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**Original Paper**

# Sea Water Acidification Affects Osmotic Swelling, Regulatory Volume Decrease and Discharge in Nematocytes of the Jellyfish *Pelagia noctiluca*

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**Key Words**
Sea water acidification • RVD • Discharge • Nematocytes • Jellyfish • *Pelagia noctiluca*

**Abstract**
***Background***: Increased acidification/$P_{CO2}$ of sea water is a threat to the environment and affects the homeostasis of marine animals. In this study, the effect of sea water pH changes on the osmotic phase (OP), regulatory volume decrease (RVD) and discharge of the jellyfish *Pelagia noctiluca* (Cnidaria, Scyphozoa) nematocytes, collected from the Strait of Messina (Italy), was assessed. ***Methods***: Isolated nematocytes, suspended in artificial sea water (ASW) with pH 7.65, 6.5 and 4.5, were exposed to hyposmotic ASW of the same pH values and their osmotic response and RVD measured optically in a special flow through chamber. Nematocyte discharge was analyzed *in situ* in ASW at all three pH values. ***Results***: At normal pH (7.65), nematocytes subjected to hyposmotic shock first expanded osmotically and then regulated their cell volume within 15 min. Exposure to hyposmotic ASW pH 6.5 and 4.5 compromised the OP and reduced or totally abrogated the ensuing RVD, respectively. Acidic pH also significantly reduced the nematocyte discharge response. ***Conclusion***: Data indicate that the homeostasis and function of Cnidarians may be altered by environmental changes such as sea water acidification, thereby validating their use as novel bioindicators for the quality of the marine environment.



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## Introduction

Nematocytes are highly specialized eukaryotic stinging cells found in Cnidaria, a *phylum* comprising Hydrozoa, Scyphozoa, Anthozoa and Cubozoa. These cells contain the nematocyst organoid, which consists of a capsule wall comprising an inverted tubule and toxin-containing fluid. The nematocyte can be easily distinguished on the basis of a girdle containing the cytoplasm confined within a thin rim, while most of the cell volume is occupied by the nematocyst. The functions of nematocytes are critical for the survival of several Cnidarian species, for territory and self-defense, prey capture, substrate attachment and locomotion. An important feature of nematocytes is discharge of the tubule containing the toxin, one of the most rapid exocytosis processes ever documented [1, 2]. Nematocyte discharge occurs following an appropriate chemico-physical stimulus — beginning with the opening of the operculum and eversion of the tubule contained inside — and ending with a decrease in capsule volume and delivery of toxins [1, 3, 4].

Nematocytes can discharge either independently or within a tissue (tentacle, acontia or oral arm), or in response to mechanical and chemical stimuli from other cells, like supporting cells, surrounding the nematocytes inside the tissue [5, 6]. Chemosensitization occurs *via* binding of exogenous compounds to chemoreceptors located on cells other than the nematocytes, which can modulate the mechanosensitive apparatus (cilia). In this regard, we have recently described that the discharge of *in situ Pelagia noctiluca* nematocytes is effectively induced by the combination of mechanical and chemical (including glutamate and N-acetylated sugars) stimuli [7].

Cell volume regulation is an essential feature for the survival of many cell types [8], including nematocytes [9]. This homeostatic parameter allows cells to counteract osmotic changes in the external environment. When exposed to hyposmotic medium, cells swell within a few minutes - the osmotic phase (OP). After the OP, cells undergo regulatory volume decrease (RVD), and cell volume returns to pre-swelling values — a process that may occur rapidly or more slowly depending on the species and cell type. On the other hand, cells shrink in response to hypertonicity. In this case, cells restore their original volume by activation of regulatory volume increase (RVI) mechanisms. Both RVD and RVI have mostly been investigated in mammalian cells [10], but also in cells of lower vertebrates and invertebrates [11-13]. With regard to invertebrates, we have previously demonstrated cell volume regulation in response to both hyposmotic and hypertonic stress in isolated nematocytes, and suggested the involvement of transport systems for multiple ions (namely $Cl^-$ and $K^+$ for RVD and $Na^+$ for RVI) and aquaporins in this regulation [9, 14-17].

