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ORIGINAL ARTICLE

From egg to maturity: a closed system for complete life cycle studies of the holopelagic jellyfish *Pelagia noctiluca*

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Despite its wide spatial distribution and its high abundance in the Mediterranean Sea, the biology and the ecology of the scyphozoan species *Pelagia noctiluca* remain poorly understood. This is mainly due to difficulties related to sampling and its maintenance in laboratory conditions. Thus, only a few studies exist on the ecophysiology of this jellyfish species under laboratory conditions. As an example, the maximum sizes of individuals obtained in previous culturing systems were not comparable to the ones found in the environment and the authors could not obtain a second generation. Here we present an improved rearing system for *P. noctiluca* employing a new enclosed system running with artificial seawater. The monitoring of the jellyfish in this new system highlights the importance of the quality of the food sources provided to the cultures, as well as the volume available for jellyfish growth. We obtain adults similar in size to the ones found in the open ocean (>11 cm), and we were able to obtain a second generation, 140 days after the first one. Our system is both less time-consuming and less stressful for the jellyfish.

KEYWORDS: jellyfish; closed culturing system; growth rate; *Pelagia noctiluca*

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INTRODUCTION

Gelatinous zooplankton are recognized as important members of the zooplankton although they have been long considered as harmful because of their negative ecological (trophic shut down) and socio-economic impacts (reducing fish stock, stinging, damaging nets and clogging of power plants; Richardson *et al.*, 2009). In the Mediterranean Sea, the scyphozoan *Pelagia noctiluca* (Forsskal, 1775) aggregates in large blooms that can have substantial consequences on human leisure and economic activities (Pang and Schwartz, 1993; Ayed *et al.*, 2011; De Donno *et al.*, 2014). Since the 1970s, this jellyfish is considered as one of the most abundant species in the basin (Goy *et al.*, 1989; Malej and Malej, 1992; Mariottini *et al.*, 2008; Canepa *et al.*, 2014). Recent studies conducted in the Northwestern Mediterranean Sea highlighted that the distribution and intensity of jellyfish blooms largely depend on wind direction, shelf topography (Sabatés *et al.*, 2018) and food concentration. In the Ligurian sea, blooms of *P. noctiluca* occur generally offshore, with coastal intrusions led by marine currents, the latter being directly driven by wind (Ferraris *et al.*, 2012; Berline *et al.*, 2013) and its growth depends on food concentration though it can cope with extended food-limited periods surviving on its own reserve (Lilley *et al.*, 2014a). Along the Catalan coast, geostrophic forces and shelf topography induce upwelling events, which drive the jellyfish to rise up along marine canyons toward the surface layers (Benedetti-Cecchi *et al.*, 2015).

Although *P. noctiluca* can be seen has a hazard (Mariottini *et al.*, 2008), it has the potential to be an ideal pluridisciplinary model organism for research spanning from biology to evolution and ecology. Firstly, *P. noctiluca* has a holoplanktonic life cycle as its polyp phase was lost throughout evolution (Russell, 1970). Therefore, unlike most scyphozoan species, its development is completed without any fixed scyphistoma stage (Krohn, 1855). Secondly, the female can produce several thousands of eggs per spawn, on a daily basis, depending on the size of individuals (Lilley *et al.*, 2014a) which, once fertilized, develop and directly turn into the juvenile stage. In other words, these characteristics permit the production of a lot of individuals in a shorter time, which is crucial for scientific experiments.

Despite its promising characteristics, the rearing of *P. noctiluca* under laboratory conditions remains a challenge. An initial system was proposed to culture *P. noctiluca* (Lilley *et al.*, 2014b) but the post-fecundation jellyfish obtained after 500 days did not present sizes comparable to those observed in the environment. The authors speculated that the culture tanks inhibited jellyfish growth due to the stirring motion, collisions with the container walls that increased the rate of nematocysts discharge, the inability to undertake vertical migrations or limited prey availability. The culturing system proposed in the previous study was composed of 5–15 L containers, depending on organism size, and motorized PVC paddles rotating to resuspend younger individuals (Fig. 1a). This method potentially caused a lot of stress to the organisms. In addition, this method was highly time-consuming because it required frequent changes of seawater (every 1 or 2 days). Subsequent generations of jellyfish were not monitored, and therefore the sustainability of the culture system remained untested. Many researchers and aquariologists improved the methods to keep gelatinous species in laboratory and some are successfully being cultured (Raskoff *et al.*, 2003). Today, all systems are based on "planktonkreisel" (Greve, 1968) that maintain organisms in suspension in the water column thanks to circular currents (Widmer, 2008), but the shape of the containers are modified according to the species studied. The main impediments for culturing gelatinous zooplankton are the choice of food sources, the shape of tanks and the current inside them. All these elements can impact each development stage of the jellyfish in its own manner (Raskoff *et al.*, 2003).

