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From frustules to medusae: A new culture system for the study of the invasive hydrozoan *Craspedacusta sowerbii* in the laboratory

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Abstract

The invasive freshwater jellyfish *Craspedacusta sowerbii*, native from East Asia (Yangzi Jiang River), was introduced in Europe for the first time in the basins of the Royal Botanic Garden in London. From the beginning of the 20th century, worldwide reports of the presence of *C. sowerbii* have been increasing (USA, Canada, Europe, Australia, Russia). Despite its now worldwide distribution, *C. sowerbii* has rarely been the subject of recent studies. Moreover, *C. sowerbii* is difficult to rear in the laboratory, and individuals generally from the wild have only been reared for short periods. Many aspects of laboratory cultures have proven to be problematic for all stages of the life cycle (from frustule to medusae): lack of optimal growing system (circular current or not), water quality (physical and chemical conditions), diet (the type of food), and temperature. In this article, we present a technique for culturing all life stages of *C. sowerbii*, from polyps to medusae, to study its life history in the laboratory. To demonstrate the success of our culture protocol, the growth of polyps was measured for 80 days at 19 and 29°C. Colony growth increased at both temperatures in our culture system, and data were similar to those presented in the literature, illustrating the success of this protocol. Medusae were cultured for 70 days, and their bell diameter increased from 0.60 ± 0.08 mm (Day 0) to 9.0 ± 2.1 mm (Day 32). We developed a closed culture system that allowed specimens (i.e., polyps and medusae) to be maintained for more than 2 months (80 days for polyps, 72 days for medusae). This culture system will allow researchers in the future to study more precisely the metabolism (growth, ingestion, longevity) of polyps and medusae to understand life-history characteristics important to this species' ecology (periods of medusae production, predation, and diet).

KEYWORDS

culturing method, freshwater, jellyfish

1 | INTRODUCTION

Nonindigenous species introduction constitutes a major source of ecological disturbance (Boudouresque & Verlaque, 2012). As some become invasive, they often have strong ecological and economic impacts such as modifying the diversity of communities, changing interactions in food webs (e.g., competition, predation), and affecting human activities (e.g., fisheries, industrial complex, and tourism; Bampfylde et al., 2010; Cambray, 2003; Gallardo et al., 2016). Among those invasive species, gelatinous zooplankton organisms have been

an important player. Marine and freshwater gelatinous zooplankton are recognized as important members of zooplankton and constitute a large part of the pelagic biomass. In some regions, their abundance has increased significantly because of climate change or anthropogenic disturbances (Hays et al., 2018). Because of their high aggregations, socioeconomic impacts (e.g., decrease in fish stocks, stings, net clogging, and fouling of powerplants; Bosch-Belmar et al., 2017; Gucu, 2002; Purcell et al., 2001; Richardson et al., 2009) have been observed. Despite their low nutritional value, which means that large quantities must be consumed by a predator to satisfy its metabolic needs, the contribution of jellyfish to the energy balance of predators is important because of the speed of digestion, low capture costs, and availability (Hays et al., 2018).

Because of their direct and cascading effects on ecosystems and biodiversity, invasive species in aquatic environments have attracted public and scientific attention (Barz & Hirch, 2005; Daskalov et al., 2007; Smith & Alexander, 2008). However, to better understand the role of invasive species in the function and structure of aquatic ecosystems, it is important to understand how environmental features affect their physiology (Di Santo & Lobel, 2016; Folino-Rorem et al., 2016; Ko et al., 2014; Mayor et al., 2015; Pauly & Cheung, 2018 Zhang et al., 2015). Therefore, improved culture methods are needed to study the full life cycle and physiology of invasive species in the laboratory. One such species is the freshwater medusa *Craspedacusta sowerbii* Lankester 1880.

The invasive freshwater medusa *C. sowerbii* (phylum Cnidaria, class Hydrozoa, family Olindiidae) is native to East Asia (Didžiulis, 2006; Didžiulis & ŻŻurek, 2013; Parent, 1982). *Craspedacusta sowerbii* was first observed and described by William Sowerby in 1880, in the basins of the Royal Botanic Garden in London. From the beginning of the 20th century, worldwide reports of *C. sowerbii* have been increasing (Arbačiauskas & Lasutiene, 2005; Boothroyd et al., 2002; El Moussaoui & Beisner, 2017; Lewis et al., 2012; Marchessaux et al., 2020; Thomas, 1951). Because the pelagic phase (i.e., medusa) is sporadic and present only during summer, it is difficult to study all stages of the life cycle of *C. sowerbii*.

The life cycle of *C. sowerbii* has two reproductive phases (Figure 1): an asexual phase (benthic polyp stage) and a sexual phase (pelagic medusa phase). The complete life cycle from the egg to the pelagic free medusa lasts between 34 and 51 days (Acker & Muscat, 1976; Colin & Delahaye, 1995; Didžiulis & ŻŻurek, 2013; de Larambergue, 1945; Wang et al., 2006). Asexual reproduction is carried out by budding, in which a polyp (measuring 1–2 mm) will bud either frustules that separate from the initial polyp and form a new

