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Chronic exposure of the model organism *Aurelia coerulea* (Scyphozoa, Cnidaria) to "first generation" artificial sweeteners

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ABSTRACT

Artificial sweeteners (ASs) are low-calories additives which utilization has increased in the last decades, reaching an economic revenue of ca. 26 billion US dollars in 2024. Through direct discharge or inadequate treatment of wastewater, they can spread in soil and reach the marine environment. Although the concrete risk of pollution deriving from these substances, their influence on coastal benthic communities is still unknown. We tested the effects of two ASs (aspartame and saccharin) through a chronic toxicity test on the cnidarian model species *Aurelia coerulea* polyps. We exposed polyps to a solution of aspartame and saccharine with filtered seawater (100 mg/l according to the REACH legislation for xenobiotics (Regulation 1272, Supplement No. 1, 2008), and measured their health status, asexual reproduction, somatic growth and mortality rates at 3-days intervals for ca. 60 days. Spectrophotometric analysis showed the degradation of aspartame, while saccharine remained unvaried indicating stability in water solution. Saccharin induced a moderate slowdown of growth, not impacting reproduction and health, while aspartame affected polyps by arresting growth, asexual reproduction, and triggering regression process. Afterwards, a full regeneration was reported with registered values similar to control group, suggesting acclimatization. Even though no mortality was observed, a delay in budding and growth rates could result in a carry-over effect on ephyrae production and adult populations. Since polyps are recognized as primary responsible driving jellyfish outbreaks, their altered physiological processes could affect the population dynamics of planktonic life-stages and consequently the trophic relationships of the habitat both at small and large scale.

1. Introduction

Food selection and consumption by humans is strongly influenced by the taste of ingredients and the dietary recommendations for a healthy lifestyle. The preferences on offered products directly condition the food industry and the marketing strategies (Sørensen et al., 2003). Artificial sweeteners (ASs) are low-caloric additives that mimic the taste of sugar in recipes and prepared foods, being suitable for hypocaloric diets (Chattopadhyay et al., 2014). Thanks to their sweetening power, up to 300 times stronger than sugar, they are widely used to control health diseases such as diabetes and obesity (Whitehouse et al., 2008). Moreover, artificial sweeteners are employed as additives in animal feeds, pharmaceuticals, and care products, reaching a revenue of approximately 25.6 billion USD in 2024 (Statista, 2024). The first synthesized artificial sweetener was saccharin by Remsen and Fahlberg in 1879 (Weihrauch and Diehl, 2004) to overcome the sugar shortage during World War II (Mishra et al., 2015). In the following years, saccharin started to be used to reduce calories in food. Since then, many other compounds were synthetized to improve efficiency and taste of food, being defined sweeteners of "first generation" (saccharin, cyclamate,

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aspartame) and of "new generation" (acesulfame-K, sucralose, alitame, neotame) (Mishra et al., 2015; Weihrauch and Diehl, 2004). Their use has exponentially increased over the second half of the 20th century to offer low-calorie foods by maintaining the original taste (Chattopadhyay et al., 2014). Following the massive utilization of these compounds and their worldwide distribution, studies on their effects on the human organism and on the environment have been implemented. In 1970, cyclamate has been banned by the U.S. Food and Drug Administration (FDA), opening the way to animal experiments and trials to test the carcinogenic potential of these substances (Mishra et al., 2015). Some trials highlighted negative impacts on animal models, including the alteration of metabolic pathways and the dysfunction of multiple biological systems. For instance, the increase in bladder cancer onset was reported in rats fed with saccharin and aspartame, together with haematopoietic and lymphoid tumours (Fukushima et al., 1983; Soffritti et al., 2014; Tibaldi et al., 2020). A large survey on more than 100, 000 human adults evidenced the increase in breast and obesity-related cancer risk due to the protracted consumption of sweetener table-tops or sugar-free drinks (Debras et al., 2022). Hepatotoxicity, low birthweight, and lymphomas occurred in breast-fed animals and humans (Whitehouse et al., 2008). On the contrary, several studies highlight the absence of correlation between cancer risk and sweeteners consumption (Armstrong and Doll, 1975; Gallus et al., 2007; Hoover and Hartge Strasser, 1980), explaining that the onset of cancer in rats was not comparable to humans due to different carcinogenic mechanisms (Mishra et al., 2015). Although their controversial and yet still debated effects on human health, several ASs are considered emerging contaminants due to their dispersion and persistence in soils and aquatic bodies (Lange et al., 2012; Lillicrap et al., 2011; Praveena et al., 2019; Ren et al., 2016; Sang et al., 2014). ASs have been detected in freshwaters, grounds and sediments of North America, Europe and Asia mostly because of the inadequate treatment of urban wastewaters, such that they are employed as tracers for leakages around sewage plants (Fu et al., 2020; Spoelstra et al., 2020; Van Stempvoort et al., 2011). ASs likely enter the marine environment through rainfalls, rivers and direct discharge, reaching concentrations that range from nano-to micrograms per liter (Brumovský et al., 2017; Corada-Fernández et al., 2017; Fernandez et al., 2025; Gvozdić et al., 2023; Lange et al., 2012; Lillicrap et al., 2011; Sang et al., 2014). Despite being frequently detected in seawater in their original form, the impact of commonly used ASs on the coastal benthic organisms is largely unknown (Berset and Ochsenbein, 2012; Brumovský et al., 2017; Colín-García et al., 2022; Gvozdić et al., 2023; Kobetičová et al., 2018). Moreover, their degradation may originates by-products that could represent a potential threat. Aspartame is degraded in methanol (further degraded into toxic formaldehyde and formic acid), phenylalanine and aspartic acid (Pattanaargson et al., 2001; Sawma et al., 2025). These molecules alone have been shown to impair not only human health (*e.g.,* hormonal disease, damages to DNA and proteins), but also aquatic life, for instance causing algal blooms, reducing oxygen levels and disrupting microbial activity, leading to a cascade effect that involves nutrient cycling (Sawma et al., 2025).

Aiming at evaluating the impacts of "first generation" ASs on aquatic organisms, we tested two of the most distributed ASs, aspartame (E 951, https://www.efsa.europa.eu) and saccharin (E 954, https://www.efsa.europa.eu) in seawater solution, in the concentration of 100 mg/L following the REACH Legislation for xenobiotics ("Regulation - 1272/2008 - EN - clp regulation - EUR-Lex," n.d.). According to the present regulation, this represents the highest permissible concentration to evaluate chronic toxicity and the registration of the substance as contaminant (Kobetičová et al., 2018; Sawma et al., 2025). Moreover, such high concentration has been reported to cause a reduction of the growth rate and reproductive issues in aquatic species, being effectively harmful (Eriksson Wiklund et al., 2014; Heredia-García et al., 2019; Kobetičová et al., 2018; Sawma et al., 2025). Aspartame is a linear dipeptide methyl ester with chemical name N-L-asparty-L-phenylalanine-1-methyl ester, constituted of two naturally occurring amino acids: aspartic acid and phenylalanine. The digestive process by humans lead to the complete metabolic breakdown in these two amino acids and methanol. Saccharin stands for 1,2-benzoisothiazol-3(2H)-one-1,1-dioxide, and remains unchanged through the digestive system to excretion (Lange et al., 2012).

The potential effects due to chronicle exposure to these two ASs were evaluated on a marine benthic cnidarian species, *Aurelia coerulea* von Lendenfeld, 1884 (Cnidaria, Scyphozoa, Semaeostomeae). The genus *Aurelia* Lamarck, 1816 includes 26 jellyfish species with a worldwide distribution, facilitated by anthropic dispersion of planktonic forms mainly through ballast waters and by the transport of benthic stages attached on the bottom of vessels and other tools (*e.g.*, ropes, engines) and submerged infrastructures (Dawson and Martin, 2001). Indeed, the life cycle of the species is characterized by the alternation of planktonic (planula, ephyra and medusa) and benthic phases (polyp, strobila). Adult medusae are gonochoric and reproduce sexually, giving birth to ciliate planula larvae. Planulae settle on a substrate and metamorphose into polyps, which population can increase in number through several pathways of asexual reproduction (*e.g.*, lateral budding). With appropriate external conditions, polyps can metamorphose in strobila, a benthic form constituted by many layers each one representing an ephyra, that detaches and grows into an adult medusa (Suzuki et al., 2019). The population of polyps is closely linked to the availability of the substrate, predominantly confined in coastal areas, where it can flourish thanks to eutrophication and of the presence of submersed structures (Kim et al., 2024). Polyps play a key role in controlling the jellyfish seasonal outbreaks through strobilation. Thanks to their resistance to wide ranges of temperature, salinity and food availability, *Aurelia* species are among the most common bloom-forming species worldwide (Marques et al., 2021; Xing et al., 2020). The alteration of the polyp population could reflect on density, abundance and frequency of adult medusae blooms, leading to impacts both on pelagic trophic webs and on sea-related anthropic activities (Lucas and Horton, 2014; Marques et al., 2021).

The species herein analysed, *A. coerulea* is frequently employed as model organism by ecotoxicologists to test the effects of several anthropogenic pollutants, chemical substances, heavy metals, pesticides, etc. on different stages of its life cycle (Ringwood et al., 2025). Carry-over effects from polyps to ephyrae have been reported after short-term exposure to two pesticides (atrazine and chlorpyrifos) by Olguín-Jacobson et al. (2021). As well, polyps suffered oxidative stress that reflects on their reproduction pathway (Liu et al., 2024) and leading to mortality and regeneration-regression process when subjected to copper exposure (Roveta et al., 2020). Lucas and Horton (2014) demonstrated the mortality and deformity of polyps when treated with high concentration of copper or silver solutions, although decreasing through time and showing increasing budding rates, witnessing the tolerance of these organisms. Given the importance of the polyp stages in controlling adult populations and in the formation of blooms (Marques et al., 2021), this species was selected as experimental organism.