A new optimal enclosed system could offer new perspectives for research in multiple domains and scales, from molecular to the ecosystems. As opposed to scyphozoans, anthozoans are often favored by toxicologists studying cnidarian models because they are easier to maintain in an aquarium. For this reason, the biological and chemical characteristics of the venom produced by *P. noctiluca* such as its haemolytic power and its effect on human health (e.g. dyspnoea or generalized allergy) have attracted much less attention in spite of its interest (Mariottini and Pane, 2010). With a new rearing system, scientists could have the possibility to evaluate public health issue and thus help in preventing it. Moreover, the possibility to keep a lineage of jellyfish over several generations, in controlled conditions, would enable studying the species' genome and transcriptome. Generating the genomic map of genes expression as a function of growth in this species will help in answering questions about the development, the regeneration and the evolution of medusae (Leclere and Rottinger, 2016; Helm, 2018). The objectives of the present study were to develop and optimize a new closed-circuit system for the long-term culturing of *P. noctiluca* and offer new research perspectives. Special attention was given to obtaining a second generation of jellyfish that will help certify the durability of the method.

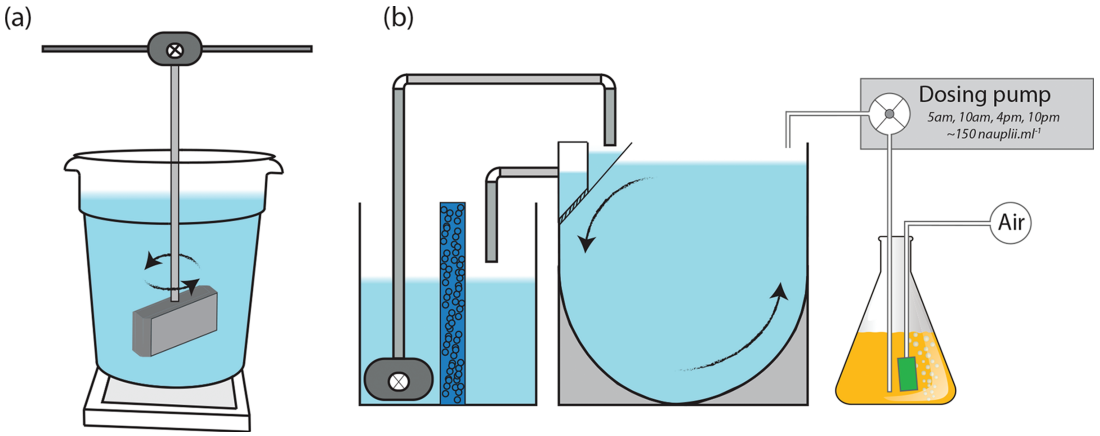

Fig. 1. Schematic diagram of closed type system for the culturing of the jellyfish *Pelagia noctiluca* used (**a**) in previous rearing (Lilley *et al.*, 2014b) and (**b**) improved in our study with an artificial seawater recycling system. The arrows indicate the water circulation inside the closed systems.

MATERIAL AND METHODS

P. noctiluca collection and rearing

Mature jellyfish were collected at the surface of the bay of Villefranche-sur-Mer (43.696 N, 7.307 E) with hand nets (1 mm mesh). Females and males were separated into 20 L plastic tanks in the laboratory. For our experiments, one male and one female were randomly selected to create the first generation. Mature males show purple gonads with transverse parallel lines of tissue whereas the females display browner gonads and mature oocytes (i.e. eggs; Lilley *et al.*, 2014b). The temperature in the culturing room was set to 18 C and a circadian cycle was programmed (12 h light/12 h night). All sexually mature jellyfish spawned daily at 11 a.m, 3 hours after the lights were switched on. Gametes were particularly visible in the tanks; unfertilized eggs presented a brown-orange color and were embedded in an amorphous mucus, while the sperm clearly whitened the seawater.