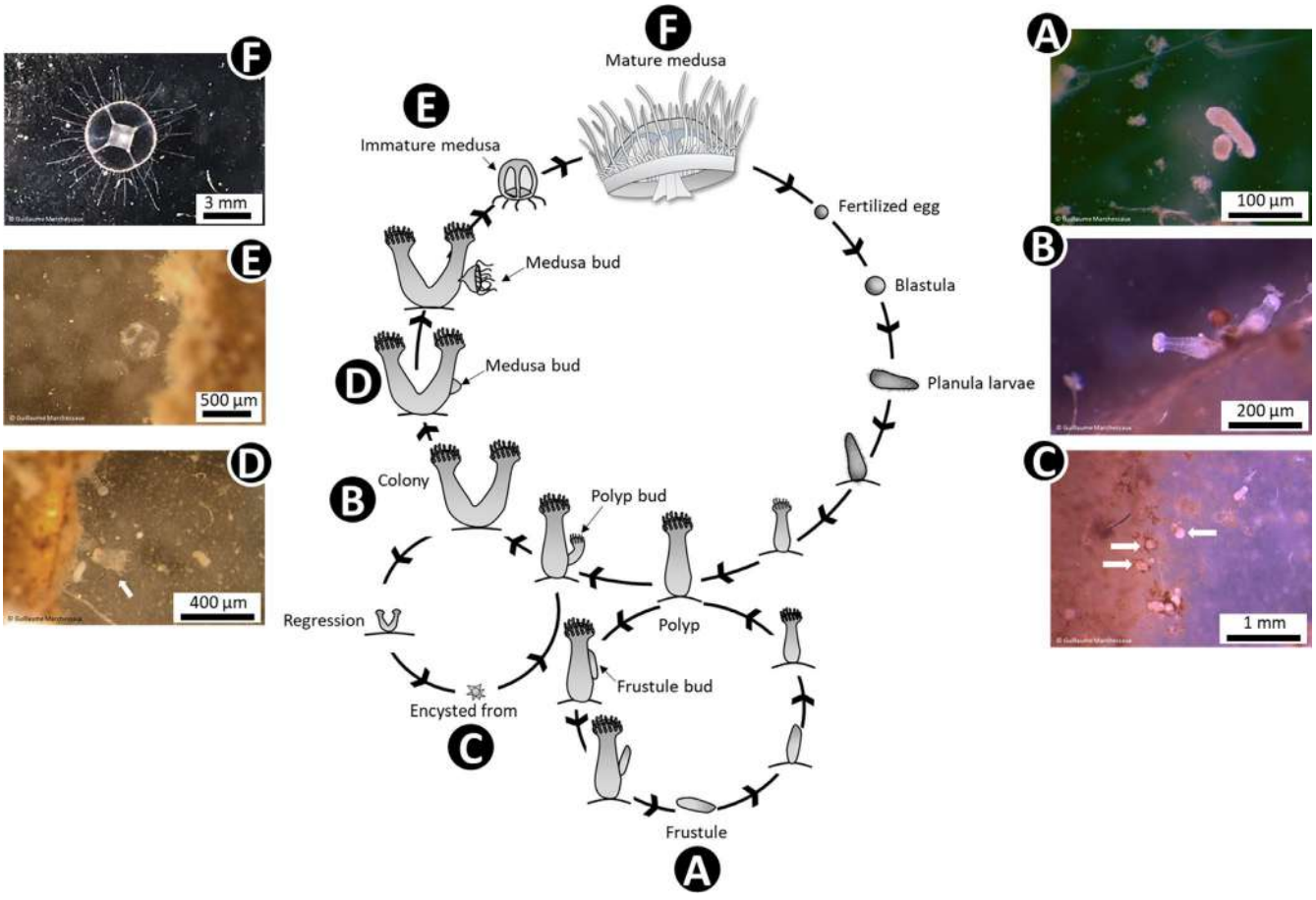

FIGURE 1 The life cycle of *Craspedacusta sowerbii*, redrawn and modified based on a drawing in Lytle (1982), and with photographs (panels A–F) of the different stages observed in our study. **A.** Frustule. **B.** Colony. **C.** Encysted form. **D.** Colony showing medusa bud. **E.** Immature medusa. **F.** Mature medusa. Letters on the drawing indicate corresponding panels

colony, or (2) immature medusae (medusa buds). Once detached, the rest of the medusa growth occurs in the pelagic phase. In *C. sowerbii*, the full mature medusa stage is reached when the diameter of individuals is between 9 and 10 mm (Colin & Delahaye, 1995). Mature individuals release their gametes in the water and after external fertilization, eggs are transformed into ciliated planula larvae which attach themselves to solid structures and metamorphose into polyps and reproducing asexually (Didžiulis, 2006; Matthews, 1966).

It is very difficult to find and sample the polyp phase (Bushnell & Porter, 1967; Duggan & Eastwood, 2012; Folino-Rorem et al., 2016; Zhang et al., 2009). There is a lack of data on the life cycle, biotic, and abiotic factors inducing production of medusae and on the species' potential predatory impact on food webs (diet and predatory pressure). The impact of *C. sowerbii* (especially polyps) on ecosystems is likely underestimated (Folino-Rorem et al., 2016; Smith & Alexander, 2008; Spadinger & Maier, 1999). Some studies showed that as predators *C. sowerbii* could have a cascading effect of primary producers (Smith & Alexander, 2008), but no data exist on the predatory impact of polyps.

Despite its worldwide distribution, *C. sowerbii* has rarely been the subject of recent studies. Moreover, individuals of *C. sowerbii* are difficult to rear in the laboratory, and specimens from the wild have only been reared for short periods. To our knowledge, only two publications on the life cycle and laboratory culturing of *C. sowerbii* have been published in English (Folino-Rorem et al., 2016; McClary, 1959). The temperature and the availability of food play a role in the asexual or sexual reproduction in *C. sowerbii* (Acker & Muscat, 1976; Boothroyd et al., 2002; McClary, 1959, 1961, 1964; Rayner, 1988; Slobodkin & Bossert, 1991). The production of frustules appears to be independent of temperature, and the development of frustules into polyps has been observed between 12 and 28°C (Folino-Rorem et al., 2016; McClary, 1959, 1961, 1964). Acker and Muscat (1976) found that between 26 and 33°C, a high concentration of food stimulated the production of medusae by polyps. Under unfavorable environmental conditions (low temperatures, little food), resistant polyps (i.e., encysted frustules) have been observed and can survive 40 years of desiccation (Bouillon & Boero, 2000; Brancotte & Vincent, 2002).

Many aspects of laboratory cultures have proven to be problematic for all stages of the life cycle (from frustule to medusa): lack of optimal growing system (circular current or not), water quality (physical and chemical conditions), diet (the type of food), and temperature (Acker & Muscat, 1976; Folino-Rorem et al., 2016; Lytle, 1961). Folino-Rorem et al. (2016) cultivated a natural population of polyps from Panama in Petri dishes, and the medusae were kept in a 9.5-L aquarium. However, even though the polyp phase was well maintained in their system, a phenomenon of eversion of the medusa bell was observed. It is therefore essential to create a functional culturing system for different life cycle stages of *C. sowerbii* to better understand its invasive capacities (i.e., sexual and asexual reproduction, growth rates, ingestion, etc.), and to produce specimens in large quantity for specific studies (e.g., genetics of polyps and medusae).

