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Polyp flats, a new system for experimenting with jellyfish polyps, with insights into the effects of ocean acidification

Alejandro Olariaga[1], Elisa F. Guallart[1], Verónica Fuentes[1], Àngel López-Sanz[1], Antonio Canepa[1], Juancho Movilla[1], Mar Bosch[1], Eva Calvo[1], and Carles Pelejero[1,2]*

[1]Institut de Ciències del Mar (ICM-CSIC), Passeig Marítim de la Barceloneta 08003, Barcelona, Catalonia, Spain
[2]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Catalonia, Spain

Abstract

Research interest on jellyfish has grown exponentially over the last years and studies focusing on the biology and ecology of the jellyfish polyp stage are being recognized as crucial in understanding jellyfish proliferations. Due to the difficulty of conducting in situ work with jellyfish polyps, laboratory experiments are the most used approach. Here, we describe the design and successful testing of a new system that allows continuous seawater renewal while keeping constant the selected physicochemical conditions of the water throughout the experiment in contrast to closed systems used previously. As a first test, we started an experiment to assess the effects of ocean acidification on the growth and development of jellyfish polyps of *Aurelia* sp. This new design demonstrated high precision in maintaining constant conditions (pH, temperature, and flow rates) among the replicates of each treatment, and ensured excellent conditions for jellyfish polyp survival. All together it has shown to be an effective platform to assess the effect of environmental variables on the growth and development of jellyfish polyps.

In recent years, reports of jellyfish blooms are increasing worldwide (Brotz et al. 2012; Purcell 2012), and there are reasons to believe that oceans are facing a more gelatinous future (Richardson et al. 2009; Purcell et al. 2012). Many anthropogenic stressors are likely to impact jellyfish development, including global warming, overfishing, nutrient additions, invasion and translocation of species or global ocean sprawl (Purcell et al. 2007, 2012; Duarte et al. 2012). Ocean acidification, the decrease in seawater pH due to the marine absorption of atmospheric CO_2 (Pelejero et al. 2010 and references therein), could also play an important role. However, the very few published studies attempting to assess the possible effect of acidification on jellyfish are still inconclusive, and it is unknown whether this group of animals will be benefited or harmed by such pressure. Even though one study has suggested increasing jellyfish population due to ocean acidification (Attrill et al. 2007), the conclusions are still unclear and even contradictory results had been shown in other studies (Richardson and Gibbons 2008).

The term jellyfish includes several taxonomic groups, but scyphozoans are the most known group that produces jellyfish blooms. Most scyphozoans have a metagenetic life cycle that includes planktonic medusa and benthic polyp generations (Fig. 1). Polyps produced from the settlement and metamorphosis of planktonic planulae larvae can increase the population by asexual reproduction, contributing to the development of jellyfish blooms (Boero et al. 2008). Monitoring and testing these polyps in the field is notoriously difficult due to their small sizes and the lack of knowledge regarding the settlement preferences of the planulae (Holst and Jarms 2007; Holst 2012). Thus, laboratory experimentation is still the most feasible approach to study the response of jellyfish polyps to natural environmental conditions and to detect the factors that might affect their survival, reproduction, and other developmental parameters (see references in Table 1). In all these studies, however, results were obtained from incubation experiments under non- or discontinuous renewal of seawater. In such conditions, it is very difficult to maintain the

*Corresponding author: E-mail: olariaga@icm.csic.es

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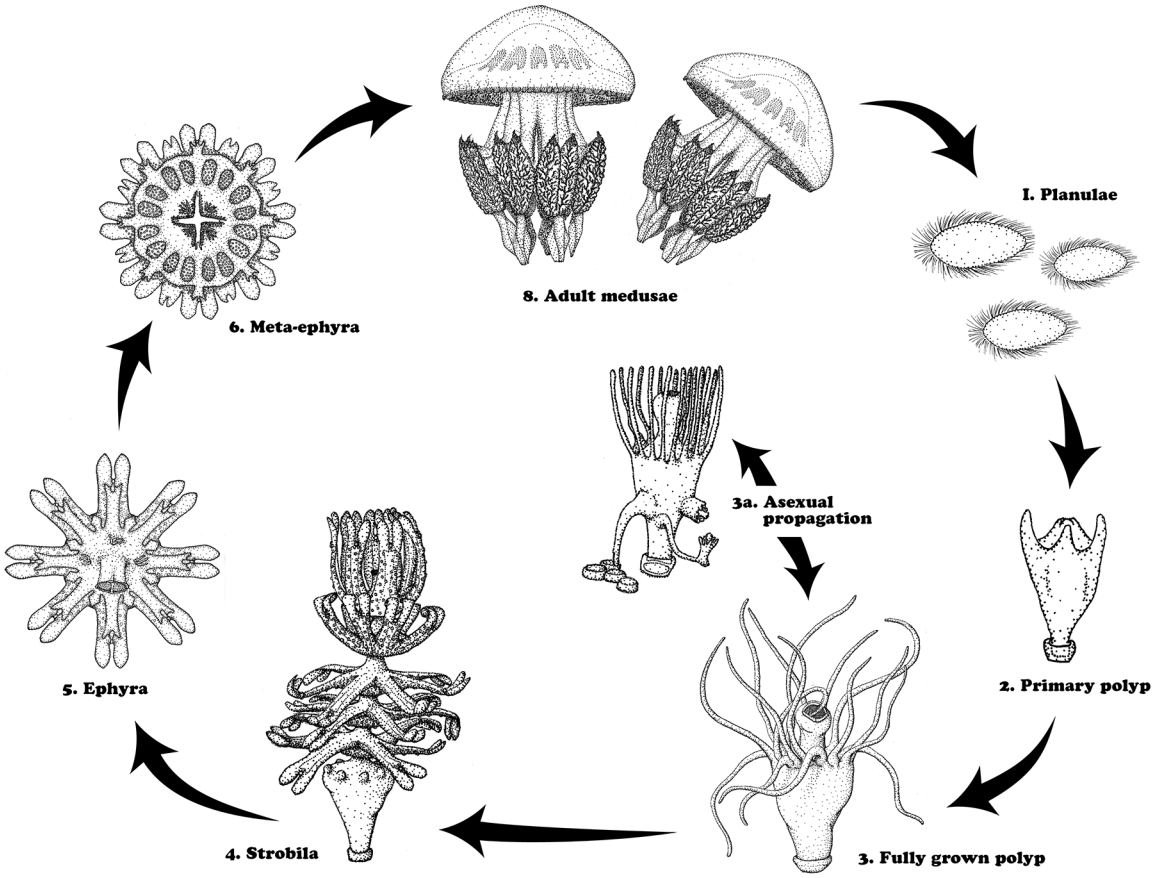