Since discharge and cell volume regulation are predictive for Cnidaria viability, and ocean acidification alters marine animal homeostasis and cell function [18], the aim of the present paper was to establish the effect of sea water acidification on the OP, RVD and discharge of nematocytes from *Pelagia noctiluca*.

## Materials and Methods

*Experimental solutions and reagents*

Normosmotic artificial sea water (ASW) had the following composition (in mM): NaCl 520, KCl 9.7, $CaCl_2$ 10, $MgCl_2$ 24, $MgSO_4$ 28, imidazole 5, pH 7.65, 1,100 mOsm/$Kg_{H2O}$. ASW was made hyposmotic (65% ASW) by reducing the NaCl concentration to 355 mM (710 mOsm/$Kg_{H2O}$). Acidification of normo- or hyposmotic ASW for RVD tests was performed by adding 1M HCl. Low- $Ca^{2+}$ ASW was (in mM): NaCl 520, KCl 9.7, $CaCl_2$ 0.01, $MgCl_2$ 24, $MgSO_4$ 28, imidazole 5, pH 7.65. $Ca^{2+}$-free ASW was (in mM): NaCl 538, KCl 9.7, $MgCl_2$ 24, $MgSO_4$ 28, imidazole 5, EGTA 0.5, pH 7.65. pH measurements were taken with an Orion pH-meter. Osmolality of solutions was measured with a Fiske osmometer. All chemicals were purchased from SIGMA (Milan, Italy).

*Nematocyte isolation*

Eurytele nematocytes, according to Mariscal's classification [19], were isolated from tentacles of *Pelagia noctiluca* (Cnidaria, Scyphozoa) collected from the Strait of Messina (Italy) as previously described [20]. Briefly, once excised from the umbrella of the jellyfish, tentacles were repeatedly washed with low-$Ca^{2+}$ ASW to remove mucus. Subsequent treatment with extrusion solution (605 mM NaSCN, 0.01 mM $CaCl_2$), followed by $Ca^{2+}$-free ASW, allowed the nematocytes to separate and detach from the tissue. Substitution with normosmotic ASW facilitated isolated nematocytes to attach to the slide surface and the tissue was finally removed. Prior to being subjected to RVD experiments, isolated nematocytes were incubated at 14-16 °C for no more than 3 h and their morphological integrity visually inspected with a light microscope (Leica DMLS, Milan, Italy, 400x magnification).

*RVD tests*

A make-shift perfusion chamber was assembled by placing double sided tape between a glass slide and coverslip containing isolated nematocytes. Either normosmotic or hyposmotic ASW, according to the experimental plan described below was completely and rapidly exchanged by adding it to one side of the coverslip and removing it at the opposite side with strips of filter paper.

With regard to control RVD tests, the experimental design consisted of three periods: period 1: pH 7.65, normosmotic ASW for 5 min; period 2: pH 7.65, hyposmotic ASW (65% ASW) for 15 min; period 3: pH 7.65, normosmotic ASW for 6 min. With regard to RVD tests in pH-modified medium, the experimental protocol consisted of four periods: period 1A: pH 7.65, normosmotic ASW for 6 min; period 1B: pH-modified (either pH 4.5 or 6.5), normosmotic ASW for 5 min; period 2: pH-modified (either pH 4.5 or 6.5), hyposmotic ASW (65% ASW) for 15 min; period 3: pH 7.65, normosmotic ASW for 6 min.

Cell volume measurements were taken from nematocytes demonstrating strong adhesion to the slide. About 30 images/nematocyte were taken, minute by minute during the whole experiment, with a phase contrast microscope (Leica DMLS, Milan, Italy) connected to a video camera (JVC model TK-1180E) and a computer equipped with suitable software (Apple Video Player, Adobe Photoshop). The cross sectional area (as an indication of cell volume) of each recorded image was successively measured (Image J, US) as a function of time. The results were then expressed as the relative area, $A/A_0$, where A and $A_0$ represent, respectively, the cross sectional area of a nematocyte at a given time, and the average of the cross sectional areas of the same nematocyte in pH 7.65, normosmotic ASW.