2. Materials and methods

2.1. Experimental procedures for Aurelia coerulea

Aurelia coerulea polyps were collected at the Aquarium of Cattolica (Italy), transferred to the Marine Zoology Laboratory of Polytechnic University of Marche and reared in seawater filtered at 0.22 μm, at constant temperature (24 ± 1°C) and salinity (38 ± 1 PSU) with 16:8 h of light:dark photoperiod (according to Gregorin et al., 2025). Polyps were acclimated to the new conditions for 30 days prior to start the exposure. During this period they were regularly fed with 3-day-old nauplii of the brine shrimp *Artemia salina* (Linnaeus, 1789) three times per week. All reagents, *i.e.,* aspartame, saccharin (Fig. 1) and KCl were

purchased from SIGMA (IT) and Water milliQ (Millipore system) was used to prepare all solutions. Artificial sweeteners are highly water-soluble and commonly tested in aquatic exposure systems (*e.g.*, Buerge et al., 2009; Wiklund et al., 2012a). The experiment was designed to test two treatments represented by artificial sweeteners solutions: 1) aspartame, "ASP" and 2) saccharin, "SAC". The control treatment was represented by filtered seawater ("FSW"). The solutions at nominal concentration of 100 mg/l in saccharin and aspartame were obtained by dissolving the appropriate quantity of commercial sweetener powder in FSW, by the use of a magnetic stirrer and by warming up the solution to 60°C to facilitate dissolution. Each commercial saccharin tablet (Saccarina Roberts, L. Manetti–H. Roberts & C. S.p.A., Italy) contained sodium bicarbonate, tartaric acid and 26 mg of saccharin, while each aspartame tablet (Suaviter Sugar Control Zero Classic, Desa Pharma S.r.l., Italy) contained lactose, aspartame (20 mg), sodium carboxymethylcellulose and leucine. To start the experiment, eighteen fully developed polyps were selected similar in mouth disk diameters and without swelling tissue indicating the formation of a lateral bud. Mouth disk diameter is the most conservative measure for *Aurelia* spp. polyps (Gambill and Jarms, 2014) and provides a similar growth status of individuals, also referring to previous experimental procedures performed on the same species (Gregorin et al., 2024). Polyps were placed in three 6 -wells-plates, one polyp per well, one plate per treatment including the control (Fig. 2). The chronical exposure of polyps to the treatments was carried out for 56 days (from June 12 to August 7, indicated as 12_06 and 7_08). The first day (12_06) was the pre-exposure sampling time, the exposure started the day after (13_06) and thereafter data were collected two times per week (sampling times, Fig. 2) by direct observation and photographs till the end of exposure (7_08). An amount of 12 ml of FSW, ASP and SAC solutions were added to the wells and replaced twice a week. During this period polyps were kept to stable external conditions, feeding was controlled by giving three *A. salina* nauplii directed on each polyp's tentacles through a micropipette (Gilson P20) after the water renewal.

2.2. Data collection procedures

The analysed response variables were: 1) Growth rate, measured by the increase of the Mouth Disk Diameter (MDD, mm), defined as the longest diameter from one point on the oral rim to the opposite, passing through the mouth (Gambill and Jarms, 2014); 2) Asexual reproduction rate (%), evaluated by the number of individuals produced via asexual reproduction, through photos and direct observations; 3) Health status, by using a reference scale assigning to each polyp the scores 1 (optimal), 0.5 (intermediate) and 0 (bad, corresponding to degenerated polyps) (Table 1). The health scale was optimized by preliminary observations; 4) Mortality rate (%). Mortality was considered when health score 0 was prolonged leading to the death of the individual, to the loss of tissue portions and complete disappearance. Photos were taken twice a week with a Nikon Z50 camera and processed in the software *ImageJ* (Rasband, 2012) for the measurements of MDDs. Simultaneously, data on asexual reproduction, health and mortality data were gathered twice a week before the water change to avoid external disturbance to individuals. The total number of sampling points was 18, from 12_06 (immediately prior to start the experiment) to 7_08 (the last observation time) (Fig. 2).

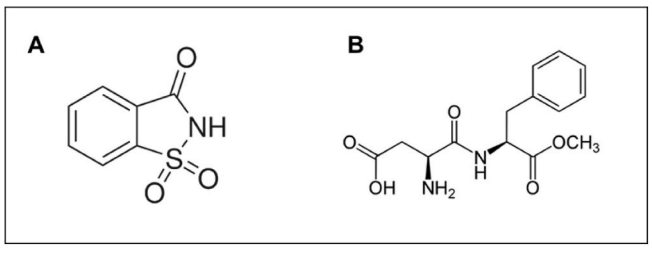

Fig. 1. Chemical structures of the investigated compounds: A) Aspartame and B) Saccharin.

2.3. Spectrophotometric analysis

Spectrophotometric analyses were run using two sweeteners in concentration 100 mg/L prepared in KCl (0.1 M) aqueous solution. The solutions were prepared in Milli-Q water and kept in the dark and at 4°C until analysis. The stability tests were run for 30 days. UV-Vis absorption spectra were recorded using an Evolution™ One Plus UV-Vis Spectrophotometer (Thermo Scientific™, Waltham, MA, USA). Samples were placed in a 1-cm-pathlength quartz cuvette and scanned from 190 to 340 nm with a scan rate of 600 nm/min. Prior to each run, the system was blanked with Milli-Q water.

2.4. Statistical analyses

The comparison of the MDDs of all polyps in pre-exposure conditions was tested through one way analysis of variance (ANOVA), after checking normal distribution (Shapiro-Wilk's test) and homoscedasticity of data (Cochran's test). Statistical analyses on data collected from the start to the end of exposure were performed by using the repeated-measures (RM) ANOVA, after checking the compliance with assumption of homogeneity of variances (Cochran's test) and sphericity (Mauchley's test). The multivariate Pillai-Barlett trace (Pillai, 1965) was used to test for between-subject effects because particularly robust to deviations from sphericity. Further planned comparisons were made to look for significant differences of specific sampling times between and within treatments via *t*-tests or Welch's tests, when different sample size did not allow for paired comparisons. The significant effects of time, treatment and their interaction were considered with p values ≤ 0.05 (95% confidence intervals). Statistica 7 (Statsoft) and R version 4.2.2 (R Core Team, 2021) were used for analyses.

3. Results

3.1. Growth rate

At the beginning of the experiment, all polyps had similar mouth disk diameters (MDDs, Fig. 3A; Fig. 4) (mean ± standard error, SE = 1.27 ± 0.02 mm, ANOVA: $F_{2,15} = 0.389$, $p = 0.685$; Fig. 3 A). Growth of polyps was influenced by the time (RM-ANOVA, Pillai's trace: $F_{17,255} = 20.058$, $p < 0.001$), by the type of treatment (RM-ANOVA, Pillai's trace: $F_{2,15} = 5.394$, $p = 0.017$) and by their interaction (RM-ANOVA, Pillai's trace: $F_{34,\ 255} = 6.396$, $p < 0.001$). Post-hoc planned comparisons highlighted differences between control (filtered sea water, FSW) and saccharin solution (SAC; $t = 2.309$; $p = 0.036$) and between FSW and aspartame solution (ASP; $t = 3.178$; $p = 0.006$) throughout the exposure period. The MDDs of the polyps of the control remained stable (from pre-exposure 1.27 ± 0.05 mm to end exposure 1.23 ± 0.02 mm, $t_{10} = 0.7559$, $p = 0.4672$). Similarly, the size of the polyps subjected to sweeteners showed comparable MDDs by pre- and end-exposure (SAC: 1.23 ± 0.04 mm to 1.20 ± 0.02 mm, $t_{10} = 0.6575$, $p = 0.5257$; ASP: 1.30 ± 0.07 mm to 1.28 ± 0.05 mm, $t_{10} = 0.0537$, $p = 0.9608$). However, both sweeteners affected polyps after ca. 10 days after the start of the exposure. The MDD showed a decline in polyps exposed both to SAC and ASP (Fig. 2 A, starting from 22_06 (FSW *vs.* SAC: $t_{10} = 2.4265$, $p = 0.03566$), becoming more evident from 26_06 (FSW *vs.* SAC: $t_{10} = 2.6356$, $p = 0.2492$; FSW *vs.* ASP: $t_{10} = 4.6382$, $p = 0.0009$). Polyps exposed to SAC showed a low peak on 29_06 (1.02 ± 0.04 mm) and start to re-increase their MDDs thereafter. Polyps exposed to ASP decreased their size to 0.85 ± 0.03 mm between 6_07 and 10_07 showing a more prolonged and heavier MDD reduction. After 10_07, the MDD start to re-increase to reach pre-exposure conditions. Polyps exposed to both ASs showed MDDs similar to those of the control by the end of the