Artificial fertilization took place in a smaller plastic tank of 10 L filled with artificial seawater (osmotic water and red sea salt was adjusted at 37‰) at a temperature of 18 C. All eggs produced by the female were added to the 10 L tank using a 0.5 cm wide glass pipette and nearly 20 mL of concentrated sperm was added. Then, a slow turbulence was applied during a few minutes to keep the mucus entire. After 3 hours, the eggs were recovered and placed in plastic tank of 10 L with non-turbulent artificial seawater. Within 4 days, eggs metamorphosed into planula larvae and ephyrae.

Once the jellyfish were able to move in the water tank, they were placed in a new closed system composed of (i) a Kreisel, (ii) a settling container, (iii) bacteriological foam, (iv) a water pump and (v) artificial seawater (Fig. 1b). As previously mentioned, the Kreisel is an aquarium designed for gelatinous zooplankton culturing made of an inlet and an outlet water that create a circular and laminar flow. The dirty artificial seawater was continually recycled in a settling tank by a bacteriological foam. Microorganisms enable to denitrify, purify and clean the water culture. Each month, 30% of the artificial seawater was changed in each Kreisels and pH and nitrogen ions concentrations were verified using a colorimeter kit (JBL test lab) to detect potential toxic conditions. The pH was kept close to 8.2 and NO_2, NO_3 and NH_4 concentrations were monitored not to exceed 0.1 mg.L^{-1}, 20 mg.L^{-1} and 0.2 mg.L^{-1}, respectively.

Diet and zooplankton prey

Two cultures from the same fecundation event were grown on different food regimes and zooplankton prey types: (i) culture 1 (~750 ephyrae) was fed with nauplii of *Artemia salina* (~697 ± 64 µm), (ii) culture 2 (~1030 ephyrae) was fed with a mix of *Paracentrotus lividus* eggs (~90 µm) and nauplii of *A. salina* for 50 days after fecundation, before switching to nauplii only because fresh sea urchin eggs were not available (Table 1). Newly hatched *A. salina* nauplii were used as alternative food sources and were obtained by culturing cysts from commercial brand in a plastic bottle filled with artificial seawater continuously oxygenated. Once the nauplii hatched (~24 h), they were concentrated, kept in a reserve at a concentration of nearly 150 nauplii.mL^{-1} and were injected in each Kreisel every day at 5 a.m., 10 a.m., 4 p.m. and 10 p.m. with a dosing pump. The food concentration was kept so all jellyfish had preys continuously in their gastric cavity, but prey concentration was visually monitored to avoid accumulation of dead

Table 1: Experimental details of cultures in the laboratory (generation, culture name, fecundation date, feeding start post-fecundation, culture end post-fecundation, volume, water type, jellyfish abundance, rearing systems and food quality)

Generation	Culture	Fecundation date	start (d)	end (d)	Volume (L)	Water	Abundance	Rearing systems	Food offered	S_{max}	k	MSE
F1												
	1	*25 January 2016*	+4	+98	17	Artificial	~750 ephyrae	new kreisel systems	*A. salina*	1.69	0.03	0.01
	2	*11 November 2016*	+4	+144	17	Artificial	~1030 ephyrae	new kreisel systems	*A. salina* and *P. lividus* eggs	1.94	0.06	0.05
	2_a	*5 January 2017*	+59	+165	35	Artificial	ND	new kreisel systems	*A. salina* and mixed zooplankton (>200 µm)	4.42	0.04	0.13
	2_a_I	*24 March 2017*	+137	+165	90	Artificial	ND	new kreisel systems	*A. salina*, mixed zooplankton (>200 µm) and fish larvae	6.37	0.03	0.18
	2_b	*20 January 2017*	+74	+137	90	Artificial	~30 adult	new kreisel systems	*A. salina*, mixed zooplankton (>200 µm) and fish larvae	9.15	0.04	0.08
	2_b_I	*17 March 2017*	+130	+165	15 (×4)	Filtered (100 µm)	~12 adult	containers without turbulence	mixed zooplankton (>200 µm) and fish larvae	9.77	0.04	0.35
	2_b_II	*17 March 2017*	+130	+167	200	Filtered (100 µm)	~14 adult	new kreisel systems	*Aurelia spp.* (adult and juvenile)	11.04	0.04	0.24
F2												
	3	*28 March 2017*	+4	+118	17	Artificial	~700 ephyrae	new kreisel systems	*A. salina* and *P. lividus* eggs	0.98	0.07	0.03
	3_a	*9 May 2017*	+40	+118	17	Artificial	~100 ephyrae	new kreisel systems	*A. salina* and *P. lividus* eggs	1.09	0.09	0.06