*In situ nematocyte discharge, tissue preparation*

Oral arm segments, containing holotrichous isorhizas nematocytes, were excised with ophthalmic scissors and collected by fire-polished silicon-coated glass Pasteur pipettes. The segments were then rinsed with low-$Ca^{2+}$ ASW to remove mucus. Tissues were then transferred to 5 ml Petri dishes previously treated with Sylgard (Dow Corning) and fixed with *Opuntia* spines, and repeatedly rinsed with normosmotic ASW, pH 7.65. Tissue was neither stretched nor slackened and was visually inspected using an inverted light microscope (Cambridge Photozoom Inverted microscope, 100x magnification) for structural integrity assessment.

*Chemical-mechanical stimulation of oral arms*

Oral arms were submitted to a combined chemical-mechanical stimulation according to [21] with appropriate changes. Test probes consisting of 2 cm segments of 0.8±0.01mm diameter nylon fishing line were coated at one end with an ~0.06mm layer of 30% (w/v) gelatine. After storage for 24 h at 4 °C and 100% humidity, the uncoated ends of the probes were inserted into glass capillary tubes and fixed to a micromanipulator (Leitz). The gelatine-coated ends were then placed into oral arm fragments previously incubated for 20 min with control (pH 7.65) or modified (pH 4.5 or pH 6.5) normosmotic ASW plus $10^{-3}$ M glutamate. The adhesion of discharged nematocysts was visually inspected using an inverted microscope (200x magnification). Single gelatine-coated probes bearing discharged nematocysts were placed in separate microtiter wells (Microtest 11, Falcon Plastics), each containing 50 µL of 1% enzyme/detergent mixture (Trizyme; Amway Products, Ada, MI, USA). After 4 h incubation at room temperature to completely dissolve the gelatine, the probes were removed. Discharged nematocysts were visually counted using an inverted microscope (400x magnification) and represent the discharge capability of the tissue.

*Statistics*

Data are shown as mean values ± standard error of the mean (S.E.M.). Each data set is derived from five individual experiments performed on cells isolated from animals collected in the same week. Significance of the differences was tested using one- or two-way analysis of variance (ANOVA), followed by Dunnet's or Bonferroni's post-hoc test, as indicated. $p<0.05$ was considered as statistically significant.

## Results

*OP and RVD in Pelagia noctiluca nematocytes*

Fig. 1 shows the changes in nematocyte volume in response to an ~35% reduction of osmolality (from 1,100 mOsm/$kg_{H2O}$ to 710 mOsm/$kg_{H2O}$; 65% ASW, pH 7.65). Hyposmotic shock significantly increased the $A/A_0$ ratio of nematocytes, and hence their cell volume, by more than 10% within 4 minutes (0 min $A/A_0$ = 1.0000 ± 0.0001, n = 5; 4 min (peak) $A/A_0$ = 1.1053 ± 0.011, n = 5, $p < 0.001$, Table 1 and Fig. 1, period 2), and represents the OP. After the peak change, $A/A_0$ fell to levels comparable to those observed before exposure to hyposmotic ASW (15 min $A/A_0$ = 1.0039 ± 0.0013, n = 5, $p < 0.001$ compared to peak $A/A_0$ and not significantly different compared to 0 min $A/A_0$, Table 1 and Fig. 1, period 2), indicating that a complete restoration of cell volume occurred within 15 min of hyposmotic shock. When the hyposmotic medium was replaced by normosmotic medium (Fig. 1, period 3), cell volume further decreased (21 min $A/A_0$ = 0.979 ± 0.013, n = 5, $p < 0.05$ compared to 15 min $A/A_0$, Table 1). Nevertheless, no RVI post-RVD was seen within 6 min of observation (Fig. 1, period 3).

*pH reduction delays OP and impairs RVD in Pelagia noctiluca nematocytes*

The effect of two different acidic pHs (4.5 and 6.5) on the volume of isolated nematocytes in both normosmotic and hyposmotic conditions is presented in Fig. 2 and Fig. 3.