Fig. 1. The life cycle of the scyphozoan *Rhizostoma pulmo*, which is typical for most scyphozoans, including *Aurelia* sp. (adapted from Fuentes et al. 2011), with kind permission of Springer Science+Business Media

different physicochemical parameters at the desired constant levels, making complex the assessment of possible environmental effects from specific stressors. Therefore, improving the available methodology towards continuously seawater renovation systems is of special interest.

In this context, we provide details on a newly developed experimental set-up that allows the manipulation of environmental parameters in individual containers with continuous water renewal. They are designed to host single jellyfish polyps under a continuous seawater flow, guaranteeing a good reproducibility amongst all containers in each treatment. Given the recently recognized potential threat of ocean acidification, this set-up has been focused on pH manipulation, but it could be well adapted to modify other relevant parameters (e.g. nutrients, salinity, temperature, oxygen, among others). This new set-up represents an important step forward in experimental research on jellyfish, with the potential to provide new information on the fate of these species in the future changing ocean.

Materials and procedures

System overview

In this article, we describe a new system that we call "polyp flats" specially designed to conduct experiments with jellyfish polyps. As a first test of its performance and suitability, we have used this system for assessing the effect of ocean acidification on jellyfish proliferations. In the following, we provide full details on the design of this system and the specific configuration that allows changing and maintaining the pH conditions of the polyp flats associated to each treatment.

Incubation chambers and main containers

The polyp flats system was designed to supply a constant flow of seawater in 18 replicates for each level of the experimental treatment, whose parts and components are depicted in Fig. 2.

A transparent acrylic closed box (40 cm long, 23 cm wide, 5 cm high; A in Fig. 2) was built and 18 circular holes (4 cm diameter each) were sawed in the upper surface of it to allow fitting the individual incubation chambers. The distribution of the holes in the box is shown in Fig. 2. In one of the vertical sides, two small circular holes are used as water inlet/outlet (B in Fig. 2) allowing the connection of several boxes to the same water bath line. This recirculating water through the acrylic boxes acts only to homogenize temperature between all the individual incubation chambers, and there is no contact between the water in the bath with that inside the incubation chambers.

Table 1. Synthesis of methods used in experimental studies conducted on jellyfish polyps. T = temperature; Temperature regulation: Water Bath (W-B), Constant running water (C-R-W), Incubators (I), not mentioned (NM); Type of chambers: Glass bowls (G-B), Culture plates (C-P), Glass Jars (G-J), Flasks (F), Better Square Dishes (S-D) and add Acrylic plates (A-P) afterwards, Acrylic Incubation Chamber (A-I-CH), Glass Bottle (G-BT); Controlled parameters: T = temperature, DO = Dissolved oxygen, F = Flow rate, L (Light), N = Nutrients, FS = Food supply.

	Methods							
Species	T regulation	Type of chamber	Nr of polyps per chamber	Volume of chambers (mL)	Water renewal	Controlled parameters	Exp. duration (weeks)	Reference
Aurelia aurita	I	C-P	1	10	NM	T*, Salinity*, DO*, FS*	4-8	Thein et al. 2012
Cotylorhiza tuberculata	I	G-B	22	150	Once a week	T*, Salinity, Photoperiod	2	Astorga et al. 2012
A. aurita, Cyanea capilata, Cyanea lamarckii, Chrysaora hizoscella	I	G-B	bulk of polyps	150	Once a week	T*, Salinity	40-80	Holst 2012
A. aurita, Rhizostoma pulmo, C. tuberculata	W-B	C-P	1	10	Every 2-4 days	T*	11-14	Purcell et al. 2012
C. tuberculata	I; W-B	F	20 to 50	NM	Once a week	T*, salinity*, L*, N*, FS*	8-10	Prieto et al. 2010
A. aurita	NM	C-P	1	5	NM	T. FS*		Kamiyama 2011
Aurelia labiata	I	G-J	1	125	Every 2-4 days	T*, pH*	17	Winans and Purcell 2010
A. aurita	I	S-D	3	160	NM	T*, FS*	4	Han and Uye 2010
A. aurita	I	C-P	1	50	Every 2-3 days	T*, L*	11	Liu et al. 2009
A. aurita	NM	G-BT	3	100	NM	DO*, T		Ishii et al. 2008
A. labiata	I	C-P	1	10	Every 2-3 days	T*, S*, L*	29	Purcell 2007
Aurelia sp.	W-B	A-P	30	NM	Every 2 days	T*, Salinity*	5	Willcox et al. 2007
Aurelia sp.	C-R-W	A-I-CH	I	35	Every minute, continuous flow	T*, pH*, F*	38	This study

*Parameters manipulated/controlled in the experiments.

The acrylic box containing the incubation chambers was constructed on top of a PVC box of the same size (C in Fig. 2). This PVC box has a transparent lid and contains 18 MSD light-emitting diodes (LED), located on the perpendicular axis of each incubation chamber. The LEDs are connected to an adjustable external power supply that allows for regulation of the light's duration and intensity, which are important factors to be considered depending on the jellyfish species under study. For example, the Mediterranean jellyfish *Cotylorhiza tuberculata* has symbiotic zooxanthellae and, therefore, light conditions are critical. In this experiment, light was used to facilitate the polyp's feeding. The LEDs were located at the bottom of the polyp flat, where the polyps are fixed. The polyps were fed with live *Artemia salina* nauplii that have positive phototaxis, facilitating their capture by the polyps.