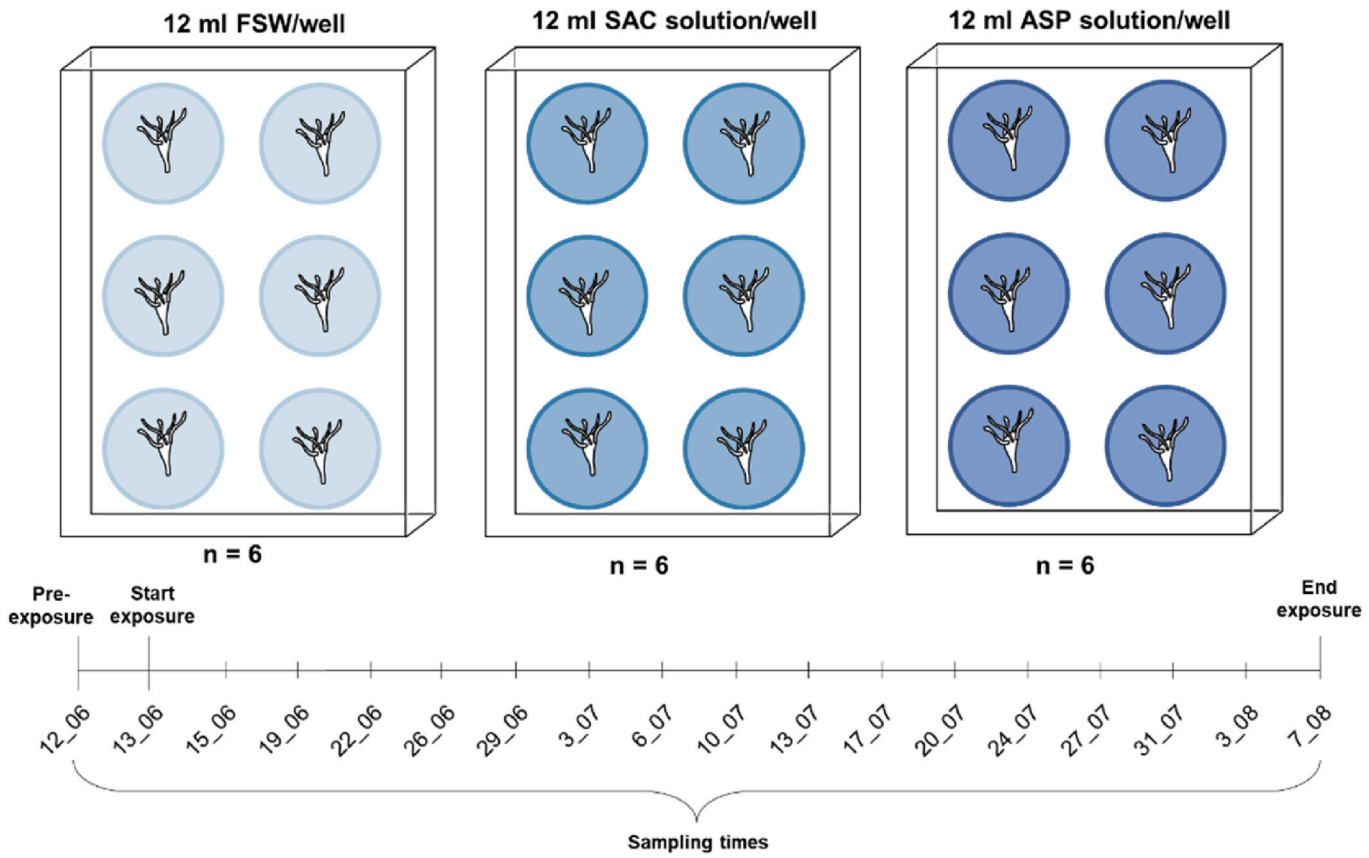

Fig. 2. Scheme of the experimental set-up: control treatment (FSW, light blue), saccharin solution (SAC, blue), aspartame solution (ASP, dark blue). Each plate had six wells containing one *Aurelia coerulea* polyp. The six polyps per treatment constituted the replicates (n = 6). Sampling dates represent the dates in which the response variables were collected, starting from the first pre-exposure sampling (12_06) to the end of the exposure two months later (7_08). The start of the exposure occurred on 13_06. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Health scale developed and used to determine the health status of *Aurelia coerulea* polyps during the exposure to artificial sweeteners.

Health scale	Description
Score 1	Optimal health status, polyps have erected column, extended tentacles, a brilliant white colouring of the tissue, and positively react to feeding, *e.g.* swallowing the *A. salina* nauplii within few seconds.
Score 0.5	Polyps have a roundish shape, calyx is shortened, tentacles are retracted, often folded upon the mouth disk, *A. salina* nauplii are swallowed with difficulty, requiring ca. 30 s. The colour of the tissue is pinkish/brownish. It indicates the initiation of the regression phase.
Score 0	Polyps are completely round, body parts are slightly or not visible (pedal disk, column, tentacles). No or weak feeding activities are registered, the nauplii are not ingested, or swallowed within hours.

experimental period.

3.2. Health status and mortality rate

The decrease in size was reflected by the health status of individuals exposed to aspartame (Fig. 3 B; Fig. 5), that declined from 1 (optimal health status) to 0.17 ± 0.11, indicating degenerative progression with shortened tentacles, retracted column, and indistinct mouth on the mouth disk. Surprisingly, after the lowest peak (on 10_07), their health status started to re-improve to reach score 1 by the end of the experimental period (Fig. 6). Conversely, saccharin did not worse the health of exposed polyps, showing score 1 for the entire duration of the exposure, comparable with the control individuals. Health was affected by time (RM-ANOVA, $F_{5,75} = 14.624$, $p < 0.0001$), by the treatment (RM-ANOVA, $F_{2,15} = 38.043$, $p < 0.0001$) and by their interaction (RM-ANOVA, $F_{10,75} = 14.624$, $p < 0.0001$). Post-hoc planned comparisons confirmed the occurrence of the effect on polyps exposed to aspartame, which significantly different both to SAC ($F_{1,15} = 57.06522$; $p < 0.0001$) and to the FSW ($F_{1,15} = 57.06522$; $p < 0.0001$). The lowest score of health (Fig. 4) was followed by complete recovery of polyps (Fig. 6). Mortality was not registered in any of the experimental replicates.

3.3. Asexual reproduction rate

The asexual reproduction of individuals, expressed as percentage of population increase via budding and production of fully developed and independent polyps, was affected by exposure to artificial sweeteners (Fig. 3 C). The RM-ANOVA highlighted overall effects of time ($F_{15,225} = 18.155$, $p < 0.001$) and of time × treatment ($F_{30,255} = 2.247$, $p < 0.0004$) while the treatment itself did not triggered differences in asexual reproduction of polyps ($F_{2,15} = 3.467$, $p = 0.058$). The number of new clones produced by the end of exposure was on average 2.17 ± 0.75 by FSW polyps, 1.83 ± 1.33 by SAC polyps and 0.83 ± 0.3 by ASP polyps. New individuals produced by polyps exposed to SAC was not different ($t_{10} = 0.5345$, $p = 0.6047$) to those produced by FSW polyps. Conversely, the number of new individuals produced by polyps exposed to ASP was significantly different from the FSW ($t_{10} = 3.0679$, $p = 0.0119$).

3.4. UV-Vis spectral comparison of aspartame and saccharin

The stability in aqueous solution of aspartame and saccharin was assessed by UV-Vis spectrophotometry. Over a 30-day observation

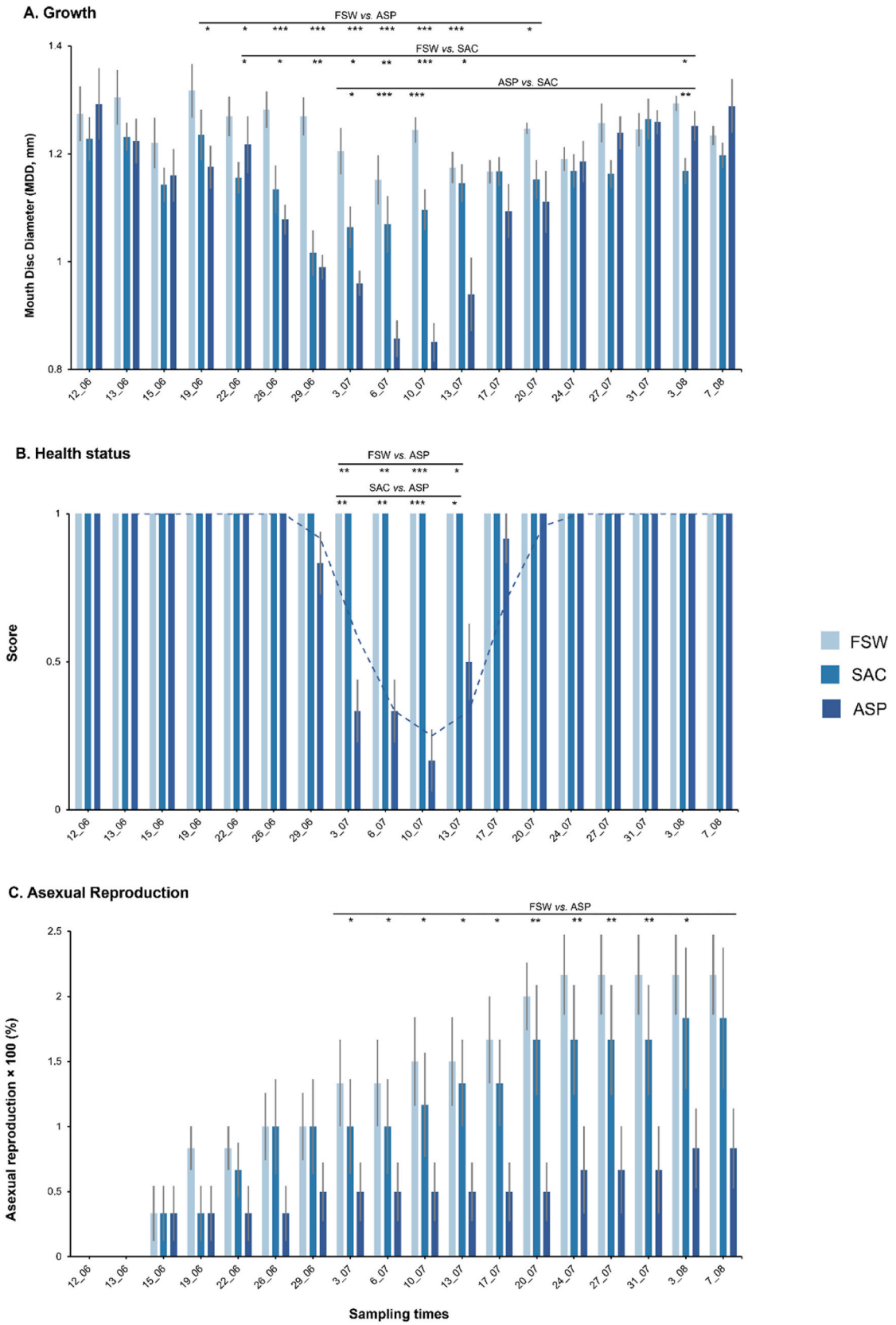

Fig. 3. Average values of A) Mouth Disk Diameter (MDD, mm), B) health status and C) asexual reproduction of *Aurelia coerulea* polyps (n = 6) in the control group (FSW, light blue) and exposed to saccharin solution (SAC, light blue) and aspartame solution (ASP, dark blue). In B, the line refers to the trend of aspartame solution and highlights the negative peak from July 3rd to July 13th. Bars refer to standard error. * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