All animals were fed several times daily but zooplankton composition from the net and assimilation efficiency were not recorded. Zooplankton prey offered was collected daily, from Villefranche-sur-Mer bay. *ND: no data recorded*

organisms in the Kreisels. At the beginning, culture 2 started in a 17 L Kreisel but, after Day 50 and the food change, growth started to slow down due to volume limitation. The jellyfish were gradually transferred to larger Kreisels (35 L then 90 L, Table 1). The jellyfish were then fed with diverse zooplankton preys (copepods, pteropods and gelatinous zooplankton >200 µm) coming from the daily oblique net sampling (0–400 m deep) in the bay of Villefranche-sur-Mer. At Day 130 post-fecundation, culture_2_b from the 90 L Kreisel was split in two (Table 1): (i) 12 specimens were placed in 4 large containers (3 specimens per container) of 15 L filled with natural seawater (culture_2_b_I), without turbulence and fed twice a day with large zooplankton preys (> 1 cm); and (ii) 14 specimens were fed with adult *Aurelia spp.* and placed at the Oceanographic Museum of Monaco in an open system of natural seawater and a large 200 L Kreisel (culture_2_b_II) to overcome the volume limitation of our laboratory. We moved all specimens to tanks of increasing volume and provided different types of food sources to test the influence of volume availability and prey quality on jellyfish growth.

Measurements and statistical analysis

To compare the jellyfish growth rates obtained through our cultures amongst themselves and to those obtained with previous studies (Lilley *et al.*, 2014b), bell diameters (BDs) were measured from lappets to lappets to monitor the size of the jellyfish. Each week, 6–10 randomly selected jellyfish were individually isolated from each culture and placed in a Petri dish with a small amount of seawater. The organisms smaller than 2 cm were measured using a dissection microscope (Zeiss Stemi SV11 equipped with an Olympus DP11 camera), calibrated with a stage micrometer for each magnification. Each specimen was photographed with a digital camera and their body size dimensions were measured by photo analyses using Adobe Illustrator CS6. For organisms larger than 2 cm, body size was measured with a ruler to the nearest millimeter. Special care was taken when deploying the umbrella and the lappets in a Petri dish. For each culture, a general logistic model was applied between jellyfish size and post-fecundation days in order to estimate the growth rate coefficient (k) and the maximum possible size (S_{max}) such as

$$S_t = {S_0.S_{max}}\Big/{S_0 + (S_{max} - S_0)\,.e^{-kt}}$$

with t representing the time step and S_0 the initial sizes ($S_0 = 0.25$ cm). Growth rates variability were estimated through bootstrapping and cross validation for each culture. This method consisted in fitting the logistic model on random sampling (size measurements) with replacement several times ($n = 1000$) and estimating a distribution of growth rates. Then, the distributions were compared between cultures using univariate variance analysis.