Each incubation chamber (Fig. 3) consists of a 6 cm high transparent acrylic cylinder (1 cm thick) with an external diameter of 4 cm and an internal volume of 35 cm^3. Nylon lids were made using a mechanical lathe and an O-ring assured the sealed connection with the acrylic cylinder. Two holes were drilled in the lids to house the inlet and outlet water tubes, ensuring water renewal, and a small 60 μm mesh covered the water outlet, acting as a filter to avoid losses of organisms (food, polyp buds, or even small polyps). The specifically designed cover of the incubation chamber is shown in detail in Fig. 4.

Positioning and maintenance of the jellyfish polyps in the polyp flat

Polyps of the scyphozoan species *Aurelia* sp. (NW Mediterranean sea population) were used on this first experiment to test the performance of the new system. The polyps were obtained from the jellyfish cultures maintained at the Experimental Aquaria Zone (ZAE) of Institut de Ciències del Mar (ICM-CSIC), which are kept at a salinity of 38 and a temperature of 21°C to ensure the typical natural strobilation in spring.

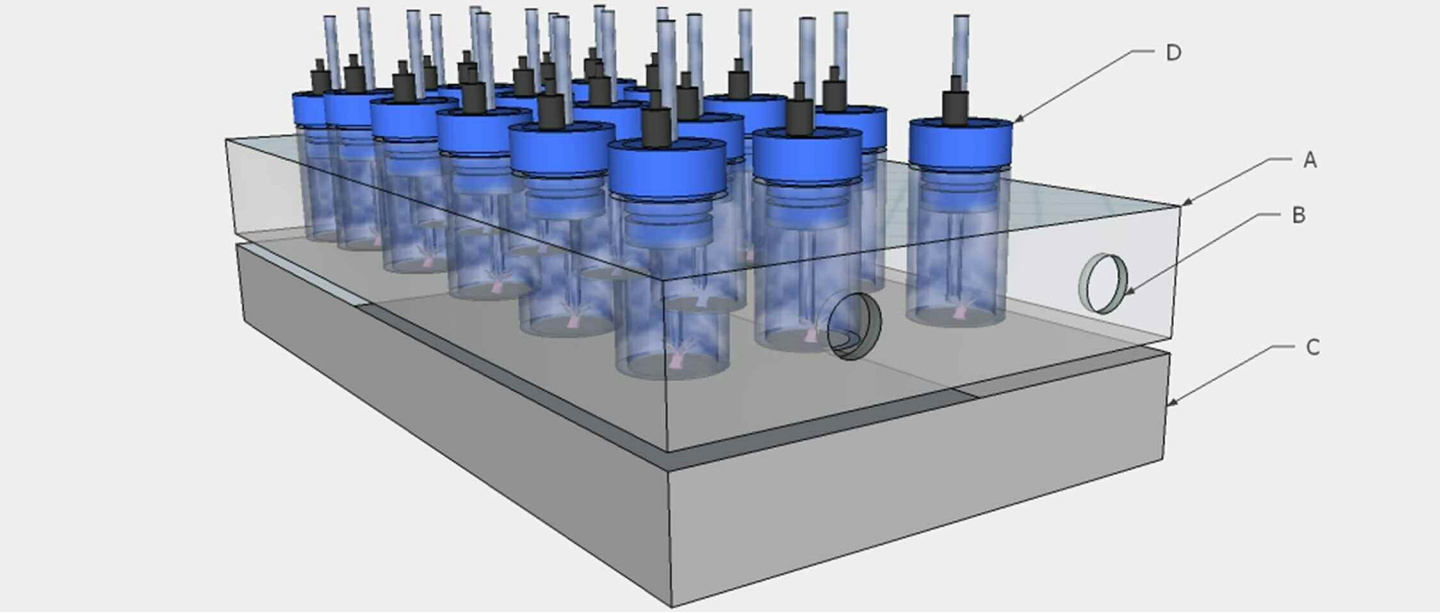

Fig. 2. Complete polyp flat for one treatment consisting in 18 individual incubation chambers submerged in a temperature controlled water bath. A) Acrylic box, B) holes for the bath water inlet and outlet, C) PVC box with a transparent lid containing the LED lights, D) incubation chambers.

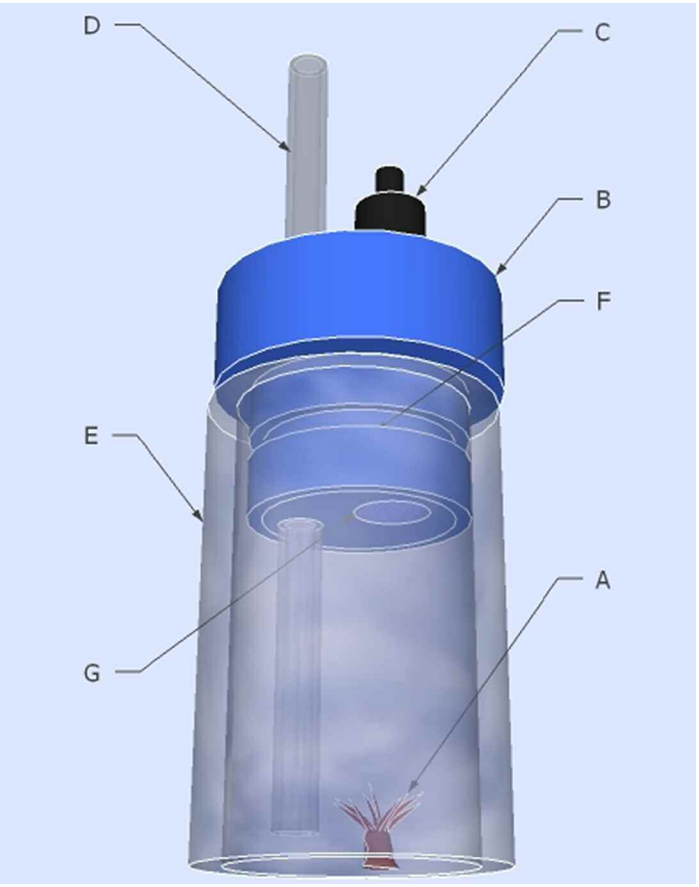

Fig. 3. Detail of an incubation chamber. A) Jellyfish polyp, B) Nylon lid, C) Water outlet, D) Water inlet, E) Incubation chamber, F) "o"-ring slot housing, G) 60 μm mesh.