We present a culture technique for *C. sowerbii* from polyps to medusae. We have developed a closed culture system that allowed specimens (i.e., polyps and medusae) to be maintained over the long term (80 days for polyps, 72 days for medusae), and we have tested this system on a population of polyps from Cinéaqua Aquarium, Paris, France.

2 | METHODS

2.1 | Culture system: Protocol of construction

To make the culture system for *C. sowerbii*, a commercial aquarium and three laboratory mouse cages (Tecniplast®, cover, tank, and metal support) were used. In addition, a pump (Sicce Micra PRM100®), an air pump (Chialstar®), microscope glass slides (Rogo Sampaic™), plexiglass plates, silicon, and 20- and 60-μm meshes were used.

The global system consisted of a 128-L commercial glass aquarium (80 × 40 × 40 cm, length × width × height) filled with 50 L of reverse-osmosis filtered water produced by a triple-filtration technique using the Dennerle® Osmose Professional 190 system (Figure 2A,D,E). A transparent cover was made using a polycarbonate plate (80 × 40 × 1.6 cm) to close the system and limit water evaporation losses (Figure 2A,D).

A pump (Sicce Micra PRM100®, 5.7 × 4.3 × 5.2 cm) was placed in the left corner of the aquarium, allowing the water to circulate with a low current (50 ml/s) through a plastic pipe (2 cm diameter, 2 m long; Figure 2A,D,E). To measure the speed of the current, an empty 2-L bottle was placed in the circulating water and the time to fill was recorded. A tap was placed at the end of the hose to change the flow of water (Figure 2A,D,E). To oxygenate the water, a drip system (one drip per second for polyps, one drip every 5 s for medusae) and an air pump (Chialstar®, 5.2 × 2.4 cm) were installed.

The natural water filtration system was created using the tank of a laboratory mouse cage (Tecniplast®). The mouse cage was composed of a plexiglass cover (30 × 21 ×8 cm, volume 5.04 L), a tank (30 × 21 × 20 cm, volume 6.10 L), and a metal support (30 × 21 × 8 cm). A 20-μm mesh was glued with silicon to the front of the cover. Approximately 4 L of sand (grain size 5–10 mm) was placed in the tank. Sand (1 L) and Java moss, *Taxiphyllum barbieri*, were placed in the cover, which was placed upside down in the tank (Figure 2D,E). The installation was placed on the metal support in the aquarium. The natural filtration system consisted of the passage of water through moss, sand, and the 20-μm mesh (Figure 2A,D,E). This type of filtration made water changes unnecessary. A neon light (Ferrara®, two tubes, T8 type, 36 W, length 120 cm) was placed 1.5 m from the cover on a 12:12 hr day: night cycle.

Polyps were placed in 51-ml culture tanks (7.6 × 2.6 × 2.6 cm) made from glass microscope slides (Rogo Sampaic™) assembled with silicon (Figure 2B). The polyp tanks were placed in a second cover of a mouse cage, which was upside down on a metal support in the aquarium (Figure 2A,D,E). A 60-μm mesh (35 × 21 cm), glued with silicon to the front of the cover, allowed water exchange (Figure 2A,D,E).

To culture medusae, a 19-L cube (12.5 × 12.5 × 12.5 cm; Figure 2C) was constructed using transparent plexiglass plates assembled with silicon. In the center of two opposing sides of the medusa cube, a 2-cm-diameter hole was pierced using a soldering iron,

