growth and reproduction of spoilage microorganisms. In general, seaweed processed with a freeze/thaw cycle increases protein precipitation and doubles the total protein yield (Kadam et al. 2015; Abdollahia et al. 2019; Obluchinskaya and Daurtseva 2020). Indeed, freezing causes the formation of crystals inside the cell and cell membranes to rupture, leading to the release of intracellular fluid. Thus, the freeze-thaw process can facilitate the release of phagostimulants in the water. Similarly, the high response observed in our experiments from urchins exposed to fragmented and freeze-dried *Ulva* could be driven by the substances released into the water following the transformation of the biomass.

Although drying processes can alter the levels of certain natural nutrients in seaweed (Wong and Cheung 2001; Choi et al. 2012), this mechanism was not observed when the freeze-dried *Ulva* was used in our trials. On the other hand, when the freeze-dried spirulina was used, it resulted as the most effective stimulus, acting in a dose-dependent manner. The response to spirulina should be further investigated to identify if the strong activation of the tube feet, pedicellaria, and spines is an attractive or repulsive response. Recent studies confirmed several benefits of Spirulina-enriched diets which improved gonadic growth and gamete production in *P. lividus* (Cirino et al. 2017) and enhanced the content of astaxanthin, a carotenoid with antioxidant properties and beneficial effects for various degenerative diseases, in the egg of *Arbacia lixula* (Galasso et al. 2018).

Of the other compounds tested, sea urchins responded to glucose, but not to its isomer fructose, while sucrose resulted ineffective. Among the amino acids tested, glycine and glutamine at the highest dosages, and alanine, produced a significant response highlighting a concentration–response relationship.

This study indicates that the *P. lividus* exhibits a chemosensory selectivity towards different trophic stimuli, indicating that the response is not related to the nutritional value of a single compound. The ability of this species to selectively detect some components rather than others might have an ecological meaning. New studies using two-choice treatments (Campbell et al. 2001) could help to identify the role of *Ulva* in the animal's feeding choice (and the ingestion rate), in our case the sea urchin *P. lividus*, but also other reared species.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10811-023-02925-0.

Acknowledgements The author wishes to thank the Coordinator of COST Action 20106 Prof. Muki Shpigel.

Author contribution All of the experiments, data analysis, and manuscript preparation were conducted by P.S., P.A.; V.P.; Data curation, P.S., P.A., V.P., A.A. Writing – Original Draft Preparation, P.S., P.A.; Writing – Review & Editing, P.S., V.P., P.A., A.A, V.M, D.M. All the authors reviewed and approved the manuscript.

Funding Open access funding provided by Università degli Studi di Cagliari within the CRUI-CARE Agreement. These research activities were carried out under the support of the project EU COST Action 20106 (Seawheat), Academic fund from the University of Cagliari (project Aquaviva).

Data availability All data generated or analysed during this study are included in this published article and its supplementary information file.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest There are no conflicts of interest to declare in this work.

References

Abdollahia M, Axelssona J, Carlssona N-G, Nylundb GM, Albersc E, Undelanda I (2019) Effect of stabilization method and freeze/thawaided precipitation on structural and functional properties of proteins recovered from brown seaweed (*Saccharina latissima*). Food Hydrocoll 96:140–150

Akakabe Y, Kajiwara T (2008) Bioactive volatile compounds from marine algae: feeding attractants. J Appl Phycol 20:661–664

Atema J (2012) Aquatic odour dispersal fields: opportunities and limits of detection, communication, and navigation. In: Bronmark C, Hansson LA (eds) Chemical ecology in aquatic systems. Oxford University Press, Oxford, pp 1–18

Bargmann CI (2006) Comparative chemosensation from receptors to ecology. Nature 444:295–301

Burke RD, Angerer LM, Elphick MR, Humphrey GW, Yaguchi S, Kiyama T, Liang S, Mu X, Agca C, Klein WH, Brandhorst BP, Rowe M, Wilson K, Churcher AM, Taylor JS, Chen N, Murray G, Wang D, Mellott D, Olinski R, Hallböök F, Thorndyke MC (2006) A genomic view of the sea urchin nervous system. Dev Biol 300:434–460