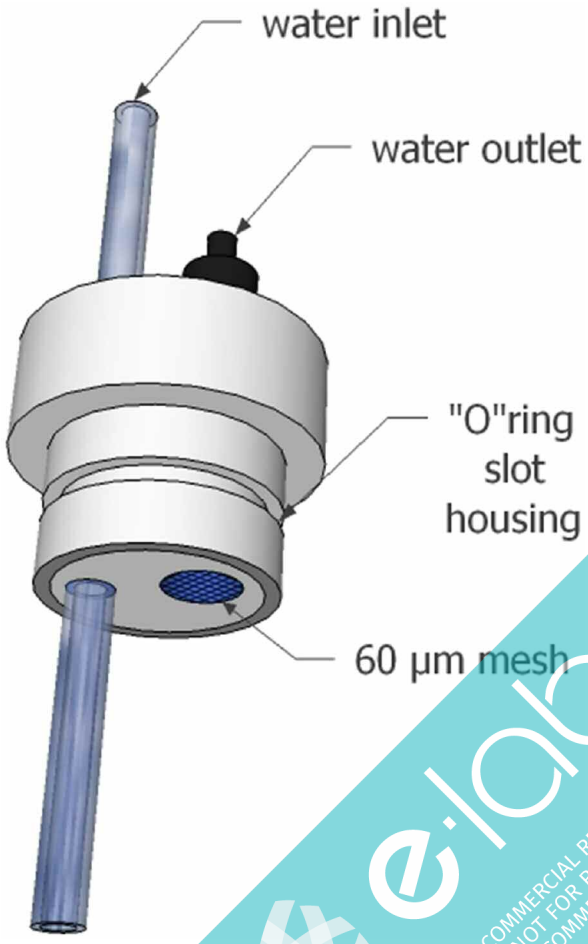

Fig. 4. Detail of the designed lid of each incubation chamber.

Single polyps of similar sizes were selected from these cultures and positioned at the base of each incubation chamber (Fig. 3). Before starting the experiment, all polyps were kept in their chambers with a very low water flow to ensure and facilitate their proper attachment. Once the polyps were totally attached, the water flow was increased to achieve a proper water renewal (~30 mL/min) so approximately the whole volume of the chamber was renewed every minute.

Once a week, the polyps were checked for survival, asexual reproduction (production of buds), and strobilation (production of ephyrae) following Purcell et al. (2012). The produced buds were removed periodically while maintaining the original polyp in the incubation chamber, allowing the tracking of individual specimens. The polyps were fed twice a week with newly hatched *A. salina* nauplii by closing the seawater flow-through during one hour to ensure a proper feeding. After feeding, the chambers were cleaned using a fine brush to gently scrap away all dirt and food remains.

Experimental setup and CO_2 system manipulation

Filtered seawater (through a 20 μm cartridge filter) was continuously supplied to four 150 L tanks (A in Fig. 5), and their pH was gradually adjusted to four selected values of ~8.10, ~7.80, ~7.50, and ~7.20 units (total scale). To achieve these pH levels, seawater in each tank was bubbled with CO_2 (99.9% purity; C in Fig. 5) or CO_2-free air (using a filter filled with soda lime absorber, Sigma Aldrich; F in Fig. 5). Seawater pH was monitored continuously by glass electrodes (LL Ecotrode plus –Metrohm; K in Fig. 5) connected to two pH controllers (Consort R316, Topac Inc.; E in Fig. 5), which automatically opened and closed the solenoid valves of CO_2 or CO_2-free air when needed (J and G in Fig. 5, respectively). To avoid drifts in the pH measurements, glass electrodes were calibrated on a daily basis with a Tris buffer, following standard procedures (SOP6a of Dickson et al. 2007). In addition, small volumes of water were taken periodically to analyze total alkalinity (TA) by potentiometric titration (Perez and Fraga, 1987; Perez et al. 2000) and pH using spectrophotometry (Clayton and Byrne 1993), which provides better precision than glass electrodes. Water from the 150 L tanks flowed continuously (1.8 L per hour) by means of individual silicone tubing (not shown) through each incubation chamber where organisms were maintained. The pH-manipulative experimental set-up was installed inside a thermostatic room, ensuring constant temperature values during the whole experiment.

The objective of this manipulation experiment, whose scientific results and implications will be published elsewhere,