Instantaneous growth rates (μ; d^{-1}) were calculated over the consecutive times periods. BDs were converted into carbon weight (CW) following the relationship of Lilley *et al.* (2014b):

$$CW = 0.235.BD^{3.115}$$

and μ was estimated, such as

$$\mu = {\ln(CW2/CW1)}\Big/{t_2 - t_1}$$

RESULTS

Culture 1 was conducted over 97 days post-fecundation while culture 2 was extended up to 161 days post-fecundation (Fig. 2a). In culture 1, the jellyfish grew exponentially (k = 0.03; MSE = 0.01) reaching an equilibrium average size ~1.69 cm according to the fitted logistic model. The oral morphology characteristics, such as gastric cavity and oral arms, changed continuously from the ephyra stage. The first gastric filaments were observed 29 days after fecundation when mean jellyfish diameter was of 0.52 ± 0.7 cm. Meanwhile, the primary tentacles appeared 49 days after fecundation when the jellyfish measured $\sim 0.72 \pm 0.1$ cm. Unfortunately, no gonad and consequently no egg production could be observed. The jellyfish of culture 2 showed exponential growth as well (k = 0.06; MSE = 0.05) reaching an equilibrium size at 74 days post-fecundation, with an average size of 1.8 ± 0.4 cm. The average growth rate was slightly greater than under the conditions of culture 1, even if the number of ephyrae in culture 2 was higher ($P < 0.05$, Fig. 2a). This result could be due to the fact that *P. lividus* eggs were added with the nauplii in culture 2, potentially bringing additional nutritive values that enable a better development. In the latter case, the first gastric filament and oral development were observed 18 days after fecundation when the average jellyfish size was 0.41 ± 0.06 cm while tentacles started budding 38 days after fecundation, when average jellyfish size was $\sim 0.96 \pm 0.11$ cm. Here again, no gonad and egg production were observed.

In culture 2_a (Kreisel of 35 L), jellyfish size increased and reached an equilibrium at 4.2 ± 0.4 cm (Fig. 2b), 120 days after fecundation. Therefore, compared to culture