Pre-exposure of isolated nematocytes for 5 min to pH 4.5, normosmotic ASW (Fig. 2, period 1B) did not significantly alter the $A/A_0$ ratio (-4.5 min $A/A_0$ = 1.008 ± 0.001, n = 5, not statistically different from 0 min $A/A_0$ = 0.9935 ± 0.008, n = 5, Table 1). However, nematocytes exposed to pH 4.5 hyposmotic ASW for 15 min (Fig. 2, period 2), did not reach peak $A/A_0$ values until 9 min, thus suggesting that acidic pH delayed the OP. $A/A_0$ ratios after 2 min following exposure to the hyposmotic ASW were compared to establish if the osmotic response of nematocytes within the OP was altered by acidic pH. This value (1.0571 ± 0.006, n = 5, Table 1) was significantly reduced with respect to that measured 2 min following exposure to pH 7.65, hyposmotic ASW (1.0864 ± 0.012, n = 5, $p < 0.05$, Table 1), therefore showing that the OP was also compromised by acidic pH. It is likely that the delayed OP is a consequence of reduced water permeability of the cell membrane, hypothetically occurring *via* inhibition of aquaporins [17].

Statistic comparison of peak $A/A_0$ ratios and that measured after 15 min of hyposmotic stress was performed to evaluate a possible effect of ASW acidification on RVD. $A/A_0$ measured 15 min following exposure to pH 4.5, hyposmotic ASW (1.0922 ± 0.007, n = 5) was not significantly different compared to the peak $A/A_0$ ratio (1.0986 ± 0.004, n = 5, Table 1), thus signifying that pH 4.5 had completely abrogated the RVD seen in nematocytes treated with pH 7.65, hyposmotic ASW. The $A/A_0$ ratio remained at a plateau until the cells were returned to normosmotic ASW (Fig. 2, period 2). The fact that $A/A_0$ returned to levels similar to those observed prior to the hyposmotic challenge (21 min $A/A_0$ = 0.9826 ± 0.007, n = 5, not statistically different compared to 0 min $A/A_0$, Table 1) in pH 7.65, normosmotic ASW (Fig. 2, period 3) suggests, however, that the osmotically-induced water efflux through presumable aquaporins [17] was not irreversibly affected by acidic pH.

When the same procedure was repeated with pH 6.5 ASW (Fig. 3), the increase of the $A/A_0$ ratio due to the OP was again delayed, reaching its peak after 5 min of hyposmotic challenge (Fig. 3, period 2). $A/A_0$ values measured 2 min following exposure to pH 6.5, hyposmotic ASW (1.0055 ± 0.005, n=5) was not different from that measured at 0 min (1.000

**Table 1.** Comparison of $A/A_0$ ratios in control (pH 7.65), pH 4.5 or pH 6.5-treated nematocytes at different time points during RVD tests. Data (mean ± S.E.M) are from Figs. 1-3. pHs indicated in the first column refer to period 2 (hyposmotic). Statistically significant differences between $A/A_0$ ratios within the same column (i.e. at the same time point) were determined by one-way ANOVA, followed by Dunnet's post-hoc test where $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{ns}$ is not significant relative to pH 7.65. Statistically significant differences between $A/A_0$ ratios within the same row (i.e. between the different time points) were determined by two-way ANOVA, followed by Bonferroni's post-hoc test where $*p < 0.05$, $***p < 0.001$ and n.s. is not significant. OP, osmotic phase; RVD, regulatory volume decrease

| | $A/A_0$ | | | | | |
|---|---|---|---|---|---|---|
| | *period 1 (normosmotic)* | | *period 2 (hyposmotic)* | | | *period 3 (normosmotic)* |
| | **-4.5 min** | **0 min** | **2 min (OP)** | **peak (OP)** | **15 min (RVD)** | **21 min** |
| ***pH = 7.65*** | | 1.0000±0.0001, n=5 | 1.0864±0.012, n=5 | 1.1053±0.011, n=5 (4 min) | 1.0039±0.0013, n=5 | 0.979±0.013, n=5 |
| ***pH = 4.5*** | 1.008±0.001, n=5 | $0.9935\pm0.008^{ns}$, n=5 | $1.0571\pm0.006^{\#}$, n=5 | $1.0986\pm0.004^{ns}$, n=5 (9 min) | $1.0922\pm0.007^{\#\#}$, n=5 | 0.9826±0.007, n=5 |
| ***pH = 6.5*** | 0.998±0.001, n=5 | $1.0000\pm0.001^{ns}$, n=5 | $1.0055\pm0.005^{\#\#}$, n=5 | $1.0876\pm0.005^{ns}$, n=5 (5 min) | $1.0234\pm0.001^{\#}$, n=5 | 1.010±0.005, n=5 |
| | | **0 min *vs* -4.5 min** | **2 min *vs* 0 min** | **peak *vs* 0 min** | **15 min *vs* peak** | **21 min *vs* 0 min** |
| ***pH = 7.65*** | | | *** | *** | *** | n.s. |
| ***pH = 4.5*** | | n.s. | *** | *** | n.s. | n.s. |
| ***pH = 6.5*** | | n.s. | n.s. | *** | *** | n.s. |
| | | | | | **15 min *vs* 0 min** | **21 min *vs* 15 min** |
| ***pH = 7.65*** | | | | | n.s. | * |
| ***pH = 4.5*** | | | | | *** | *** |
| ***pH = 6.5*** | | | | | * | n.s. |