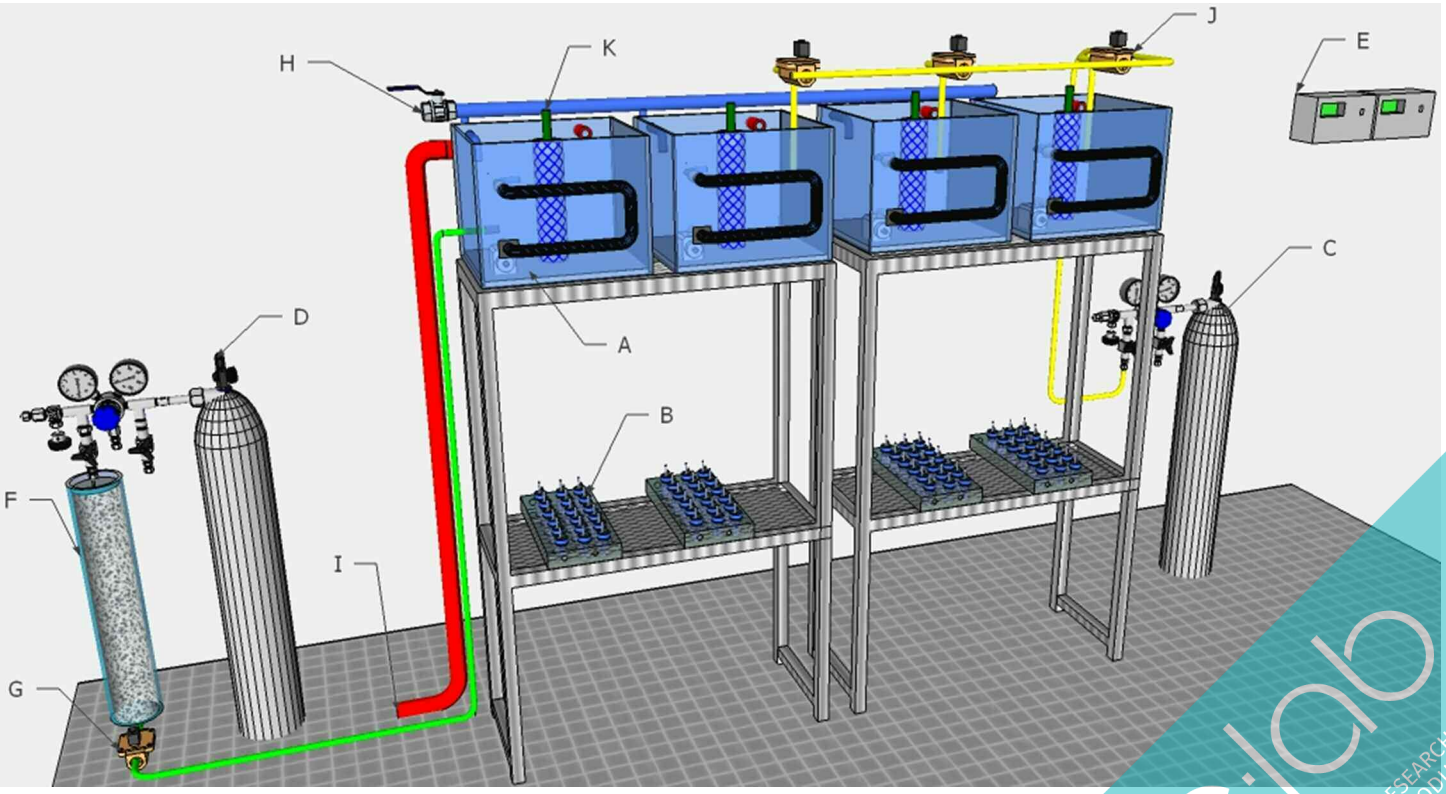

Fig. 5. Experimental setup used to control and modify seawater pH in each polyp flat. A) large 150-L tanks for seawater conditioning at pH 8.10, 7.80, 7.50, and 7.20; B) polyp flats containing 18 isolated polyps in each incubation chamber; C) 50 kg CO_2 cylinder; D) 50 kg compressed air cylinder; E) pH controller and data logger; F) soda lime filter; G) CO_2-free air solenoid valve; H) filtered seawater inlet line; I) drain outlet line; J) CO_2 solenoid valve; K) glass electrodes for pH and PT100 probes for temperature measurements. For simplicity, we have not shown all tubing connecting the conditioning tanks to each polyp flat, but note that all tubing is connected to the lower part of the U-shaped black pipeline depicted in A.

was to investigate the effects of different acidified conditions on the physiology, structure, performance, and reproduction of jellyfish polyps.

pH manipulation experiment and tests of system performance

In order to test the efficiency of the system in maintaining stable conditions of seawater pH, temperature, and flow rate in all replicates of each treatment, periodic measurements were taken in each of the 72 incubation chambers after adjusting the seawater pH to the four desired levels (Tables 2-4). To conduct these measurements, small amounts of water (~25 mL) draining from each incubation chamber were collected in plastic test tubes, and pH (total scale), temperature (°C), and salinity were measured. Afterwards, the flow rate (mL/min) through the polyp flat was measured using a chronometer and a volumetric flask. The whole set of measurements (pH, temperature, salinity, and flow rate) took approximately 1 min per polyp flat, fast enough to ensure unchanged conditions in the sampled water. Sensors and equipment used in these measurements were a Metrohm 826 pH mobile with a glass electrode Aquatrode Plus from Metrohm (± 0.003pH units) calibrated with a TRIS buffer for pH (total scale), an Ebro TFX-410 Pt1000 temperature probe (± 0.1 °C) for temperature, and a YSI 30 probe (± 0.1) for salinity.

Assessment

Accuracy and precision

As outlined above, the primary objective of this continuous-flow system was to overcome the problems of maintaining

Table 2. First example of measurements of pH (total scale), temperature (T, in °C), salinity (S), and flow rate (in mL/min). Each parameter was measured in each of the 18 polyp flats (1 to 18) in the four pH treatments (T1 toT4). Data are given in separated panels for each treatment, which are divided into eighteen boxes which identify each polyp flat. Each polyp flat box contains the four measured parameters ordered as in the panels' headers. Headers for each treatment contain the average of each parameter over the 18 polyp flats ± the maximum difference from the mean that we considered acceptable for the proper performance of the system. This maximum difference is used to color code the measured values in the following way: Values marked in white are those that are closer to the average of the eighteen measurements whereas values marked in dark gray are the most distant from the average. Values outside the ± maximum difference are all in dark gray.

pH 8.06 ± 0.1 T 15.7 ± 0.5 S 37.6 ± 0.2 Flux 28 ± 10		
T1		
18	17	16
8.07	8.06	
15.8	15.7	
37.6	37.4	
33	22	
13	14	15
8.06	8.06	8.06
15.8	15.7	15.5
37.8	37.7	37.6
31	26	27
12	11	10
8.07	8.06	8.05
15.7	15.7	15.8
37.8	37.8	37.6
28	31	23
7	8	9
8.05	8.05	8.06
15.5	15.6	15.8
37.5	37.7	37.6
27	27	24
6	5	4
8.06	8.07	8.06
15.5	15.8	15.6
37.4	37.6	37.6
22	33	30
1	2	3
8.06	8.06	8.07
15.8	15.4	15.5
37.5	37.5	**37.4**
26	27	31