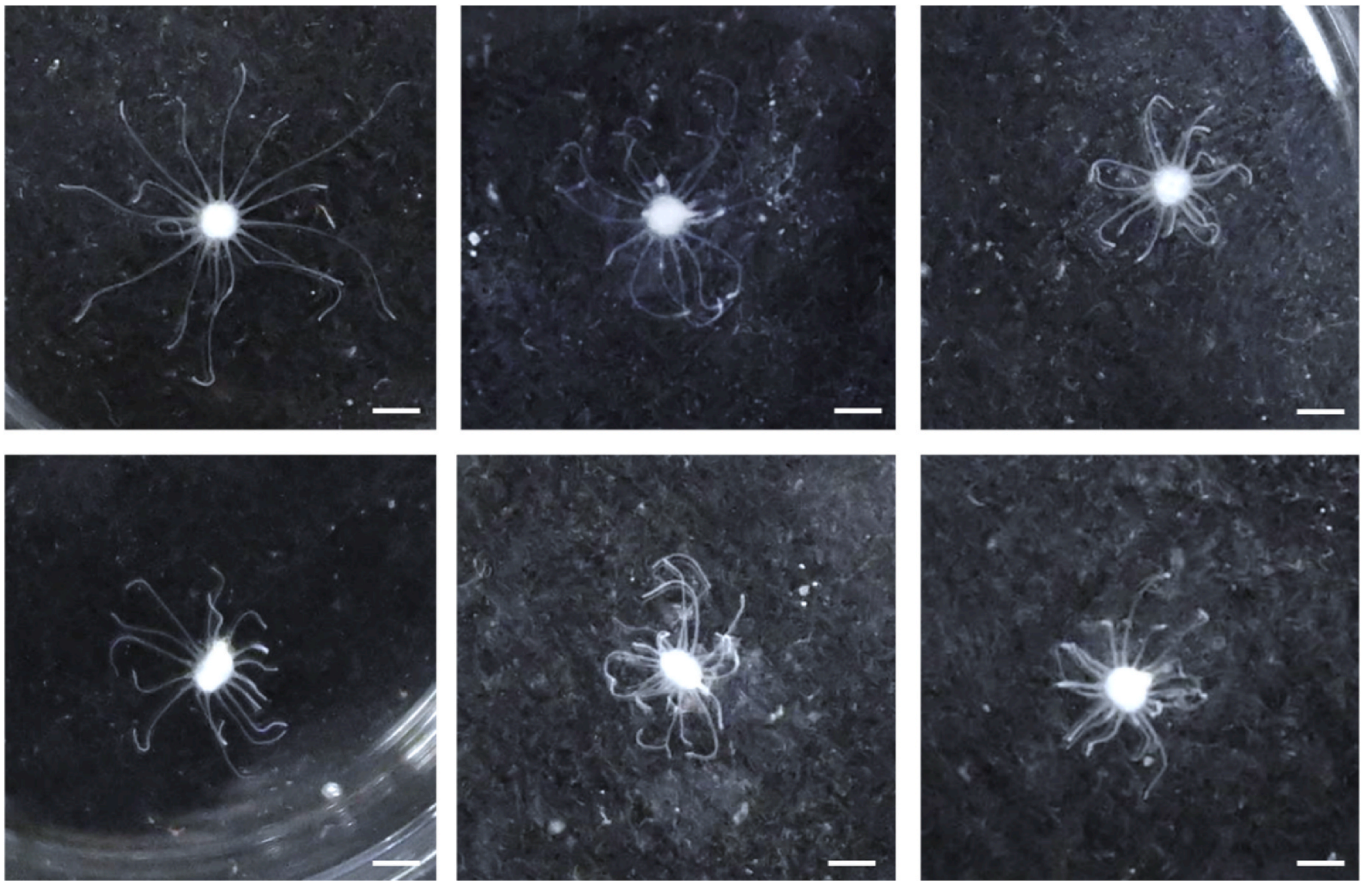

Fig. 4. Six polyps randomly photographed prior to start the experiment (on 12_06) and to be assigned to their experimental group. Each group was formed by 6 individuals ($n_{FSW} = n_{SAC} = n_{ASP} = 6$) for a total of 18 tested polyps.

period, the aspartame solution (ASP) (Fig. 7 A) showed progressive changes in its absorption spectrum, mainly in the 240-260 nm range, corresponding to the $\pi \rightarrow \pi^*$ transitions of the aromatic ring of L-phenylalanine (Lakowicz, 2006) which is one of the compounds that make up aspartame (Fig. 1). The main absorption peak gradually decreased in intensity. This trend follows the known hydrolytic processes that break down aspartame into L-phenylalanine and other products like L-aspartic acid and methanol (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2013). In contrast, the saccharin solution (SAC) (Fig. 7 B) analysed between day 1 and day 30 showed no major spectral shifts or signal intensities: the main absorption band, also attributable to the aromatic ring, remained constant at approximately the same wavelength (roughly 250-260 nm; Lakowicz, 2006), and no new peaks or signal decreases indicative of degradation products were observed. Overall, these data suggest that under the conditions tested, room temperature and no light shielding, aspartame undergoes partial hydrolysis already visible from the day 7, whereas saccharin remains substantially stable over the time period analysed.

4. Discussion

Benthic fauna inhabiting coastal areas is increasingly exposed to contaminants and pollutants of anthropogenic origin (Sánchez-Bayo et al., 2011). Altered natural conditions may interfere with the life-cycle, reproduction, and metabolic pathways of organisms, potentially leading to vast mortality events (Loizeau and Tusseau-Vuillemin, 2014). Contaminants can hinder ecology, physiology and evolutionary pathways of sessile species, that have a physical limitation and must implement a wide spectrum of strategies to face emerging threats, whose effects can persist for long after the source has been identified and eliminated (Mayer-Pinto et al., 2020).

Our results showed that the prolonged exposure to aspartame negatively affected the fitness of *A. coerulea* polyps, impacting their growth, asexual reproduction, and health. Negative effects appeared after 14 days, leading to a ~30-day-degeneration period and followed by a slow but complete recovery. By the end of the experiment, growth and reproduction rates were comparable to pre-exposure and controls. Saccharin had milder effects on *A. coerulea* polyps compared to aspartame, leading to a slowdown of the growth rate with shorter duration, no polyp degeneration and no deterioration of the health status. Indeed, despite being slightly smaller, polyps produced a similar number of buds compared to the control polyps by the end of the exposure. No mortality was recorded by the end of the experiment in any treatment.

The alteration of *A. coerulea* life-history stages is one of the first processes being affected by chronical exposure to contaminants, often leading to the delay of buds' production and strobilation (Liu et al., 2024; Lucas and Horton, 2014). Even without records of mortality, the delayed asexual reproduction and growth could lead to a cascade effect influencing the population dynamics both directly, acting on the number of polyps within a population and their density on the substrate, and indirectly on the birth of ephyrae and adult jellyfish, profoundly altering the trophic dynamics and ecology of the marine ecosystem (Lucas and Horton, 2014; Suzuki et al., 2019). The asexual reproductive processes are strictly connected to the external conditions, among which the food availability, that provides the required energy for the creation of polyps and strobila (Di Camillo et al., 2010; Suzuki et al., 2019). It has been observed that sub-lethal concentrations of contaminants could stimulate the production of buds, probably as a first response to stress (Lucas and Horton, 2014). However, when the contaminants concentration exceeds