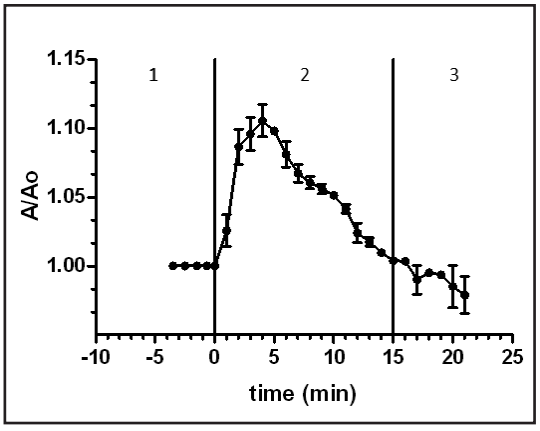


**Fig 1.** Effect of osmolarity reduction on nematocyte $A/A_0$ ratios. The $A/A_0$ ratios in isolated nematocytes were monitored for 1) 5 min in normosmotic ASW, pH 7.65; 2) 15 min in hyposmotic ASW, pH 7.65 and 3) 6 min in normosmotic ASW, pH 7.65. Symbols represent the mean ± S.E.M. of 5 nematocytes.

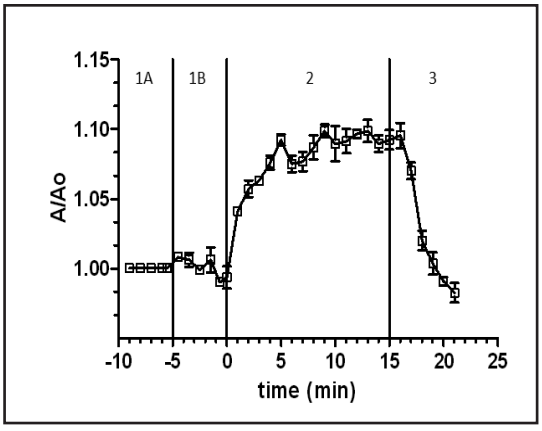


**Fig 2.** Effect of pH 4.5 ASW on nematocyte $A/A_0$ ratios. The $A/A_0$ ratios in isolated nematocytes were monitored for 1A) 5 min in normosmotic ASW, pH 7.65; 1B) 5 min in normosmotic ASW, pH 4.5; 2) 15 min in hyposmotic ASW, pH 4.5 and 3) 6 min in normosmotic ASW, pH 7.65. Symbols represent the mean ± S.E.M. of 5 nematocytes.

± 0.001, n = 5, Table 1) and was significantly reduced with respect to that determined 2 min following exposure to pH 7.65, hyposmotic ASW (1.0864 ± 0.012, n = 5, $p < 0.01$, Table 1), showing that OP was again severely compromised. Moreover, the $A/A_0$ ratio measured 15 min following exposure to pH 6.5, hyposmotic ASW (1.0234 ± 0.001, n = 5) was significantly reduced compared to the peak $A/A_0$ value (1.0876 ± 0.005, n = 5, $p < 0.001$, Table 1), thus signifying that RVD was not completely abrogated. However, this ratio was significantly increased relative to that measured 15 min following exposure to pH 7.65, hyposmotic ASW (1.0039 ± 0.0013, n = 5, $p < 0.05$, Table 1). In addition, the cellular volume measured in normosmotic ASW was not completely restored (compare 15 min $A/A_0$ to 0 min $A/A_0$, pH 6.5, $p < 0.05$ Table 1), suggesting that RVD mechanisms were in fact compromised by lowering the pH by ~1 unit.