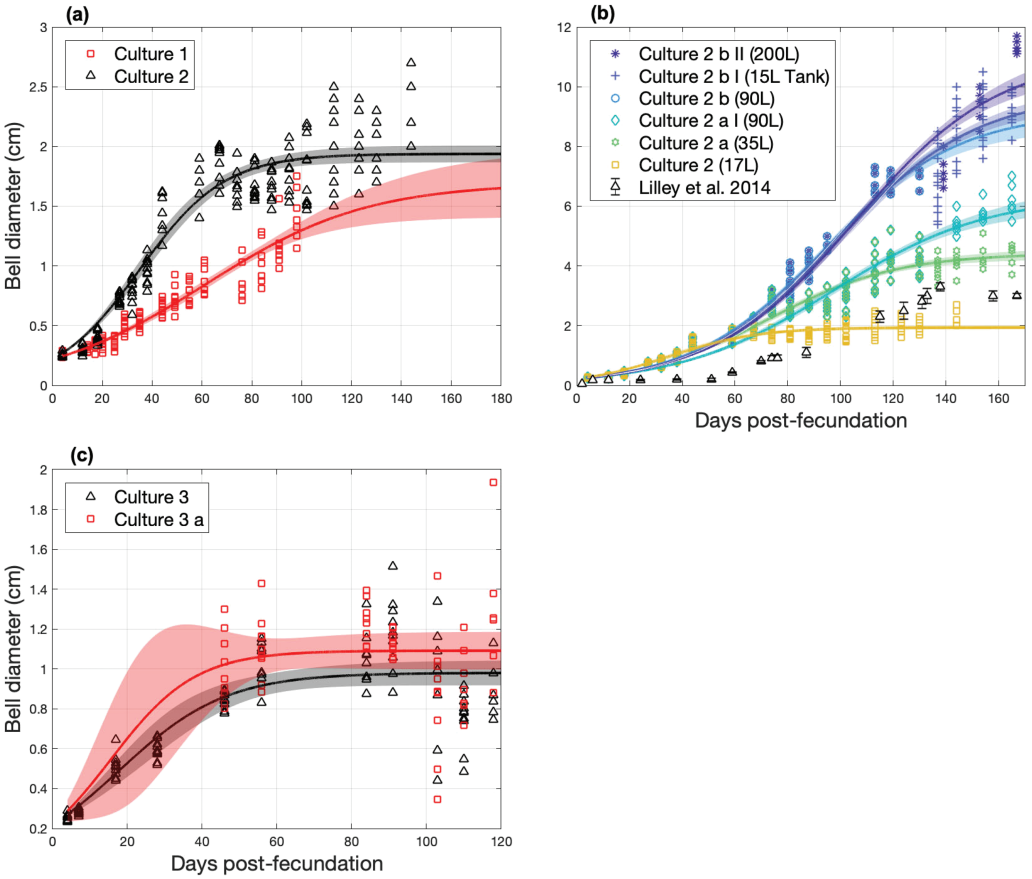

Fig. 2. Changes in the diameter of the bell of the jellyfish *Pelagia noctiluca* cultured in Kreisel for (**a** and **b**) the first generation with different volume capacities (17, 35, 90, 200 L and tanks without turbulence) and food types (Table 1) and (**c**) the second laboratory jellyfish generation. The shaded area represents 95% confidence limits.

2 (Kreisel of 17 L), the doubling of the volume capacity and the addition of fresh zooplankton prey clearly had a positive effect on jellyfish size. Considering the periods of positive growth, the average growth rate in culture 2_a was ~0.08 ± 0.06 d^{-1}. At the 130th day post-fecundation, when culture 2_a was split and transferred in the 90 L Kreisel to become culture 2_a_I, growth rate started to increase exponentially again at an average rate of 0.03 ± 0.01 d^{-1}. Nevertheless, no egg production and gonad formation were observed in these two cultures. In culture 2_b (Kreisel of 90 L) the size equilibrium was observed ~6.9 ± 0.3 cm, 119 days after fecundation. When this last culture was split to create cultures 2_b_I and 2_b_II (Table 1), jellyfish growth increased to achieve a maximum size of 10.5 cm and 11.7 cm at Day 150 and 164 days post-fecundation, respectively. The growth rate *versus* BD regression was performed for the periods of positive growth from all cultures 2 (Fig. 3). New coefficients relationship from the equation developed by Lilley *et al.* (2014b) were estimated such as $\mu = 0.105.BD^{-0.25}$ ($R^2 = 0.28$, $n = 58$).

In cultures 2_b_I and 2_b_II, gonad formation and egg production were observed when jellyfish reached 8 cm, ~140 days after fecundation. At this stage, eggs and sperm were released daily at 11 a.m.. The gametes (eggs and sperm) were viable and the growth of a second-generation culture was monitored (Fig. 2c). The culture 3 began in a Kreisel of 17 L where 700 ephyrae were added. At the 40th day after fecundation, the second generation was split in two different 17 L Kreisels: one with 100 ephyrae (culture 3_a) and the other with 600 ephyrae, both fed with nauplii of *A. salina*. The jellyfish born from this second generation reached sizes that are similar to ones from the previous cultures (Fig. 2c), therefore demonstrating the replicability of the experiment. All first and second generation cultures were stopped the 21 April 2017 and the 20 July 2017(i.e. 165 and 118 days post-fecundation, respectively).