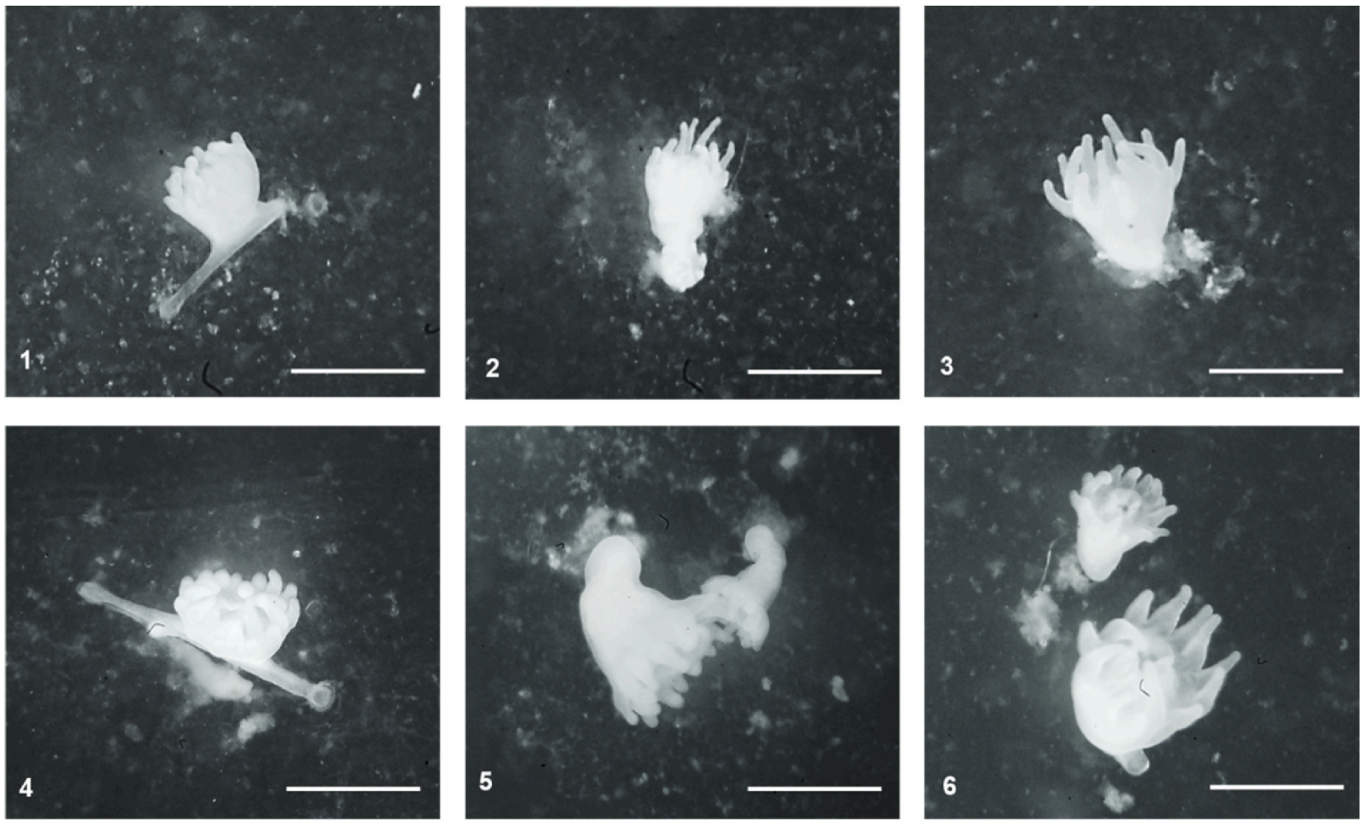

Fig. 5. Polyp (and newborn in photos 5 and 6) exposed to aspartame sweetener (ASP) after ca. 20 days after start of exposure, on 6_07. All polyps showed a health score of 0.5 and 0, presenting shortened tentacles and column, and mouth not well distinguishable on the mouth disk. Scale bar = 1 mm.

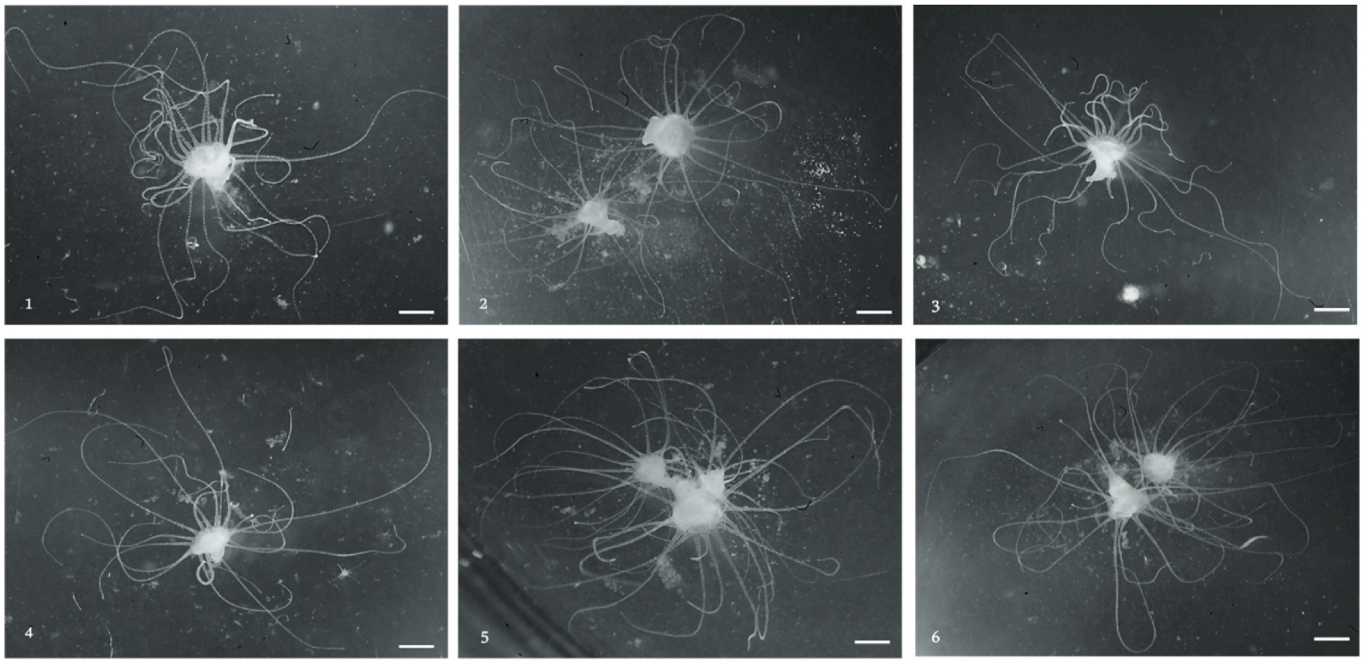

Fig. 6. Polyps (and newborn in 5 and 6) exposed to aspartame solution (ASP) at the end of the experimental period (on 07_08). All polyps recovered and showed a health score of 1. Scale bar = 1 mm.

a certain threshold, one of the first responses is the arrest of budding, probably due to the reallocation of energy that polyps may use to face the emergency and/or to get used to the modified traits of their habitat. Such impairments of benthic life-cycle of *Aurelia* spp. have been reported in controlled conditions under the exposure to heavy metals (copper, silver, cadmium) even though the effects were mitigated by the antagonistic action of two coupled elements (Liu et al., 2024; Lucas and Horton, 2014; Roveta et al., 2020). Similarly, the exposure to pesticides

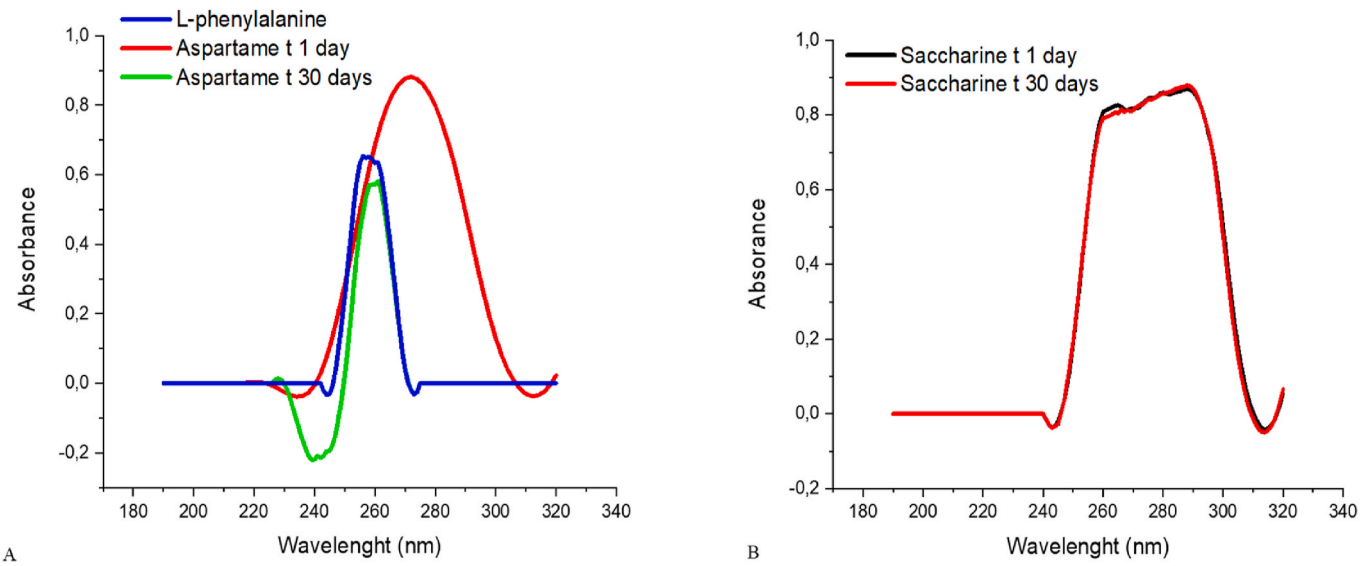

Fig. 7. A) UV-Vis spectra of ASP (day 0 in red, day 30 in blue) and reference L-phenylalanine (green). B) UV-Vis spectra of SAC (day 1 in black, day 30 in red). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

induced a polyp-to-ephyra carry-over effect in two metabolites involved in neurotransmission and osmoregulation (Olguín-Jacobson et al., 2021). The deterioration of the health status and the regression-regeneration cycle of polyps induced by ASs (here, aspartame) could be induced by the oxidative stress through the production of reactive oxygen species. In fact, Aspartame is metabolized into phenylalanine, aspartic acid, and methanol (Shaher et al., 2023). Methanol is further metabolized into formaldehyde, which is known to induce oxidative stress at cell level (Çadirci et al., 2020; Zerin et al., 2015). Studies in animals have shown that aspartame consumption can lead to oxidative stress in various organs, including the brain, liver, and kidneys (Çadirci et al., 2020; Griebsch et al., 2023). Oxidative cell damage has been registered in the liver of the freshwater fish *Carassius auratus* (Linnaeus, 1789) due to exposure of degraded products of acesulfame (Ren et al., 2016) and was responsible of an increase in the occurrence of malformations and mortality of *Danio rerio* (Hamilton, 1822) (zebrafish) early life-stages exposed to sucralose (Colín-García et al., 2022). Similarly, sucralose induced biochemical oxidative responses in organs and blood cells, and at a neuronal level in the freshwater fish *Cyprinus carpio* (Linnaeus, 1789) and crustacean *Daphnia magna* (Straus, 1820), respectively (Eriksson Wiklund et al., 2014; Heredia-García et al., 2019; Saucedo-Vence et al., 2017). In addition to the direct impacts of ASs or their by-products, these substances can couple with other contaminants, enhancing their toxicity and persistence in the environment. Sucralose and acesulfame, highly resistant and persistent in waters, chelate to heavy metals contained in sediments, making them biologically available for aquatic organisms (Khayatzadeh and Abbasi, 2010).