pH 7.83 ± 0.1 T 15.7 ± 0.5 S 37.6 ± 0.2 Flux 27 ± 10		
T2		
18	17	16
7.83	7.82	7.82
15.6	15.6	15.8
37.4	37.5	37.8
24	23	31
13	14	15
7.83	7.83	7.82
15.8	15.7	15.6
37.7	37.6	37.6
26	30	33
12	11	10
7.83	7.83	7.83
15.9	15.7	15.6
37.8	37.7	37.6
25	30	26
7	8	9
7.83	7.83	7.83
15.8	15.8	15.6
37.8	37.7	37.6
22	30	26
6	5	4
7.82	7.82	7.84
15.7	15.6	15.7
37.6	37.5	37.6
21	30	29
1	2	3
7.82	7.83	7.82
15.8	15.7	15.6
37.4	37.5	37.5
32	24	24

pH 7.54 ± 0.1 T 15.7 ± 0.5 S 37.7 ± 0.2 Flux 33 ± 10		
T3		
18	17	16
7.54	7.53	7.53
15.9	15.9	16.0
37.4	37.4	37.6
28	36	38
13	14	15
7.53	7.54	7.53
15.5	15.6	15.8
37.8	37.8	37.9
36	35	33
12	11	10
7.54		7.54
15.4		15.8
37.7		37.5
32		36
7	8	9
7.54	7.54	7.54
15.9	15.9	15.8
37.6	37.6	37.5
32	27	34
6	5	4
7.54	7.54	7.54
15.4	15.7	15.6
37.6	37.8	37.7
37	35	32
1	2	3
7.54	7.54	7.54
15.8	15.6	15.6
37.8	37.7	37.7
26	36	28

pH 7.26 ± 0.1 T 15.7 ± 0.5 S 37.7 ± 0.2 Flux 31 ± 10		
T4		
18	17	16
7.25	7.26	7.25
15.7	15.8	15.7
37.8	37.8	37.8
32	33	36
13	14	15
7.26	7.25	7.25
15.7	15.6	15.6
37.8	37.6	37.6
28	31	29
12	11	10
7.26	7.26	7.26
15.7	15.6	15.9
37.8	37.6	37.8
32	31	35
7	8	9
7.27	7.26	7.26
15.9	15.8	15.7
37.8	37.8	37.6
31	35	26
6	5	4
7.27	7.27	7.26
15.9	15.9	16.0
37.8	37.8	37.4
27	30	33
1	2	3
7.25	7.24	7.25
15.6	15.4	15.9
37.8	37.7	37.8
28	24	33

Table 3. Second example of measurements of pH (total scale), temperature (T, in °C), salinity (S), and flow rate (in mL/min), following the same scheme and color coding as Table 1.

pH	8.07	± 0.1	pH	7.81	± 0.1	pH	7.51	± 0.1	pH	7.24	± 0.1
T	15.6	± 0.5	T	15.7	± 0.5	T	15.7	± 0.5	T	15.6	± 0.5
S	37.5	± 0.2	S	37.6	± 0.2	S	37.6	± 0.2	S	37.6	± 0.2
Flux	30	± 10	Flux	30	± 10	Flux	32	± 10	Flux	31	± 10
T1			T2			T3			T4		
18	17	16	18	17	16	18	17	16	18	17	16
8.06	8.06		7.80	7.80	7.81	**7.51**	7.50	7.50	7.23	7.23	7.24
15.6	15.5		15.8	15.8	15.8	**15.5**	15.4	15.4	15.9	15.9	15.4
37.5	37.5		37.7	37.7	37.7	**37.6**	37.4	37.4	37.4	37.3	37.5
35	27		26	29	35	**37**	35	33	32	34	38
13	14	15	13	14	15	13	14	15	13	14	15
8.07	8.06	8.06	7.81	7.81	7.81	7.51	7.51	7.51	7.24	7.23	7.23
15.7	15.5	15.4	15.7	15.6	15.9	15.8	15.9	15.4	15.7	15.4	15.6
37.6	37.5	37.4	37.6	37.7	37.7	37.5	37.6	37.4	37.7	37.5	37.6
38	22	32	29	35	35	36	34	34	25	34	29
12	11	10	12	11	10	12	11	10	12	11	10
8.07	8.07	8.06	7.82	7.81	7.81	7.52		7.51	7.23	7.23	7.24
15.6	15.6	15.6	15.6	15.7	15.7	15.9		15.8	15.4	15.7	15.5
37.6	37.6	37.5	37.5	37.7	37.6	37.4		37.8	37.4	37.6	37.8
34	38	26	34	26	24	31		29	32	31	35
7	8	9	7	8	9	7	8	9	7	8	9
8.07	8.07	8.07	7.83	7.82	7.82	7.52	7.51	7.51	7.24	7.24	7.24
15.7	15.7	15.7	15.7	15.7	15.7	15.9	15.9	15.7	15.5	15.4	15.4
37.5	37.6	37.5	37.7	37.6	37.6	37.7	37.8	37.6	37.7	37.8	37.8
24	32	28	31	29	33	37	22	34	32	35	27
6	5	4	6	5	4	6	5	4	6	5	4
8.07	8.07	8.07	7.83	7.82	7.82	7.52	7.52	7.52	7.25	7.25	7.26
15.7	15.4	15.8	15.7	15.8	15.5	15.7	15.6	15.5	15.6	15.4	15.8
37.6	37.6	37.5	37.6	37.7	37.4	37.6	37.8	37.8	37.5	37.6	37.8
35	22	31	23	31	33	35	30	30	27	30	34
1	2	3	1	2	3	1	2	3	1	2	3
8.06	8.07	8.07	7.82	7.81	7.82	7.52	7.52	7.51	7.25	7.25	7.25
15.8	15.6	15.7	15.4	15.5	15.6	15.5	15.6	15.9	15.8	15.7	15.8
37.5	37.3	37.5	37.4	37.5	37.6	37.7	37.8	37.8	37.7	37.6	37.8
30	26	38	35	23	23	22	35	27	29	23	33

stable water conditions, in this case temperature, pH, and water flow rate, along a long-term experiment. As mentioned, the system is fed from filtered seawater whose salinity varied naturally from 37.8 to 38.6 during the experimental period. No attempt was made to adjust and/or maintain the salinity of the water constant along the experiment. Data obtained in three different measurements performed over different days to test the system (pH, temperature, and flow rate) are shown in Tables 2-4, where a grayscale color code of measured values for each polyp flat is used to illustrate the extent of deviation of each container to the averaged values.