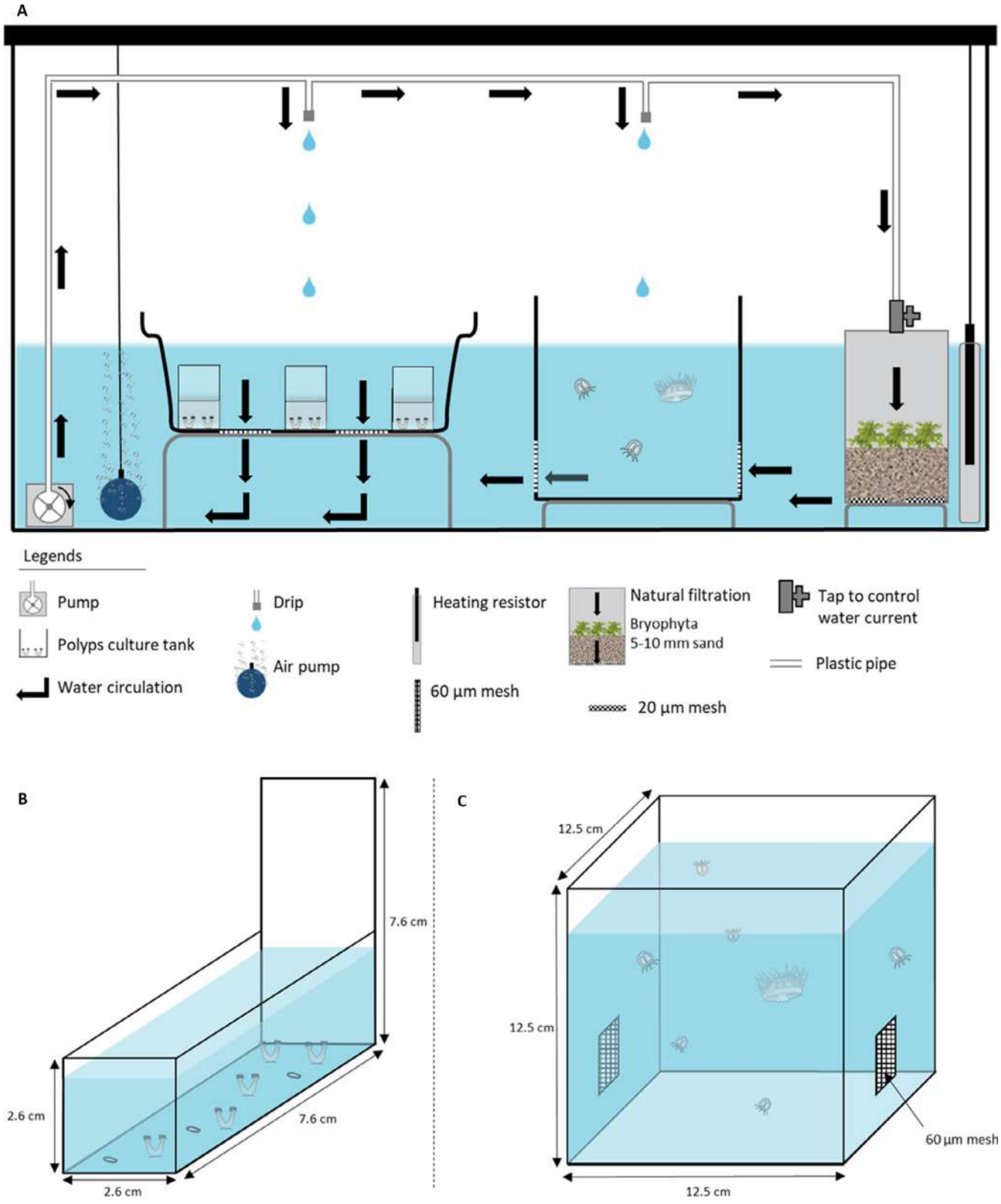

FIGURE 2 Schematic diagrams of the system for culturing *Craspedacusta sowerbii*.**A.** Global system (commercial glass aquarium, 80 × 40 × 40 cm, length × width × height; volume 128 L). **B.** Polyp tank (7.6 cm × 2.6 × 2.6 cm, volume 51 ml), constructed with glass microscope slides. **C.** Medusa cube constructed with transparent plexiglass plates (12.5 × 12.5 × 12.5 cm, volume 1.9 L). D, E. Photographic overview of the system. **D.** Front view. **E.** Top view

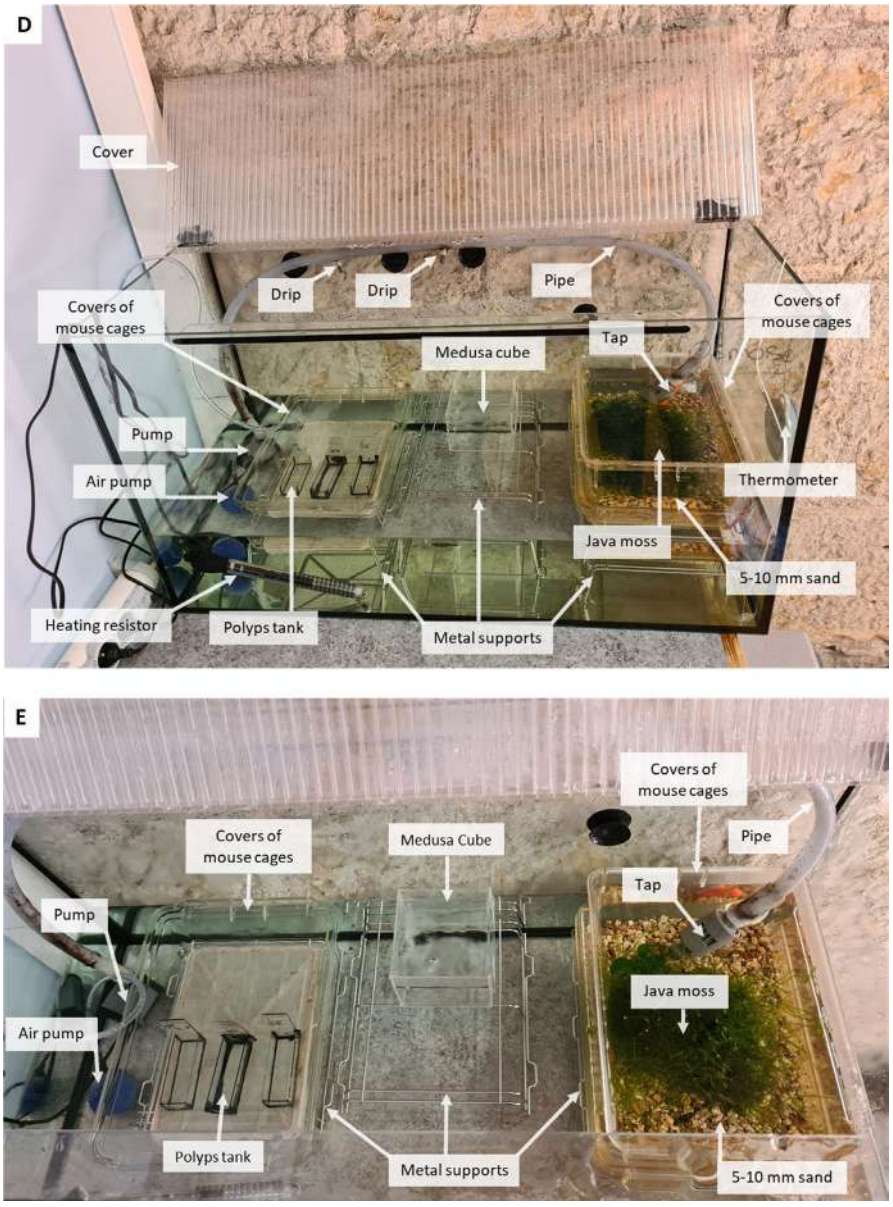

FIGURE 2 Continued

and a 60-μm mesh (l2.5 × 2.5 cm) was glued using silicon to allow water exchange. The medusa cube was placed on a mouse cage metal support in the aquarium (Figure 2A,D,E).

Water temperature was measured every 10 s using a digital thermometer (iLog® Temperature Loggers). Water chemistry variables including pH, ammonia (NH_4^+), nitrates (NO_2^-), nitrites (NO_3^-), phosphates (PO_4^{2-}), silicon dioxide (SiO_2^-), iron (Fe^{2+}), and copper (Cu^{2+}) were measured every week using JBL® ProAquaTest LAB.