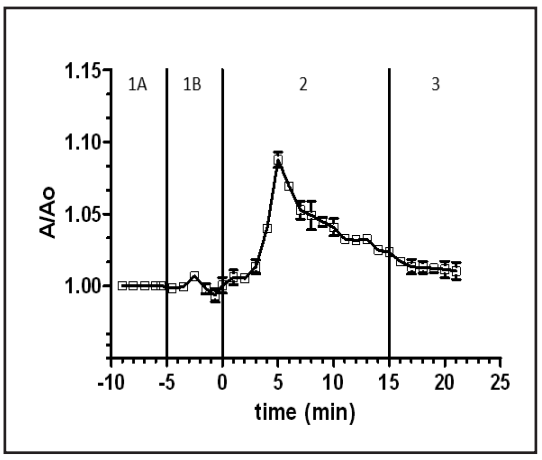


**Fig 3.** Effect of pH 6.5 ASW on nematocyte $A/A_0$ ratios. The $A/A_0$ ratios in isolated nematocytes were monitored for 1A) 5 min in normosmotic ASW, pH 7.65; 1B) 5 min in normosmotic ASW, pH 6.5; 2) 15 min in hyposmotic ASW, pH 6.5 and 3) 6 min in normosmotic ASW, pH 7.65. Symbols represent the mean ± S.E.M. of 5 nematocytes.

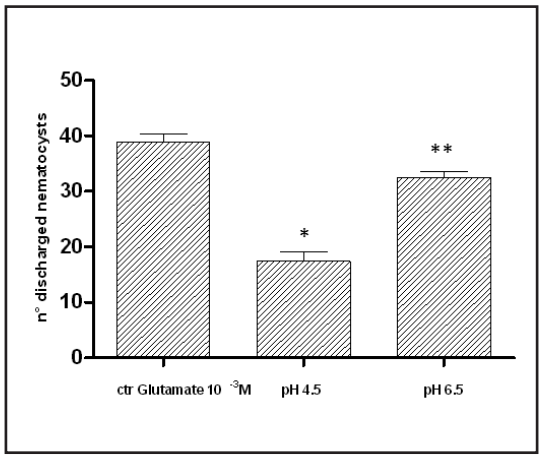


**Fig 4.** Effect of pH on nematocyte discharge. Oral arms were bathed for 20 min in normosmotic ASW either pH 7.65, pH 4.5 or pH 6.5 and subjected to chemical-mechanical stimulation. Bars represent the mean ± S.E.M. of 6 experiments, where *p<0.05 as determined by one-way analysis of variance (ANOVA), followed by Bonferroni's post-hoc test. *values statistically different respect to the control; ** values statistically different respect to the control and respect to pH 4.5.

The results support the conclusion that a pH reduction from 7.65 to 6.5 or 4.5 caused a significant change in the onset of the OP at both acidic pH values, and a partial reduction or complete inactivation of RVD at pH 6.5 and 4.5, respectively (Fig. 3 and Fig. 2). The peak $A/A_0$ values were not affected by ASW acidification (Table 1).

*pH reduction negatively affects in situ discharge of Pelagia noctiluca nematocytes*

The effect of pH on the discharge of holotrichous isorhiza nematocytes from oral arms (as opposed to eurytele nematocytes isolated from tentacles) is shown in Fig. 4. Chemical-mechanical –stimulated discharge of oral arms bathed for 20 min in normosmotic ASW of pH 4.5 and pH 6.5 was significantly reduced compared to that in oral arms bathed in normosmotic ASW, pH 7.65 ($p < 0.05$; Fig. 4). Nematocyst discharge observed in pH 4.5, normosmotic ASW was significantly reduced compared to that observed in pH 6.5, normosmotic ASW ($p<0.05$; Fig. 4). Thus, acidic ASW not only altered the OP and RVD in isolated nematocytes, but also severely blocked their discharge.