The accuracy and precision of the polyp flat system for maintaining the measured variables constant in time but different among treatments were statistically tested using a hierarchical linear model (two-way nested ANOVA). To avoid problems with repeated measurements (longitudinal data), a specific error structure was added to the model, leaving individual incubation chamber variation in time to be nested inside each treatment (Logan 2010), allowing for comparisons of selected parameters among treatments and also among consecutive days. In order to pass normality assumptions, rank transformations were applied to the data. All analyses were performed using the free statistical platform R (R Core Team 2012).

Statistical analysis showed differences in pH levels among treatments ($F_{3,204}$ = 119.43, P < 0.01) but no differences were detected among experimental days ($F_{2,204}$ = 0.231, P = 0.794). These results indicate a proper reproducibility of achieved pH levels in all incubation chambers of each treatment (Fig. 6A). Regarding those variables that were set fixed among the different pH treatments and experimental days, statistical analysis showed no differences for temperature and flow rate between treatments ($F_{3,204}$ = 0.31, P = 0.82 and $F_{3,204}$ = 0.65, P = 0.59, respectively) nor between days ($F_{2,204}$ = 2.42, P = 0.091 and $F_{2,204}$ = 0.81, P = 0.45, respectively) (Fig. 6 B and C). These results give strength to the robustness of this system to maintain constant the selected conditions throughout the experiment. Regarding salinity, however, stronger deviations from the mean were measured (color code in Tables 2-4). Because salinity was the last parameter to be taken, interpretation of these data has to be made with caution, given that vessels were open several times during measurements, and different extents of evaporation could have increased the uncertainty in the salinity

Table 4. Third example of measurements of pH (total scale), temperature (T, in °C), salinity (S), and flow rate (in mL/min), following the same scheme and color coding as Table 1.

pH	8.06	± 0.1	pH	7.81	± 0.1	pH	7.52	± 0.1	pH	7.23	± 0.1
T	15.6	± 0.5	T	15.8	± 0.5	T	15.6	± 0.5	T	15.5	± 0.5
S	37.8	± 0.2	S	37.7	± 0.2	S	37.8	± 0.2	S	37.8	± 0.2
Flux	31	± 10	Flux	32	± 10	Flux	32	± 10	Flux	30	± 10
T1			T2			T3			T4		
18	17	16	18	17	16	18	17	16	18	17	16
8.06	8.06		7.82	7.81	7.81	7.50	7.51	7.52	7.23	7.23	7.23
15.7	15.7		15.8	15.9	15.9	15.7	15.9	15.8	15.6	15.6	15.6
37.8	37.8		37.8	37.5	37.5	37.6	37.8	37.6	37.8	37.6	37.6
38	27		26	26	36	24	33	32	31	35	36
13	14	15	13	14	15	13	14	15	13	14	15
8.07	8.06	8.07	7.81	7.81	7.81	7.53	7.52	7.52	7.22	7.23	7.23
15.6	15.5	15.6	15.9	15.8	15.9	15.7	15.7	15.6	15.5	15.4	15.5
37.9	37.8	37.8	37.8	37.7	37.5	37.8	37.8	37.7	37.6	37.7	37.7
32	27	37	28	35	37	35	32	33	26	34	29
12	11	10	12	11	10	12	11	10	12	11	10
8.07	8.07	8.07	7.80	7.80	7.81	7.52		7.53	7.23	7.23	7.23
15.7	15.7	15.5	15.8	15.8	15.9	15.5		15.7	15.6	15.6	15.7
37.8	37.8	37.8	37.8	37.8	37.8	37.6		37.8	37.8	37.7	37.8
30	33	34	34	28	38	29		35	31	27	34
7	8	9	7	8	9	7	8	9	7	8	9
8.07	8.07	8.06	7.82	7.82	7.81	7.53	7.52	7.53	7.23	7.23	7.23
15.4	15.7	15.6	15.8	15.9	15.8	15.6	15.5	15.6	15.4	15.5	15.6
37.9	37.8	37.9	37.8	37.8	37.8	37.8	37.8	37.8	37.8	37.9	37.9
38	30	24	32	29	32	34	33	34	30	35	28
6	5	4	6	5	4	6	5	4	6	5	4
8.07	8.07	8.07	7.83	7.83	7.82	7.52	7.52	7.53	7.23	7.24	7.24
15.7	15.6	15.9	15.6	15.9	15.7	15.5	15.6	15.5	15.5	15.5	15.5
37.9	37.9	37.8	37.8	37.5	37.8	37.9	37.8	37.8	37.9	37.9	37.9
32	25	32	26	37	32	35	34	27	28	30	33
1	2	3	1	2	3	1	2	3	1	2	3
8.06	8.06	8.06	7.82	7.82	7.82	7.53	7.53	7.52	7.24	7.25	7.25
15.6	15.6	15.8	15.9	15.6	15.7	15.5	15.4	15.5	15.5	15.4	15.5
37.8	37.7	37.8	37.6	37.8	37.8	37.6	37.8	37.8	37.7	37.7	37.9
30	25	38	30	38	26	29	34	23	24	24	33