2.2 | Quantifying the growth of colonies and medusae

To evaluate the success of this culture system, population growth from frustules to medusae was measured at 19 and 29°C. The initial population of polyps used for this experiment came from the Cinéaqua Aquarium (Paris, France), native from Kamo, Japan. Fifteen new frustules produced by the initial population were gently transferred using a micropipette under a stereomicroscope and placed in a polyp tank. Three polyp tanks were placed in a closed culture aquarium for 80 days (Figure 2A,D,E). We measured budding activity as the number of polyps present rather than the number of colonies (McClary, 1959). The budding of polyps was measured under a stereomicroscope, and the total number of polyps, the number of polyps per colony, the number of frustules per polyp, and the medusa buds per polyp were determined every 2 days for 80 days.

The new medusae (40 specimens) produced in 24–36 hr were placed in the medusa cube. Every day, all specimens were delicately isolated, and the bell diameter was measured under a

stereomicroscope. The growth of medusae was measured for 32 days. Because the handling of individuals can induce damage (risk of tearing the umbrella, stress, etc.), we decided to stop making measurements once medusae reached 10–11 mm in diameter. In addition, to measure the success of the medusa cube, we recorded mortality among the 40 specimens daily until all individuals had completely disappeared.

2.3 | Feeding and zooplankton prey

Nauplii of *Artemia salina* (Ocean Nutrition™, nauplii length 430 μm) were used to feed the specimens. Nauplii were produced daily from dried eggs in a 1-L beaker containing 500 ml of saltwater (25 psu) at 25°C with on a 12:12 hr day: night cycle. To eliminate salt, nauplii were rinsed three times on a 60-μm sieve with reverse-osmosis filtered freshwater. Nauplii were cut in half (~215 μm) by use of a scalpel under a stereomicroscope to facilitate ingestion by polyps and medusae < 1 mm diameter. To feed medusae with bell diameter > 1 mm, nauplii were given intact. Polyps were fed every 3 days (one nauplius per polyp), according to methods from previous studies, which allowed suitable production of polyps (Acker & Muscat, 1976; Folino-Rorem et al. 2016; Lytle, 1961). Medusae in the cube were fed daily with a known number of nauplii per medusa (30, 50, 80, or 100 nauplii per medusa for bell diameter <1 mm, 1–3 mm, 3–6 mm, or >6 mm, respectively.

2.4 | Data analysis

The mean and the standard deviation (*SD*) were calculated for data of colony growth, production of frustules and medusa buds. To test the significance of differences in the number of polyps per colony at both temperatures, analyses of variance (ANOVA) were calculated using SigmaPlot 12.5 software. If the overall ANOVA results were significant ($p < .05$), Bonferroni pairwise comparisons were performed to test among combinations.

To quantify the change in the number of polyps per colony at both temperatures, experimental measures were analyzed by linear and nonlinear regression analysis using SigmaPlot 12.5 software. The best representations were chosen on statistical criteria (coefficient of determination (R^2), significance level (p value), the sum of squared errors, and residual).

3 | RESULTS

3.1 | Identifying the types of asexual reproduction

The polyps of *C. sowerbii* produced asexual frustules by direct budding (Figure 1). After attaching to the substrate, the frustule differentiated into a polyp forming a new colony in 2 (29°C) or 3 (19°C) days. From this polyp, the colony grew through the production of polyps by direct budding on the stolon (2, 3, 4, or 5 polyps). Production of encysted frustules was less common (1%) in our experiments, and all encysted frustules remained encysted. The production of medusae by budding from polyps was also observed.

3.2 | Colony growth

The water parameters were constant in time (Table 1) with a neutral pH equal to ~7 and nutrient concentrations (ammonia, nitrites, nitrates) <0.1 mg/L. Metal ions (Fe^{2+} and Cu^{2+}) were also present in very low concentrations.

Two (29°C) to 3 days (19°C) after adding frustules into the polyps tanks (15 frustules at the beginning), the frustules differentiated into polyps at one or both ends and were then considered colonies. The frustule bulbs present on the polyps were not yet considered as new colonies. For both temperatures, the total number of polyps increased from 4 ± 0 polyps (Day 3) to 31 ± 6 polyps (Day 80) at 19°C, and from 2 ± 1 polyps (Day 2) to 316 ± 13 polyps (Day 80) at 29°C (Figure 3A,B).

The mean number of polyps per colony followed an exponential growth relationship for both temperatures (Figure 3). At 19°C, the number of polyps per colony increased significantly from 1 ± 0 (Day 2) to 3.1 ± 0.2 polyps per colony (Day 27) and remained constant (3.0 ± 0.2 polyps per colony) over time (Figure 3A). At 29°C, the same trend was observed: from 1 polyp per colony (Day 2) to 2.8 ± 0.1 polyps per colony (Day 14), 2.2 ± 0.2 polyps per colony (Day 16 to Day 80; Figure 3B). The number of polyps per colony was significantly higher (ANOVA, Bonferroni test, $p < .05$) at 19°C (3.1 ± 0.2 polyps per colony) than at 29°C (2.2 ± 0.2 polyps per colony).

3.3 | Frustule production and medusa buds

Frustule production was sporadic and highly variable for both temperatures: from no frustules to 0.51 ± 0.18 frustules per polyp at 19°C (Figure 4A), and from no frustules to 0.28 ± 0.02 frustules per polyp at 29°C (Figure 4B). At 19°C, the first production of frustules

TABLE 1 Water chemistry variables (mean ± *SD*) from a system to culture *Craspedacusta sowerbii*, measured with a JBL® ProAquaTest LAB every week for 80 days at 19 and 29°C

Water variable	19°C	29°C	Number of samples
pH	7.23 ± 0.18	7.28 ± 0.17	13
Ammonia (NH_4^+, mg/L)	0.07 ± 0.02	0.06 ± 0.02	13
Nitrites (NO_2^-, mg/L)	0.02 ± 0.01	0.03 ± 0.04	13
Nitrates (NO_3^-, mg/L)	0.62 ± 0.22	0.65 ± 0.24	13
Phosphates (PO_4^{2-}, mg/L)	0.41 ± 0.24	0.54 ± 0.25	13
Silicon dioxide (SiO_2^-, mg/L)	0.61 ± 0.49	0.80 ± 0.70	13
Iron (Fe^{2+}, mg/L)	0.02 ± 0.00	0.02 ± 0.00	13
Copper (Cu^{2+}, mg/L)	0.05 ± 0.00	0.04 ± 0.01	13