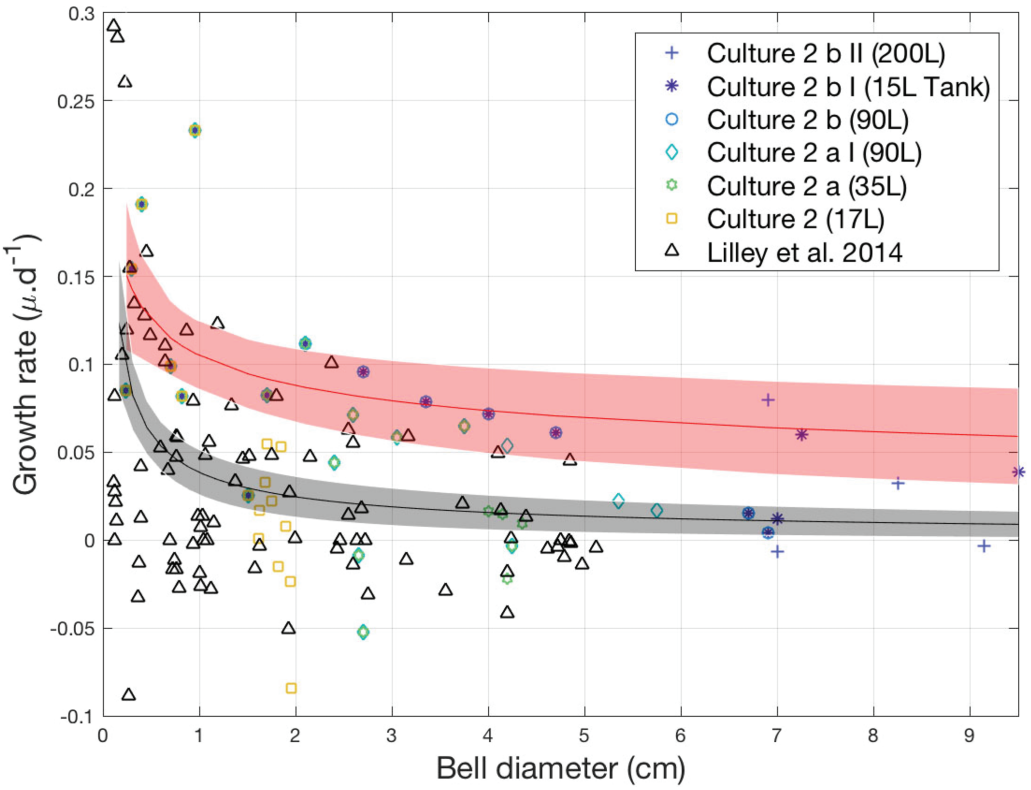

Fig. 3. Instantaneous growth rate of the first laboratory jellyfish generation in the closed systems with different volume capacities (17, 35, 90, 200 L and tanks without turbulence) and food types (Table 1) from ephyra to reproductive adults. The red (this study) and grey (Lilley *et al.*, 2014b) decrease in growth rate with BD represent a power relationship according to positive instantaneous growth rates. Each shaded area represents 95% confidence limits of their respective culture.

DISCUSSION

Culturing optimization

This study shows how to achieve a perennial culture of *P. noctiluca* in a controlled and enclosed system with artificial seawater. To our knowledge, it is the first study showing that *P. noctiluca* can be cultured in an enclosed systems filled using artificial seawater. Moreover, our culture system enabled production of organisms >11 cm, which is a clear improvement compared to the previous study (Lilley *et al.*, 2014b) where jellyfish reached sizes ~3 cm, 164 days after fecundation. Contrary to the initial system of Lilley *et al.* (2014b), our experiment produced jellyfish with sizes that are comparable to the ones observed *in situ*, as the median and the maximum BD values recorded for adults of *P. noctiluca* are ~9 and 21 cm in the Ligurian Sea, respectively (Lilley *et al.*, 2014b; Rosa *et al.*, 2013). The maximal sizes reached by jellyfish (>10 cm) in the 15 L tanks compared to the culture 2_b_II suggest that the size of the rearing tanks does not influence the growth when they are mature. Nevertheless, numerous other factors in both cultures could also account for this result: (i) the food quality (mixed zooplankton vs juvenile/adult *Aurelia spp.*); (ii) the rearing systems (with or without turbulence); and (iii) the shape of tanks, which could promote jellyfish fixation to aquarium walls and reducing energy consumption.

Maintaining water circulation in the Kreisels was essential to avoid the damages from the collisions with the walls and to maintain the jellyfish in suspension. The main limitation of our culturing system resided in the adjustment of prey concentration to feed jellyfish *ad libitum* while avoiding the accumulation of waste from the unassimilated food items. During the first stages of development (from ephyra to juvenile), sea urchin eggs mixed with nauplii of *A. salina* seemed to be enough to obtain a better growth, as they potentially bring additional nutritive values enabling a faster development. It has been demonstrated that rotifers were particularly appreciated by the ephyrae of jellyfish (Widmer, 2008) and could be another additional food source. In the same way, previous studies have shown that for zooplankton (Hamburger and Boëtius, 1987) as well as other scyphozoans such as *A. aurita* (Costello and Colin, 1994; Graham and Kroutil,