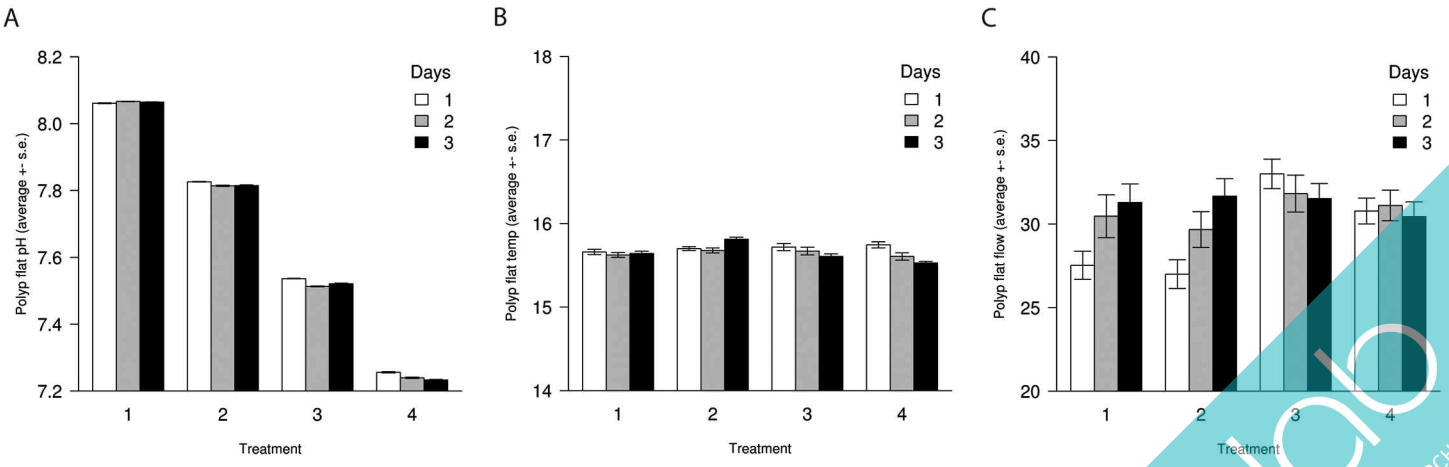

Fig. 6. Results of A) pH, B) temperature (in °C), and C) flow rate (in mL/min) averaged over all the polyp flats of each treatment measured at three different days during the experiment.

results. Therefore, it is possible that the salinity range to which these organisms were subjected within the polyp flats was lower than that measured in the open plastic containers.

Health status of the jellyfish polyps

During the experimental period, all polyps remained attached to their individual incubation chamber and devel-

oped properly, indicating that the polyp flat constituted an adequate system for the maintenance and experimentation of individual specimens. After 268 days of experiment, the total mortality was only 14% (10 out of a total of 72 polyps).

Discussion

A number of experiments to determine the effect of different environmental parameters (especially temperature and salinity) on jellyfish polyps have been conducted in the past. In all these studies (summarized in Table 1), water was renewed manually at discrete intervals of time. In most of these cases, the water was renewed when the polyps were fed, once or twice a week. It was mentioned by some of the authors that the physico-chemical conditions of the seawater changed along the experiment at different levels due to the low water renewal rate (e.g. Winans and Purcell 2010). Based on our previous experience, low water renewal posed problems for maintaining constant physicochemical conditions also due to the small volumes of the incubation chambers. As can be seen in Table 1, the jellyfish polyps (very small in size, from 1 to 2 mm high) were always placed in small containers, from 5 to 160 mL. Using small test volumes with inefficient seawater renewal might lead to unfavorable conditions for experimentation (Connor and Wilson 1972) because of accumulation of waste products, depletion of dissolved oxygen, changes in pH or because of bacterial communities composition. To solve these problems, a new design of incubation chamber was necessary in which seawater could run constantly through an open system, ensuring the necessary water renewal and maintaining constant the experimental conditions. To facilitate a broad utilization by the scientific community, the new system had to be simple and economically affordable.

Regarding the environmental stress of ocean acidification, on which research has focused recently, experiments of pH manipulation on jellyfish published so far are limited to a single study (Winans and Purcell 2010). This study focused on *Aurelia labiata*, a species typical for the west coast of North America and revealed that pH values affected the size of the statoliths, small calcium sulphate crystals (Becker et al. 2005) enclosed in the statocysts, the equilibrium organs in jellyfish. However, the pH adjustment in this study was performed by adding HCl (also affecting alkalinity), whereas in our case, we bubbled CO_2, a method that maintains the alkalinity constant and provides a more realistic approach.

At the time of writing this paper, our new system had been in use for 268 days, showing to be accurate and adequate for experimentation on jellyfish polyps. It is also easy to maintain, and therefore, suitable for long-term studies on the response of these organisms to environmental stressors such as ocean acidification. The mortality of polyps during our experiment was only 14% of the total number of polyps, which represents a very low mortality rate compared with other studies (Purcell et al. 2012). In general, mortality events in this kind of experiments occur due to the polyp manipulation and water evaporation, which increases salinity (Purcell et al. 2012). Overall, we conclude that this new system ensures a very good survival rate compared with previous studies. The performance assessment presented in this study attests to the suitability and reliability of this system to meet the current needs of environmental laboratory research on jellyfish polyps, taking advantage of the benefits of working in a continuous flow system. This prototype adds to the current pool of newly designed devices (e.g., Fangue et al. 2010; McGraw et al. 2010; Bockmon et al. 2013; Hoffmann et al. 2013) aimed at providing better tools for testing the effects of acidification and other environmental stressors in marine organisms, a discipline that has recently been identified as research front number one in Ecology and Environmental Sciences (King and Pendlebury 2013).

Comments and recommendations

In order to conduct experimental work with jellyfish polyps, several key aspects need to be considered. The polyps need some time to re-attach to the bottom of the incubation chamber. It is important to ensure that the animals are fixed before turning on the running water system. From our experience, depending on the species, the attaching process can take from a few days to one week, if polyps are in good conditions. The size of the mesh covering the water outlets of the incubation chambers has to be chosen based on the size of the polyps and the food that needs to be provided. In our experiment with *Aurelia* sp. polyps, which were fed with nauplii of *A. salina*, 60 μm represents an adequate mesh size. As future improvements to the system, peristaltic pumps could be used to facilitate the reproducible regulation of the flow rates of seawater to the incubation chambers. In addition, in order to allow the automatic and continuous measurement of selected physicochemical parameters, specific sensors could be implemented.

Overall, this study presents a relatively simple and affordable system that can greatly improve the results obtained from testing the effects of a range of environmental pressures on the eco-physiology of jellyfish polyps.

The authors have provided a multimedia Web Appendix where a multidirectional view of the full system, with details and close-up views of specific parts, will help researchers interested in reproducing it.

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