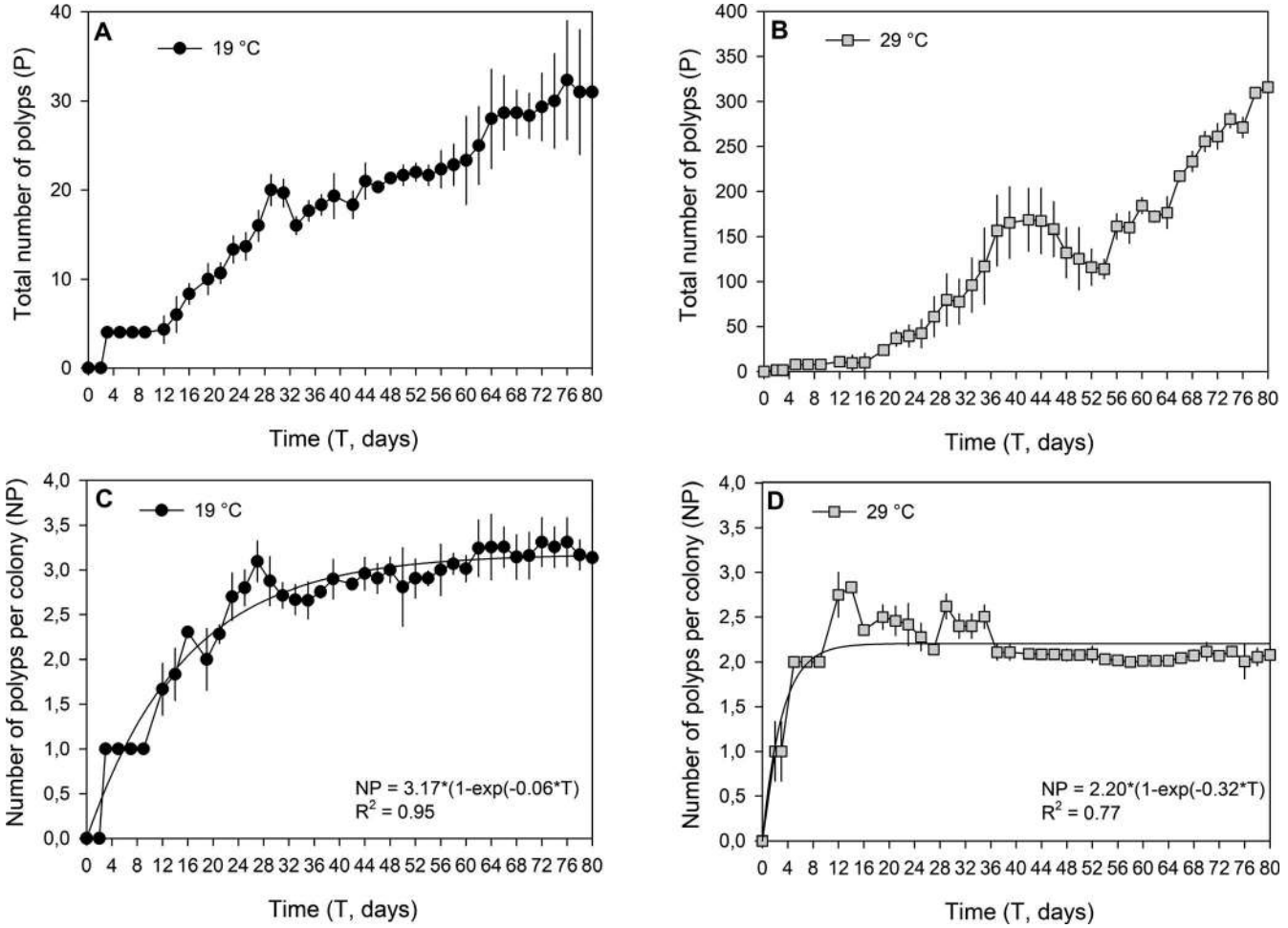

FIGURE 3 Number of polyps (mean ± *SD*) of *Craspedacusta sowerbii* over time cultured at different temperatures. **A, B.** Total number of polyps, over 80 days, at 19°C (A) and at 29°C (B). **C, D.** Number of polyps per colony, over 80 days, at 19°C (C) and at 29°C (D). Line fitting was performed using exponential regressions

appeared 14 days after the first formation of polyps. At 29°C, it was earlier (10 days).

Medusa budding was observed only at 29°C (Figure 4B). Medusa budding appeared 48 days after the beginning of the experiment, increased from 0.01 ± 0.01 buds per polyp (Day 48) to 0.05 ± 0.04 medusa buds per polyp (Day 52), and was variable at later time points (from 0 [Day 64] to 0.11 ± 0.02 medusa buds per polyp [Day 69]). Production of medusae induced a decrease of frustule production, from 0.14 ± 0.07 frustules per polyp (Day 29) to 0.01 ± 0.02 frustules per polyp (Day 46).

3.4 | Growth and survival of medusae

The newly liberated medusae measured <1 mm, and 100% had eight tentacles. All 40 specimens survived for 46 days in the medusa cube (Figure 5A). However, 50% of the specimens had died by the 56th day of the experiment, and 100% of the specimens had died after 70 days (average bell diameter: 10.1 ± 2.0 mm). The bell diameter increased from 0.60 ± 0.08 mm (Day 0) to 9.0 ± 2.1 mm (Day 32; Figure 5B), and the average growth rate was 0.30 ± 0.22 mm per day. Gonad development was present in 95% of individuals within a mean of 11.5 ± 3.0 days (n = 28; size = 3.4 ± 0.5 mm; Figure 5B). Velum development was also observed but not quantified.