Aspartame, which showed a removal efficiency from aquatic ecosystems of around 70% (Subedi and Kannan, 2014), negatively affected the growth rate of the freshwater plant *Lemna minor* L., while saccharin induced a growth stimulation to the same species (Kobetičová et al., 2018). In addition, sucralose induced modifications in the swimming behaviour of *D. magna* and on the efficiency of respiration and hunting of the crustacean *Gammarus* spp., potentially reflecting on their overall life history traits (Wiklund et al., 2012b).

As many other pollutants, ASs can enter the environment in their original and intact form without undergoing metabolization processes (Colín-García et al., 2022). Or, they can be degraded through the digestion processes, leading to the onset and release of by-products. Aspartame is almost completely metabolized in the small intestine of consumers into the two constituent amino acids (Lewis and Tzilivakis, 2021). Its stability highly depends on moisture levels, being stable at dry cool conditions (moisture <8%). In aqueous solution, its hydrolysis depends on temperature, pH, time and luminosity. Spectrophotometric analysis of the solution after 30 days in filtered seawater revealed the appearance of a peak corresponding to L-phenylalanine, likely resulting from aspartame degradation. This signal was associated with the aromatic structure of L-phenylalanine, confirming the breakdown of aspartame under the tested conditions. L-phenylalanine is an essential amino acid that has been isolated and artificially synthesized to produce aspartame, with the aim of improving the taste of the substance when combined with L-aspartic acid (Shibui et al., 2014). The appearance of phenylalanine peak coinciding with the physiological and morphological deterioration of polyps in aspartame solution suggests that these effects could be induced by the amino acid rather than by the AS itself. Excessive levels of this amino acid could in fact influence the metabolic pathway of aquatic organisms, such as the increased rate of intestinal cell apoptosis, a process usually related to inflammatory process, in the largemouth bass *Micropterus salmoides* Lacépède (1802); Yi et al. (2023). The toxicity tests performed by Vieira (2011) on zebrafish embryos exposed to amino acids (aspartic acid and glutamic acid), as to simulate nutritional imbalance, resulted in dose- and time-dependent delay of hatching, growth arrest, malformation and mortality, with the first signals after 24 h. As well, aspartic acid and methanol could alter the metabolism of model organisms. L-aspartic acid undermined some hematological parameters, kidneys functioning and serum biochemistry in rats during 90-days-exposure, even if no abnormalities and mortality were recorded (Tada et al., 2008). Neurotoxic effects of L-aspartic acid in newborn mice were recorded by Schainker and Olney (1974) and Stegink (1976) although these results were not confirmed by the studies on infant monkeys and on humans that did not report neuronal diseases (Meldrum, 1993; Reynolds et al., 1980).

In conclusion, the two tested ASs indicate different effects in *A. coerulea* polyps, with aspartame being more impacting, since it induced a deterioration of the health status and the consequent arrest of budding and feeding. Saccharin induced a slight slowdown of the growth rate but did not influence health status and asexual reproduction. Even though all polyps survived and fully recovered, we demonstrated how aspartame hindered the health status of *A. coerulea* polyps, reflecting on their asexual reproduction and growth. Despite never being tested on cnidarians so far, ASs have been reported to impair the physiological processes of many model organisms. To the best of our knowledge, aspartame has never been tested on marine organisms so far,

while saccharin general toxicity was evaluated through on the brine shrimp *A. salina*, with insignificant effects (Frija et al., 2019). Even if the concentrations of ASs registered in aquatic environments are well below the tested experimental conditions, the results obtained in this study could reflect a realistic scenario. The increase of extreme climatic events could lead to higher rainfall intensity, further impacting the flow from inland freshwaters (carrying contaminants) to the sea. No literature data are available so far to address this hypothesis. However, the increase of freshwater input and sediment resuspension during extreme meteorological events is known to cause a concentration of contaminants of one or two order of magnitude (Corada-Fernández et al., 2017). The similarities observed between our study and previous reports are interesting since they pool animals belonging to both vertebrate and invertebrate taxa. Hypothetically, the effects of an overdosage of this substance and its essential amino acid could involve pathways that are common among these phyla, needing more studies for this investigation. These findings could be useful to evaluate the inclusion of these elements, whose utilization is far from being reduced, in the European Legislation for contaminants (EU).

CRediT authorship contribution statement

Chiara Gregorin: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Anna Annibaldi:** Conceptualization, Methodology, Supervision, Writing – review & editing. **Federico Domenichelli:** Resources, Writing – review & editing. **Matteo Feliziani:** Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Stefano Gridelli:** Resources, Writing – review & editing. **Luigi Musco:** Funding acquisition, Supervision, Writing – review & editing. **Sabina Susmel:** Supervision, Writing – review & editing. **Margherita Vit:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Stefania Puce:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

References

Çadirci, K., Tozlu, Ö., Türkez, H., Mardinoğlu, A., 2020. The in vitro cytotoxic, genotoxic, and oxidative damage potentials of the oral artificial sweetener aspartame on cultured human blood cells. Turk. J. Med. Sci. 50, 448–454. https://doi.org/10.3906/sag-2001-113.

Armstrong, B., Doll, R., 1975. Bladder cancer mortality in diabetics in relation to saccharin consumption and smoking habits. J. Epidemiol. Community Health 29, 73–81. https://doi.org/10.1136/jech.29.2.73.

Berset, J.-D., Ochsenbein, N., 2012. Stability considerations of aspartame in the direct analysis of artificial sweeteners in water samples using high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). Chemosphere 88, 563–569. https://doi.org/10.1016/j.chemosphere.2012.03.030.

Brumovský, M., Bečanová, J., Kohoutek, J., Borghini, M., Nizzetto, L., 2017. Contaminants of emerging concern in the open sea waters of the Western Mediterranean. Environ. Pollut. 229, 976–983. https://doi.org/10.1016/j.envpol.2017.07.082.

Buerge, I., Buser, H.-R., Kahle, M., Müller, M.D., Poiger, T., 2009. Ubiquitous occurrence of the artificial sweetener Acesulfame in the aquatic environment: an ideal chemical marker of domestic wastewater in groundwater. Environ. Sci. Technol. 43 (12), 4381–4385. https://doi.org/10.1021/es900126x.

Chattopadhyay, S., Raychaudhuri, U., Chakraborty, R., 2014. Artificial sweeteners – a review. J. Food Sci. Technol. 51, 611–621. https://doi.org/10.1007/s13197-011-0571-1.

Colín-García, K., Elizalde-Velázquez, G.A., Gómez-Oliván, L.M., Islas-Flores, H., García-Medina, S., Galar-Martínez, M., 2022. Acute exposure to environmentally relevant concentrations of sucralose disrupts embryonic development and leads to an oxidative stress response in *Danio rerio*. Sci. Total Environ. 829, 154689. https://doi.org/10.1016/j.scitotenv.2022.154689.

Corada-Fernández, C., Candela, L., Torres-Fuentes, N., Pintado-Herrera, M.G., Paniw, M., González-Mazo, E., 2017. Effects of extreme rainfall events on the distribution of selected emerging contaminants in surface and groundwater: the Guadalete River basin (SW, Spain). Sci. Total Environ. 605–606, 770–783. https://doi.org/10.1016/j.scitotenv.2017.06.049.

Dawson, M.N., Martin, L.E., 2001. Geographic variation and ecological adaptation in *Aurelia* (Scyphozoa, Semaeostomeae): some implications from molecular phylogenetics. In: Purcell, J.E., Graham, W.M., Dumont, H.J. (Eds.), Jellyfish Blooms: Ecological and Societal Importance. Springer, Netherlands, Dordrecht, pp. 259–273. https://doi.org/10.1007/978-94-010-0722-1_21.

Debras, C., Chazelas, E., Srour, B., Druesne-Pecollo, N., Esseddik, Y., Edelenyi, F.S. de, Agaësse, C., Sa, A.D., Lutchia, R., Gigandet, S., Huybrechts, I., Julia, C., Kesse-Guyot, E., Allès, B., Andreeva, V.A., Galan, P., Hercberg, S., Deschasaux-Tanguy, M., Touvier, M., 2022. Artificial sweeteners and cancer risk: results from the NutriNet-Santé population-based cohort study. PLoS Med. 19, e1003950. https://doi.org/10.1371/journal.pmed.1003950.

Di Camillo, C.G.D., Betti, F., Bo, M., Martinelli, M., Puce, S., Bavestrello, G., 2010. Contribution to the understanding of seasonal cycle of *Aurelia aurita* (Cnidaria: Scyphozoa) scyphopolyps in the northern Adriatic Sea. J. Mar. Biol. Assoc. U. K. 90, 1105–1110. https://doi.org/10.1017/S0025315409000848.