## Discussion

The present work describes the effect of sea water acidification on cell volume regulation and discharge of *Pelagia noctiluca* nematocytes — two homeostatic responses essential for survival of both Scyphozoa and Anthozoa [9, 22]. Since such responses are essential for Cnidarian survival, it is plausible that they may be used to indirectly assess the quality of marine water. Sea water acidification is caused by the uptake of additional $CO_2$ from the atmosphere, and has already been shown to affect biodiversity *via* physiological effects on growth, survival, reproduction, immunology, global distribution and species abundances [23]. A further reduction in ocean pH is expected by the year 2100, due to increasing $CO_2$

emissions of industrial origin [24]. The biological activity of venom delivered from isolated *Pelagia noctiluca* nematocysts is also inhibited by acidic pH [25], thereby altering the prey/predatory relationship and contributing to unbalanced predation events [23, 26].

Survival of corals and invertebrates with calcareous exoskeletons represents a more widely accepted model to study the impact of ocean acidification, since pH alters the deposition of calcium carbonate in these organisms [26-28]. Much less is known regarding the consequences of ocean acidification on non-calcifying marine species, including fish and non-calcifying invertebrates [29]. The need of additional information concerning the effect of ocean acidification on these latter specimens has been underscored in recent reports [30]. Since Cnidaria specimens are abundant worldwide due to blooming events, the use of these animals to assess the impact of ocean acidification may be encouraged [31, 32].

The present investigation provides new information on the impact that ocean acidification may have on physiological features of jellyfish. Acid-base balance in cells is directly related to the intracellular concentration of free $H^+$, which is finely regulated to avoid cellular damage. Therefore, pH is a relevant homeostatic parameter, and the relationship between pH maintenance and cell function is well established in mammals, especially in neuronal cells [33-35].

It was demonstrated that acidic pH delayed the onset of the OP by doubling the time to reach peak height $A/A_0$ ratios (compare Fig. 2 and 3 with Fig. 1) following exposure of nematocytes to hyposmotic ASW. The fact that the OP was delayed in nematocytes exposed to acidic pH may depend on aquaporins, since it was previously shown that the OP in nematocytes was blocked following exposure to the selective aquaporin water channel inhibitor, $HgCl_2$ [17]. Also, there is evidence from mammalian aquaporin channels that pH modulates their activity [36]. Even more pronounced was the action of low pH on the RVD phase, which was completely obliterated when nematocytes were exposed to pH 4.5 (Fig. 2). It was previously shown from this laboratory that nematocyte RVD is mediated by $K^+$ channels and co-transporters with $Cl^-$ [9, 17]. In this regard and on the basis of what was described in the present investigation, we speculate that the impairment of RVD by acidic pH is likely due to alterations in the activities of these ion channels and/or cotransporters [37], a possibility to be further evaluated in the future.

As mentioned above, acidic pH diminished the discharge capability of *Pelagia noctiluca* nematocytes. $Ca^{2+}$ and surface receptor integrity have been shown to be essential for nematocyte discharge [38-40]. Therefore, acidic pH may negatively affect either $Ca^{2+}$ channel function or the binding of discharge-inducing compounds, like glutamate, to their receptors. The inhibition of discharge capability, underlying Cnidaria survival, is in line with previous reports describing that prey/predator relationships may be compromised by marine water pollution [23]. In fact, the role of glutamate in synaptic transmission in the nucleus of the solitary tract (NTS) is dependent on pH [35]. It has also been demonstrated that acidity causes the nematocyst wall to collapse, which may contribute to decreased discharge capacity [41].

## Conclusion

In conclusion, this study has shown that in *Pelagia noctiluca* nematocytes, both the OP and RVD are impaired, if not abolished, by acidic ASW, and that the discharge capability of nematocytes *in situ* is severely compromised. It is not clear yet, and it would be interesting to further investigate, whether there is a causal relationship between the two major effects (RVD and discharge inhibition). The present findings show that nematocyte cell volume regulation and discharge capability are affected by acidification of the external medium, which establish the jellyfish nematocyte as a good model to test environmentally caused changes in the proton concentration of sea water.

## Conflict of Interests

The Authors have no conflicts of interest to disclose.

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