Cai J, Lovatelli A, Aguilar-Manjarrez J, Cornish L, Dabbadie L, Desrochers A, Diffey S, Garrido Gamarro E, Geehan J, Hurtado A, Lucente D, Mair G, Miao W, Potin P, Przybyla C, Reantaso M, Roubac R, Tauati M, Yuan X (2021) Seaweeds and microalgae: an overview for unlocking their potential in global aquaculture development. FAO Fisheries and Aquaculture Circular No. 1229. FAO, Rome

Campbell AC (1983) Form and function of pedicellariae. Echinoderm Stud 1:139–167

Campbell AC, Coppard S, D'Abreo C, Tudor-Thomas R (2001) Escape and aggregation responses of three echinoderms to conspecific stimuli. Biol Bull 201:175–185

Campbell I, Kambey C, Mateo J, Rusekwa S, Hurtado A, Msuya F, Stentiford GD, Cottier-Cook E (2019) Biosecurity policy and legislation for the global seaweed aquaculture industry. J Appl Phycol 32:2133–2146

Choi J-S, Lee B-B, An S, Sohn JH, Cho KK, Choi S (2012) Simple freezing and thawing protocol for long-term storage of harvested fresh *Undaria pinnatifida*. Fisheries Sci 78:1117–1123

Cirino P, Ciaravolo M, Paglialonga A, Toscano A (2017) Long-term maintenance of the sea urchin *Paracentrotus lividus* in culture. Aquac Rep 7:27–33

COST Action (20106) CA20106 - Tomorrow's 'wheat of the sea': *Ulva*, a model for an innovative mariculture (seawheat). https://www.cost.eu/actions/CA20106/

Cyrus MD, Bolton JJ, Scholtz R, Macey BM (2015) The advantages of *Ulva* (Chlorophyta) as an additive in sea urchin formulated feeds: effects on palatability, consumption and digestibility. Aquacult Nutr 21:578–591

Etwarysing L, Bolton JJ, Beukes DR, Macey BM, Cyrus MD (2017) Feed stimulants from green alga *Ulva* for sea urchin *Tripneustes gratilla*. Phycologia 56:49

Galasso C, Orefice I, Toscano A, Vega Fernandez T, Musco L, Brunet C et al (2018) Food modulation controls astaxanthin accumulation in eggs of the sea urchin *Arbacia lixula*. Mar Drugs 16:186

Kadam SU, Álvarez C, Tiwari BK, O'Donnell CP (2015) Processing of seaweeds. In: Tiwari BK, Troy DJ (eds) seaweed sustainability. Food and non-food applications. Elsevier, London, pp 61–78

Kamio M, Derby CD (2017) Finding food: how marine invertebrates use chemical cues to track and select food. Nat Prod Rep 34:514–528

Kwon MY, Byung HJ, Hyung GK, Jeong HK (2021) Feeding behaviors of a sea urchin, *Mesocentrotus nudus*, on six common seaweeds from the east coast of Korea. Algae 36:51–60

Lawrence JM (2013) Sea urchins: Biology and ecology. Developments in aquaculture and fisheries science. Elsevier, Amsterdam

Malavasi V, Sciuto K, Wolf MA, Soru S, Secci M, Addis P, Sfriso A. (2020) First assessment of algal diversity in Santa Gilla lagoon (Sardinia, Italy) in the framework of the aquaculture industry. Riunione Scientifica Annuale Società Botanica Italiana (SBI) Gruppo di Lavoro per l'Algologia. https://www.societabotanicaitaliana.it/Media?c=c754881a-8097-40b7-a86f-dd75da40932e

Mercier A, Hamel J-F (2009) Endogenous and exogenous control of gametogenesis and spawning in echinoderms. Adv Mar Biol 55:1–302

Middleton CA, Nongthomba U, Parry K, Sweeney ST, Sparrow JC, Elliott CJH (2006) Neuromuscular organization and aminergic modulation of contractions in the *Drosophila* ovary. BMC Biol 4:17

Mollo E, Garson MJ, Polese G, Amodeo P, Ghiselin MT (2017) Taste and smell in aquatic and terrestrial environments. Nat Prod Rep 34:496–513

Obluchinskaya E, Daurtseva A (2020) Effects of air drying and freezing and long-term storage on phytochemical composition of brown seaweeds. J Appl Phycol 32:4235–4249

Pereira L (2016) Edible seaweeds of the world. CRC Press, Boca Raton, 463 pp

Pisut DP (2004) The distance chemosensory behavior of the sea urchin *Lytechinus variegatus*. MSc Thesis, Georgia Institute of Technology, Georgia, USA

Pusceddu A, ikhno M, Giglioli A, Secci M, Pasquini V, Moccia D, Addis P (2021) Foraging of the sea urchin *Paracentrotus lividus* (Lamarck, 1816) on invasive allochthonous and autochthonous algae. Mar Env Res 170: 105428.