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2013. Scientific Opinion on the re-evaluation of aspartame (E 951) as a food additive. EFSA J. 11, 3496. https://doi.org/10.2903/j.efsa.2013.3496.

Eriksson Wiklund, A.-K., Adolfsson-Erici, M., Liewenborg, B., Gorokhova, E., 2014. Sucralose induces biochemical responses in *Daphnia magna*. PLoS One 9, e92771. https://doi.org/10.1371/journal.pone.0092771.

Fernandez, R., Ojito, S., PaJaro, V., Gutierrez, C., Bolivar-Anillo, H., Hampel, M., Anfuso, G., 2025. Artificial sweeteners in aquatic ecosystems: occurrence, sources and effects. https://doi.org/10.20944/preprints202508.0839.v1.

Frija, L.M.T., Ntungwe, E., Sitarek, P., Andrade, J.M., Toma, M., Śliwiński, T., Cabral, L. S., Cristiano, M.L., Rijo, P., Pombeiro, A.J.L., 2019. In vitro assessment of antimicrobial, antioxidant, and cytotoxic properties of saccharin–tetrazolyl and –Thiadiazolyl derivatives: the simple dependence of the pH value on antimicrobial activity. Pharmaceuticals 12, 167. https://doi.org/10.3390/ph12040167.

Fu, K., Wang, L., Wei, C., Li, J., Zhang, J., Zhou, Z., Liang, Y., 2020. Sucralose and acesulfame as an indicator of domestic wastewater contamination in Wuhan surface water. Ecotoxicol. Environ. Saf. 189, 109980. https://doi.org/10.1016/j.ecoenv.2019.109980.

Fukushima, S., Arai, M., Nakanowatari, J., Hibino, T., Okuda, M., Ito, N., 1983. Differences in susceptibility to sodium saccharin among various strains of rats and other animal species. GANN Jpn. J. Cancer Res. 74, 8–20. https://doi.org/10.20772/cancersci1959.74.1_8.

Gallus, S., Scotti, L., Negri, E., Talamini, R., Franceschi, S., Montella, M., Giacosa, A., Dal Maso, L., La Vecchia, C., 2007. Artificial sweeteners and cancer risk in a network of case–control studies. Ann. Oncol. 18, 40–44. https://doi.org/10.1093/annonc/mdl346.

Gambill, M., Jarms, G., 2014. Can *Aurelia* (Cnidaria, Scyphozoa) species be differentiated by comparing their scyphistomae and ephyrae? Eur. J. Taxon. https://doi.org/10.5852/ejt.2014.107.

Gregorin, C., Domenichelli, Gridelli, S.F., Vega Fernández, T., Puce, S., Musco, L., 2025. The detection of specific prey cues triggers distinct predatory behaviour in *Aurelia coerulea* polyps (Cnidaria: scyphozoa). Eur. Zool. J. 92 (1), 906–924. https://doi.org/10.1080/24750263.2025.2534154.

Gregorin, C., Fernández, T.V., Spano, D., Gridelli, S., Domenichelli, F., Furfaro, G., Musco, L., Puce, S., 2024. Collective exploitation of large prey by group foraging shapes aggregation and fitness of cnidarian polyps. Mar. Biol. 171, 196. https://doi.org/10.1007/s00227-024-04519-x.

Griebsch, L.V., Theiss, E.L., Janitschke, D., Erhardt, V.K.J., Erhardt, T., Haas, E.C., Kuppler, K.N., Radermacher, J., Walzer, O., Lauer, A.A., Matschke, V., Hartmann, T., Grimm, M.O.W., Grimm, H.S., 2023. Aspartame and its metabolites cause oxidative stress and mitochondrial and lipid alterations in SH-SY5Y cells. Nutrients 15, 1467. https://doi.org/10.3390/nu15061467.

Gvozdić, E., Bujagić, I.M., Đurkić, T., Grujić, S., 2023. Untreated wastewater impact and environmental risk assessment of artificial sweeteners in river water and sediments of the Danube River Basin in Serbia. Environ. Sci. Pollut. Res. 30, 84583–84594. https://doi.org/10.1007/s11356-023-28348-5.