4 | DISCUSSION

Laboratory culture of gelatinous zooplankton is difficult, and a specific culture system is required for each species studied (Ramondenc et al., 2019; Widmer, 2008). Specimens of the family Olindiidae (i.e., *C. sowerbii*,*Gonionemus vertens*, and *Olindias phosphorica* are the best known) are not common and are difficult to rear in aquariums, but some Japanese aquariums present Olindiidae species to the public (Lange & Tai, 2015). The rarity of these species in aquariums is due to the difficulty of growing them; their habits differ from those of common pelagic jellyfish (i.e., no circular movements in aquariums, feeding behavior between the bottom and the surface). In addition, polyps are difficult to collect because they are often too small to be found in situ (Bushnell & Porter, 1967; Carlton, 2003; Duggan

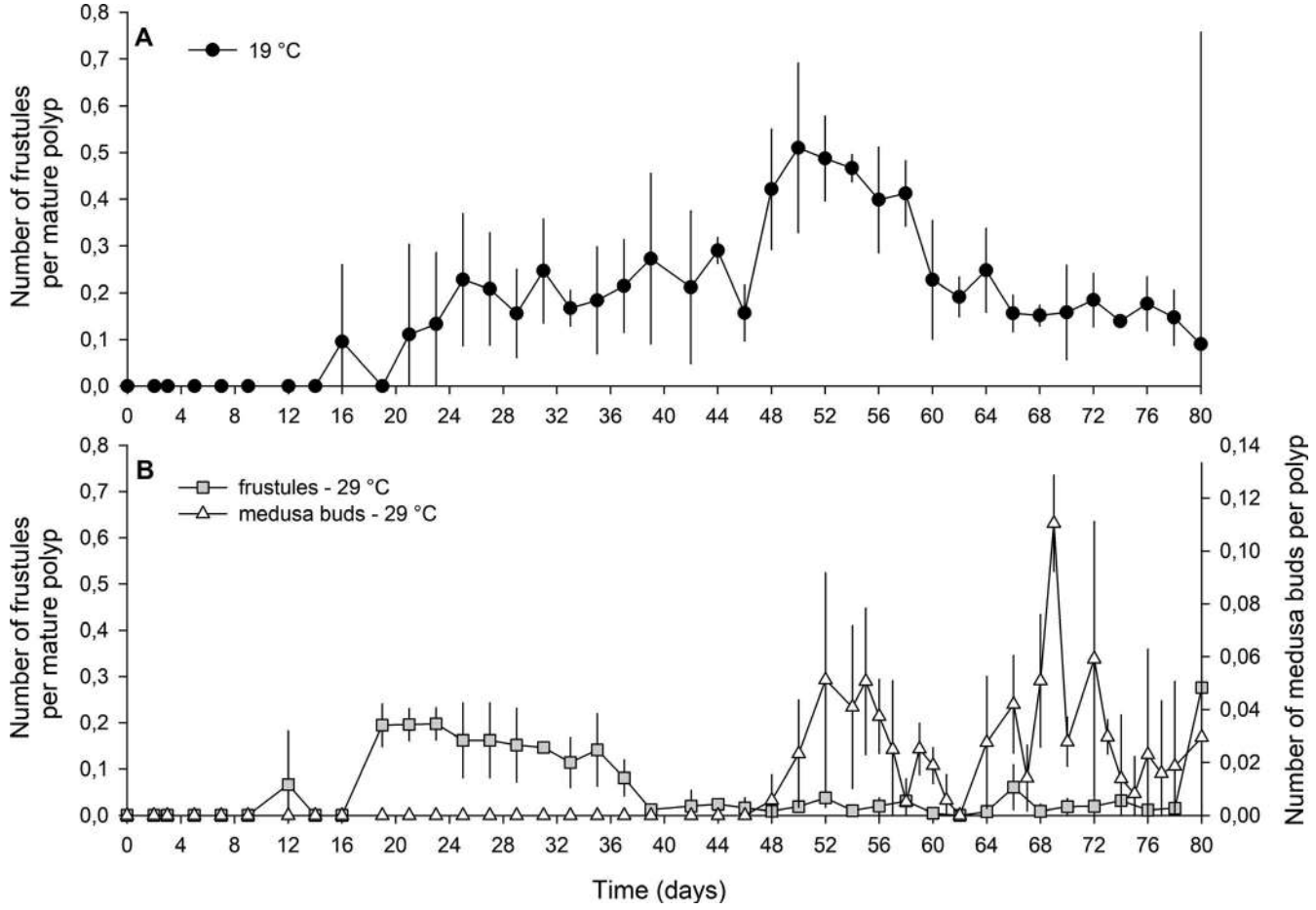

FIGURE 4 Production of frustules and medusae (mean ± *SD*) in cultures of *Craspedacusta sowerbii* over an 80-day period. **A.** Number of frustules per polyp per day at 19°C. **B.** Number of frustules per polyp and the number of medusa buds per polyp per day at 29°C

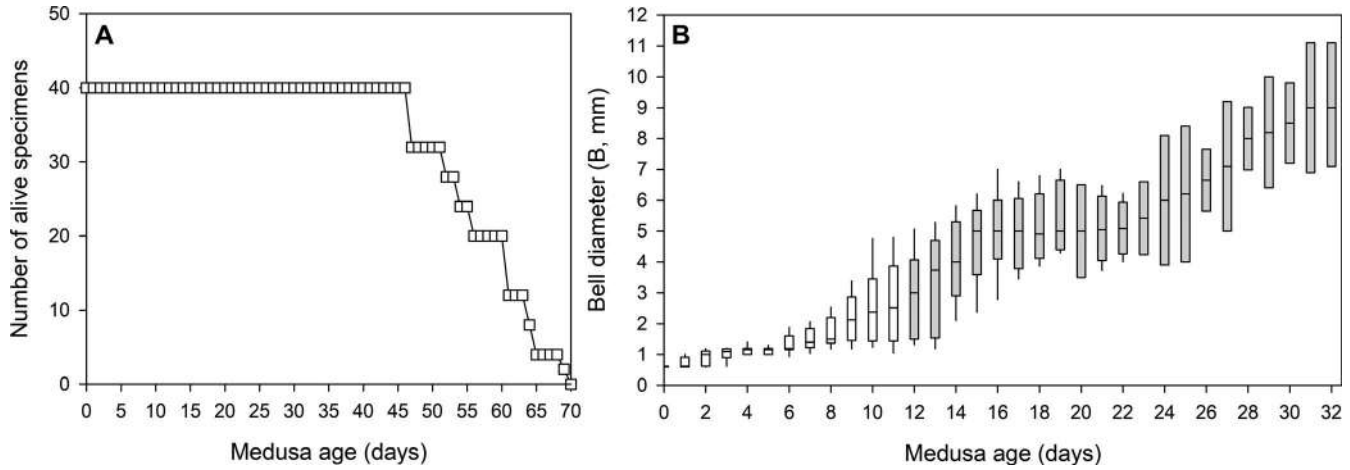

FIGURE 5 Growth and mortality in cultures of *Craspedacusta sowerbii*.**A.** Number of live specimens over a 70-day period. **B.** Boxplots of mean bell diameter of medusae over time (black lines in each box indicate mean). White boxplots indicate specimens with no gonads; gray boxplots indicate specimens with gonads