Prato E, Fanelli G, Angioni A, Biandolino F, Parlapiano I, Papa L et al (2018) Influence of a prepared diet and a macroalga (*Ulva* sp.) on the growth, nutritional and sensory qualities of gonads of the sea urchin *Paracentrotus lividus*. Aquaculture 493:240–250

Raible F, Tessmar-Raible K, Arboleda E, Kaller T, Bork P, Arendt D et al (2006) Opsins and clusters of sensory G-protein-coupled receptors in the sea urchin genome. Dev Biol 300:461–475

Reuter KE, Levitan DR (2010) Influence of sperm and phytoplankton on spawning in the echinoid *Lytechinus variegatus*. Biol Bull 219:198–206

Škaloud P, Rindi F, Boedeker C, Leliaert F (2018) Chlorophyta: Ulvophyceae. In: Büdel B, Gärtner G, Krienitz L, Schagerl M (eds) Freshwater flora of Central Europe – Süßwasserflora von Mitteleuropa, vol 13. Springer, Berlin, 288 pp

Sakata K, Tsuge M, Kamiya Y, Ina K (1985) Isolation of a glycerolipid (DGTH) as a phagostimulant for a seahare, *Aplysia juliana*, from a green alga. Ulva Pertusa Agric Biol Chem 49:1905–1907

Secci M, Ferranti MP, Giglioli A, Pasquini V, Sicurelli D, Fanelli G, Prato E, Secci M, Ferranti MP, Giglioli A, Pasquini V (2021) Effect of temperature and duration of immersion on the stability of prepared feeds in echinoculture. J Appl Aquacult 33:150–164

Shpigel M, Shauli L, Odintsov V, Ben-Ezra D, Neori A, Guttman L (2018) The sea urchin, *Paracentrotus lividus*, in an Integrated Multi-Trophic Aquaculture (IMTA) system with fish (*Sparus aurata*) and seaweed (*Ulva lactuca*): Nitrogen partitioning and proportional configurations. Aquaculture 490:260–269

Sloan NA, Campbell AC (1982) Perception of food. In: Jangoux M, Lawrence JN (eds) Echinoderm Nutrition. AA Balkema, Rotterdam pp 3–23

Snyder N, Snyder H (1970) Alarm response of *Diadema antillarum*. Science 168:276–278

Solari P, Melis M, Sollai G, Masala C, Palmas F, Sabatini A, Crnjar R (2015) Sensing with the legs: contribution of pereiopods in the detection of food-related compounds in the red swamp crayfish *Procambarus clarkii*. J Crustac Biol 35:81–87

Solari P, Sollai G, Masala C, Loy F, Palmas F, Sabatini A, Crnjar R (2017) Antennular morphology and contribution of aesthetascs in the detection of food-related compounds in the shrimp *Palaemon adspersus* Rathke, 1837 (Decapoda: Palaemonidae). Biol Bull 232:110–122

Solari P, Pasquini V, Secci M, Giglioli A, Crnjar R, Addis P (2021a) Chemosensitivity in the sea urchin *Paracentrotus lividus* (Echinodermata: Echinoidea) to food-related compounds: An innovative behavioral bioassay. Front Ecol Evol 9:749493

Solari P, Sollai G, Palmas F, Sabatini A, Crnjar R (2021b) A method for selective stimulation of leg chemoreceptors in whole crustaceans. J Exp Biol 224:jeb243636

Vadas RL (1977) Preferential feeding: an optimization strategy in sea urchins. Ecol Monogr 47:337–371

Van Alstyne KL, Wolfe, GV, Freidenburg TL, Neill A, Hicken C (2001) Activated defense systems in marine macroalgae: evidence for an ecological role for DMSP cleavage. Mar Ecol Prog Ser 213:53–65

Weissburg MJ (2000) The fluid dynamical context of chemosensory behavior. Biol Bull 198:188–202

Wong KH, Cheung PC (2001) Influence of drying treatment on three *Sargassum* species. J Appl Phycol 13:43–50