Hamilton, F., 1822. An Account of the Fishes Found in the River Ganges and Its Branches. Archibald Constable.
Heredia-García, G., Gómez-Oliván, L.M., Orozco-Hernández, J.M., Luja-Mondragón, M., Islas-Flores, H., SanJuan-Reyes, N., Galar-Martínez, M., García-Medina, S., Dublán-García, O., 2019. Alterations to DNA, apoptosis and oxidative damage induced by sucralose in blood cells of *Cyprinus carpio*. Sci. Total Environ. 692, 411–421. https://doi.org/10.1016/j.scitotenv.2019.07.165.
Hoover, R., Hartge Strasser, P., 1980. Artificial sweeteners and human bladder cnacer: preliminary results. Lancet 837–841. https://doi.org/10.1016/S0140-6736(80)91350-1. Originally published as Volume 1, Issue 1980 315.
Khayatzadeh, J., Abbasi, E., 2010. The effects of heavy metals on aquatic animals. 1st Int. Appl. Geol. Congr. 26–28.
Kim, K.Y., Youn, S.H., Choi, S.Y., Park, W., 2024. Massive outbreak of *Aurelia coerulea* in geoje Bay, Korea. Water 16, 2846. https://doi.org/10.3390/w16192846.
Kobetičová, K., Mocová, K.A., Mrhálková, L., Petrová, Š., 2018. Effects of artificial sweeteners on *Lemna minor*. Czech J. Food Sci. 36, 386–391. https://doi.org/10.17221/413/2016-CJFS.
Lacépède, B.G.E., 1802. Histoire naturelle des poissons, 4. Plassan.
Lakowicz, J.R. (Ed.), 2006. Principles of Fluorescence Spectroscopy. Springer, US, Boston, MA. https://doi.org/10.1007/978-0-387-46312-4.
Lange, F.T., Scheurer, M., Brauch, H.-J., 2012. Artificial sweeteners—a recently recognized class of emerging environmental contaminants: a review. Anal. Bioanal. Chem. 403, 2503–2518. https://doi.org/10.1007/s00216-012-5892-z.
Lewis, K., Tzilivakis, J., 2021. Review and synthesis of data on the potential environmental impact of artificial sweeteners. EFSA Support. Publ. 18, 6918E. https://doi.org/10.2903/sp.efsa.2021.EN-6918.
Lillicrap, A., Langford, K., Tollefsen, K.E., 2011. Bioconcentration of the intense sweetener sucralose in a multitrophic battery of aquatic organisms. Environ. Toxicol. Chem. 30, 673–681. https://doi.org/10.1002/etc.433.
Linnaeus, C., 1789. Systema Naturae per regna tria naturae, secundum classes, ordines, genera, species; cum characteribus, differentiis, synonymis, locis, 1. apud JB Delamolliere.
Liu, Q., Li, X., Tang, Q., Liu, X., Wang, Y., Song, M., Chen, X., Pozzolina, M., Höfer, J., Ma, X., Xiao, L., 2024. Copper-induced oxidative stress inhibits asexual reproduction of *Aurelia coerulea* polyps. Ecotoxicol. Environ. Saf. 285, 117112. https://doi.org/10.1016/j.ecoenv.2024.117112.
Loizeau, V., Tusseau-Vuillemin, M.-H., 2014. Marine ecosystems under toxic pressure. In: Vulnerability of Coastal Ecosystems and Adaptation. John Wiley & Sons, Ltd, pp. 1–64. https://doi.org/10.1002/9781119007739.ch1.
Lucas, C.H., Horton, A.A., 2014. Short-term effects of the heavy metals, silver and copper, on polyps of the common jellyfish, *Aurelia aurita*. J. Exp. Mar. Biol. Ecol. 461, 154–161. https://doi.org/10.1016/j.jembe.2014.08.003.
Marques, R., Bonnet, D., Carré, C., Roques, C., Darnaude, A.M., 2021. Trophic ecology of a blooming jellyfish (*Aurelia coerulea*) in a Mediterranean coastal lagoon. Limnol. Oceanogr. 66, 141–157. https://doi.org/10.1002/lno.11593.
Mayer-Pinto, M., Ledet, J., Crowe, T.P., Johnston, E.L., 2020. Sublethal effects of contaminants on marine habitat-forming species: a review and meta-analysis. Biol. Rev. 95, 1554–1573. https://doi.org/10.1111/brv.12630.
Meldrum, B., 1993. Amino acids as dietary excitotoxins: a contribution to understanding neurodegenerative disorders. Brain Res. Rev. 18, 293–314. https://doi.org/10.1016/0165-0173(93)90014-Q.
Mishra, A., Ahmed, K., Froghi, S., Dasgupta, P., 2015. Systematic review of the relationship between artificial sweetener consumption and cancer in humans: analysis of 599,741 participants. Int. J. Clin. Pract. 69, 1418–1426. https://doi.org/10.1111/ijcp.12703.
Olguín-Jacobson, C., Pitt, K.A., Carroll, A.R., Melvin, S.D., 2021. Chronic pesticide exposure elicits a subtle carry-over effect on the metabolome of *Aurelia coerulea* ephyrae. Environ. Pollut. 275, 116641. https://doi.org/10.1016/j.envpol.2021.116641.
Pattanaargson, S., Chuapradit, C., Srisukphonraruk, S., 2001. Aspartame degradation in solutions at various pH conditions. J. Food Sci. 66, 808–809. https://doi.org/10.1111/j.1365-2621.2001.tb15177.x.
Pillai, K.S., 1965. On the distribution of the largest characteristic root of a matrix in multivariate analysis. Biometrika 405–414.
Praveena, S.M., Cheema, M.S., Guo, H.-R., 2019. Non-nutritive artificial sweeteners as an emerging contaminant in environment: a global review and risks perspectives. Ecotoxicol. Environ. Saf. 170, 699–707. https://doi.org/10.1016/j.ecoenv.2018.12.048.
R Core Team, 2021. R: the R Project for Statistical Computing [WWW Document]. URL. https://www.r-project.org/. accessed 10.15.24.
Rasband, W.S., 2012. ImageJ: image processing and analysis in Java. Astrophys. Sourc. Code Libr. ascl 1206, 13.
Regulation - 1272/2008 - EN - clp regulation - EUR-Lex [WWW Document], n.d. URL https://eur-lex.europa.eu/eli/reg/2008/1272/oj/eng (accessed September.17.2025).
Ren, Y., Geng, J., Li, F., Ren, H., Ding, L., Xu, K., 2016. The oxidative stress in the liver of *Carassius auratus* exposed to acesulfame and its UV irradiance products. Sci. Total Environ. 571, 755–762. https://doi.org/10.1016/j.scitotenv.2016.07.047.
Reynolds, W.A., Stegink, L.D., Filer Jr., L.J., Renn, E., 1980. Aspartame administration to the infant monkey: hypothalamic morphology and plasma amino acid levels. Anat. Rec. 198, 73–85. https://doi.org/10.1002/ar.1091980106.
Ringwood, A.H., Lowder, M., Provance, E., O'Dea, J., Gaspar, T., Latijnhouwers, K., Chamberland, V., Vermeij, M., 2025. Cnidarian models for toxicology. Aquat. Toxicol., 107265 https://doi.org/10.1016/j.aquatox.2025.107265.
Roveta, C., Annibaldi, A., Vagnoni, F., Pulido Mantas, T., Domenichelli, F., Gridelli, S., Puce, S., 2020. Short-term effects of environmental factors on the asexual reproduction of *Aurelia* sp. Polyps. Chem. Ecol. 36, 486–492. https://doi.org/10.1080/02757540.2020.1735375.
Sang, Z., Jiang, Y., Tsoi, Y.-K., Leung, K.S.-Y., 2014. Evaluating the environmental impact of artificial sweeteners: a study of their distributions, photodegradation and toxicities. Water Res. 52, 260–274. https://doi.org/10.1016/j.watres.2013.11.002.
Saucedo-Vence, K., Elizalde-Velázquez, A., Dublán-García, O., Galar-Martínez, M., Islas-Flores, H., SanJuan-Reyes, N., García-Medina, S., Hernández-Navarro, M.D., Gómez-Oliván, L.M., 2017. Toxicological hazard induced by sucralose to environmentally relevant concentrations in common carp (*Cyprinus carpio*). Sci. Total Environ. 575, 347–357. https://doi.org/10.1016/j.scitotenv.2016.09.230.
Sawma, M.J., Zayyat, R.M., Ayoub, G.M., Mahmassani, N., 2025. Environmental and health impacts of selected artificial sweeteners: effectiveness of treatment methods and innovative approaches for mitigation. Results Chem. 16, 102356. https://doi.org/10.1016/j.rechem.2025.102356.
Schainker, B., Olney, J.W., 1974. Glutamate-type hypothalamic-pituitary syndrome in mice treated with aspartate or cysteate in infancy. J. Neural Transm. 35, 207–215. https://doi.org/10.1007/BF01258952.
Shaher, S.A.A., Mihailescu, D.F., Amuzescu, B., 2023. Aspartame safety as a food sweetener and related health hazards. Nutrients 15, 3627. https://doi.org/10.3390/nu15163627.
Shibui, Y., Miwa, T., Kodama, T., Gonsho, A., 2014. 28-Day dietary toxicity study of L-phenylalanine in rats. Fundam. Toxicol. Sci. 1, 29–38. https://doi.org/10.2131/fts.1.29.
Sánchez-Bayo, F., van den Brink, P.J., Mann, R.M., 2011. Ecological Impacts of Toxic Chemicals. Bentham Books.
Soffritti, M., Padovani, M., Tibaldi, E., Falcioni, L., Manservisi, F., Belpoggi, F., 2014. The carcinogenic effects of aspartame: the urgent need for regulatory re-evaluation. Am. J. Ind. Med. 57, 383–397. https://doi.org/10.1002/ajim.22296.
Spoelstra, J., Schiff, S.L., Brown, S.J., 2020. Septic systems contribute artificial sweeteners to streams through groundwater. J. Hydrol. X 7, 100050. https://doi.org/10.1016/j.hydroa.2020.100050.
Sørensen, L.B., Møller, P., Flint, A., Martens, M., Raben, A., 2003. Effect of sensory perception of foods on appetite and food intake: a review of studies on humans. Int. J. Obes. 27, 1152–1166. https://doi.org/10.1038/sj.ijo.0802391.
Statista, 2024. Global: artificial sweeteners market revenue 2019-2029 [WWW Document]. Statista. URL. https://www.statista.com/statistics/1201648/revenue-artificial-sweetener-market-worldwide/. (Accessed 24 October 2024).
Stegink, L.D., 1976. Absorption, utilization, and safety of aspartic acid. J. Toxicol. Environ. Health 2, 215–242. https://doi.org/10.1080/15287397609529428.
Straus, D.F., 1820. Mémoire sur les daphnia, de la classe des crustacés. Mémoires du Muséum d'Histoire Naturelle 5, 380–401.
Subedi, B., Kannan, K., 2014. Fate of artificial sweeteners in wastewater treatment plants in New York State, U.S.A. Environ. Sci. Technol. 48, 13668–13674. https://doi.org/10.1021/es504769c.
Suzuki, K.S., Suzuki, K.W., Kumakura, E., Sato, K., Oe, Y., Sato, T., Sawada, H., Masuda, R., Nogata, Y., 2019. Seasonal alternation of the ontogenetic development of the moon jellyfish *Aurelia coerulea* in Maizuru Bay, Japan. PLoS One 14, e0225513. https://doi.org/10.1371/journal.pone.0225513.
Tada, Y., Yano, N., Takahashi, H., Yuzawa, K., Ando, H., Kubo, Y., Nagasawa, A., Uehara, S., Ogata, A., Nakae, D., 2008. Toxic effects of l-aspartic acid at high dose levels on kidneys and salivary glands in Fischer 344 rats detected in a 90-day feeding study. Food Chem. Toxicol. 46, 2789–2795. https://doi.org/10.1016/j.fct.2008.05.013.
Tibaldi, E., Gnudi, F., Panzacchi, S., Mandrioli, D., Vornoli, A., Manservigi, M., Sgargi, D., Falcioni, L., Bua, L., Belpoggi, F., 2020. Identification of aspartame-induced haematopoietic and lymphoid tumours in rats after lifetime treatment. Acta Histochem. 122, 151548. https://doi.org/10.1016/j.acthis.2020.151548.
Van Stempvoort, D.R., Roy, J.W., Brown, S.J., Bickerton, G., 2011. Artificial sweeteners as potential tracers in groundwater in urban environments. J. Hydrol. 401, 126–133. https://doi.org/10.1016/j.jhydrol.2011.02.013.
Vieira, P.E., 2011. Amino Acid Toxicity in Zebrafish [WWW Document]. URL. https://www.proquest.com/openview/228bc2072a9ca32b7b654aa952cf5ef5/1?casa_token=Ayo11sYZe6MAAAAA:MmpolJihIfKprSJl5lhS_AiPqVVHuV_Ou3pPQhk2Qo6sexdondCq44szLaPsjYVLQ1XagM_0Lyo&cbl=2026366&diss=y&pq-origsite=gscholar. (Accessed 27 February 2025).
von Lendenfeld, R., 1884. The scyphomedusae of the southern hemisphere. Part III. Proc. Linn. Soc. N. S. W. 9, 259–306.
Weihrauch, M.R., Diehl, V., 2004. Artificial sweeteners—do they bear a carcinogenic risk? Ann. Oncol. 15, 1460–1465. https://doi.org/10.1093/annonc/mdh256.
Whitehouse, C.R., Boullata, J., McCauley, L.A., 2008. The potential toxicity of artificial sweeteners. AAOHN J. 56, 251–261. https://doi.org/10.1177/216507990805600604.
Wiklund, A.-K.E., Adolfsson-Erici, M., Liewenborg, B., Breitholtz, M., 2012a. Toxicity of artificial sweeteners to aquatic organisms. Chemosphere 86, 50–55. https://doi.org/10.1016/j.chemosphere.2011.08.060.
Wiklund, A.-K.E., Breitholtz, M., Bengtsson, B.-E., Adolfsson-Erici, M., 2012b. Sucralose – an ecotoxicological challenger? Chemosphere 86, 50–55. https://doi.org/10.1016/j.chemosphere.2011.08.049.

Xing, Y., Liu, Q., Zhang, M., Zhen, Y., Mi, T., Yu, Z., 2020. Effects of temperature and salinity on the asexual reproduction of *Aurelia coerulea* polyps. J. Oceanol. Limnol. 38, 133–142. https://doi.org/10.1007/s00343-019-8337-0.

Yi, C., Liang, H., Huang, D., Yu, H., Xue, C., Gu, J., Chen, X., Wang, Y., Ren, M., Zhang, L., 2023. Phenylalanine plays important roles in regulating the capacity of intestinal immunity, antioxidants and apoptosis in largemouth bass (*Micropterus salmoides*). Animals 13, 2980. https://doi.org/10.3390/ani13182980.

Zerin, T., Kim, J.-S., Gil, H.-W., Song, H.-Y., Hong, S.-Y., 2015. Effects of formaldehyde on mitochondrial dysfunction and apoptosis in SK-N-SH neuroblastoma cells. Cell Biol. Toxicol. 31, 261–272. https://doi.org/10.1007/s10565-015-9309-6.