& Eastwood, 2012; Folino-Rorem et al., 2016; Zhang et al., 2009). To our knowledge, aside from aquarium reports in Japanese (Izawa, 2004; Masuda & Izawa, 1999) and two publications in English (Folino-Rorem et al., 2016; McClary, 1959) there are few publications on the culture of the freshwater hydrozoan *C. sowerbii* in the laboratory. Our study, therefore, proposes a culture system for the study of all life stages (from frustules to medusae) of *C. sowerbii*.

As stated by Folino-Rorem et al. (2016), the quality of water used is an important parameter to consider. Given the results obtained by Folino-Rorem et al. (2016), reverse-osmosis filtered water was

TABLE 2 Development from frustules to polyps in cultures of *Craspedacusta sowerbii*, and comparison with data available in the literature. – indicates missing data

Temperature (°C)	Mean number of days to first polyp formation	Number of days to first frustule(s) from polyps	Number of polyps per colony	Number of frustule buds per polyp	Duration of experiments (days)	References
12	–	30	5.9	1.2	90	McClary (1959)
19	3.0 ± 0.0[a]	16	3.1 ± 0.2	0.51 ± 0.18	80	Our study
20	–	18	2–4[b]	1.2	90	McClary (1959)
24	5.1 ± 0.5	15.4	2.2 ± 0.1	0.09 ± 0.10	36	Folino-Rorem et al. (2016)
25	–	18	1.8–3.8	0.7	80	McClary (1959)
26	–	–	2–6	0.08 ± 0.10	24	Folino-Rorem et al. (2016)
28	–	18	3.8	0.9	90	McClary (1959)
29	2.0 ± 0.0	12	2.2 ± 0.2	0.07 ± 0.08	80	Our study

[a]Mean ± *SD* (Folino-Rorem et al., 2016; our study).

[b]Range (McClary, 1959).

chosen. This type of water has the advantage of being free of undesirable elements such as iron, nitrates, zinc, copper, or mercury. In the natural filtration system used in our study, Java moss and bacteria in the sand absorbed excess nutrients such as nitrates and ammonia, which are unfavorable to cultures of *C. sowerbii*, and enabled us to maintain stable physicochemical parameters over time (Table 1). The polyp population developed well in water with a pH of 7.2, which is consistent with the pH in studies by Masuda and Izawa (1999), Izawa (2004), and Wang et al. (2006), but is lower than that in Folino-Rorem et al. (2016) (pH 8.1–8.4).

To demonstrate the success of our culture system, the production of polyps was measured for 80 days at 19 and 29°C. The recorded data were similar to those presented in the literature (Table 2), illustrating the success of this culture system. From frustules, new colonies composed of a single polyp formed 2 (29°C) to 3 (19°C) days after attachment, as reported by Folino-Rorem et al. (2016) (Table 2). The total number of polyps was 10 times higher at 29°C (316 ± 13 polyps) than at 19°C (31 ± 6 polyps) at the end of our experiments. As also observed by McClary (1959), the number of polyps per colony was significantly higher (ANOVA, Bonferroni test, $p < .05$) at 19°C (3.1 ± 0.2 polyps per colony) than at 29°C (2.2 ± 0.2 polyps per colony). In addition, polyps produced many more frustules at 19°C than at 29°C (Table 2). Values at 19°C were similar to those measured by McClary (1959) at 20°C (~1.2 frustules per polyp), and those at 29°C were consistent with the results of Folino-Rorem et al. (2016) at 26°C (~0.08 frustules per polyp).

At 29°C, as the number of frustules decreased, the production of medusa buds began on Day 48, increased for 6 days, and was highly variable. In contrast to McClary (1959), no medusae were produced at 19°C in our population. This was potentially due to the type of strains studied: McClary used a natural strain from Massachusetts, whereas the strain we used was from Japan and acclimated in the aquarium. We observed a decrease in the production of frustules when the first medusa buds appeared, which is consistent with Folino-Rorem et al. (2016).

We were able to grow medusae for 70 days (~2.5 months). The medusa cube provided low water flow suitable for long-term culture of medusae, as recommended by Folino-Rorem et al. (2016). The low water flow allowed for development without stress. Consequently, in our experiments, we did not observe bell eversion, in contrast to Folino-Rorem et al. (2016), and 50% of medusae lived for 50 days, with the remaining medusae living 70 days (Figure 5A). As in many species of the family Olindiidae, the feeding behavior in *C. sowerbii* has been described as individuals parachuting down through the water column, with tentacles elongated to catch prey on contact (Dodson & Cooper, 1983; Dumont, 1994; Pennak, 1989). This dropping posture creates eddies around the bell, increasing the efficiency of prey capture (Colin et al., 2006; Lucas et al., 2013). Unlike Folino-Rorem et al. (2016), we observed this feeding behavior in the medusa cube. This could be partly because the height of the water in the medusa cube allowed the specimens to move vertically to successfully catch prey.

In summary, we have managed to keep polyps and medusae of *C. sowerbii* in a closed culture system allowing specimens (i.e., polyps and medusae) to be maintained over the long term (80 days for polyps, 72 days for medusae). This culture system will allow researchers in the future to study more precisely the metabolism (growth, ingestion, longevity) of polyps and medusae to better understand the species' life history (periods of medusa production, feeding, and diet).

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