2001) or *Chrysaora plocamia* (Riascos *et al.*, 2014), increasing the quantity and the diversity of prey increases the growth responses due to the supply of multiple essential nutrients. In laboratory, the feeding behavior of *P. noctiluca* showed their preference toward a larger diversity of gelatinous prey, such as salps, chaetognaths, pyrosomes and other jellyfish species like *Aurelia spp*. These trends were also highlighted by the growth rates measured in all type 2 cultures (Fig. 3). According to both results related to prey concentration and volume capacity, we recommend beginning cultures in a volume of 17 L, by feeding them with a mix of sea urchin eggs and nauplii of *A. salina* to increase encounter rates. Once the jellyfish become larger than 2 cm, the organisms can be transferred in larger Kreisel with gelatinous prey such as juvenile and then adult *Aurelia spp.* cut in small pieces, or adult *A. salina*.

Morphological changes

This paragraph discusses the life cycle of *P. noctiluca* and its morphological changes based on observations made at 18 C by integrating our results from laboratory experiments together with the ones from previous studies (Fig. 4). The eggs of *P. noctiluca* are much larger (300 μm) than the ones of any other scyphozoans (80–123 μm for *Aurelia spp.*; Lucas and Lawes, 1998). At 24 hours post-fertilization, embryo cells grow pale; soon after, the swimming blastulae start their unipolar gastrulation. Each gastrula eventually grows into an asymmetric larva presenting a cone-shaped bell structure, on the opposite side of the future ephyra mouth opening. At the same pole, oral arm buds are formed within 3 days (Helm *et al.*, 2015). Noteworthy, from this stage called "cone larva", each individual begins to float in the water column. From the cone larva to the ephyra stage, the bell is progressively reduced, marginal lappets develop and the oral lips and rhopalia (sense organs) appear. In our cultures, the ephyra stage was reached 4 days after fecundation with individuals measuring ∼2.5 mm and the eight marginal lobes subdivided in two small lips are clearly recognizable (Fig. 4; "*Ephyrula*"). Two weeks later (18 post-fecundation day), the gastric filaments become visible and the average size of jellyfish is 4.1 mm (Fig. 4; "*Gastric filament*"). This is slightly larger than previous observations where filaments become visible at 3.5 mm (Russell, 1970). Similarly, the four primary marginal tentacles are well developed and four secondary tentacles appear at 9.6 mm (Fig. 4; "*Tentacles*") against 8 mm in the first morphological description of the species (Russell, 1970). The juvenile stage is considered to begin from the appearance of the tentacles and to end at sexual maturity. With time, the mesoglea thickens, the warts on the umbrella are organized in regular rows, the abundance of gastric filaments increases (Kramp, 1961; Russell, 1970) and the yellow color changes progressively to the pink that is typical of the adults. In our cultures, gonad formation and gametes emission were observed only for jellyfish higher than 8 cm (Fig. 4; "*Adult*"). In the northern Adriatic Sea and in the central Mediterranean Sea, mature specimens were generally observed at smaller sizes, between 4 and 5 cm (Sandrini and Avian, 1991). The authors also estimated that jellyfish spawn after nearly 5 months, which is closer to our estimation. The time lag of the morphological changes between laboratory and *in situ* observations could be explained by thermal stress (Avian *et al.*, 1991; Morand *et al.*, 1987). The morphological changes discussed here are based on observations made at 18 C, which is the perfect temperature to reduce stress of this species. Indeed, *P. noctiluca* spawning events, eggs fertilization and mobility occur mainly in an optimum temperature interval between 16 and 19 C (Avian, 1986). However, previous rearing systems also conducted at 18 C predicted the first spawning events to occur for specimens ranging between 2.5 and 5 cm (Lilley *et al.*, 2014b), and the maturation of gonads mainly depends on abundant source of prey and the feeding regime. Our results suggest that our new rearing system is less stressful for the individuals. But it could also suggest that jellyfish invest more in growth than in gonads development, and/or that preys introduced in the Kreisel were less limiting than the natural condition.

P. noctiluca as a new model organism

The cultures maintained in the laboratory showed that organisms could be developed as an experimental model, which offers many perspectives for a large range of disciplines, from academic research to industrial applications. From an ecological point of view, the trophic position of *P. noctiluca* in pelagic ecosystems remains poorly understood. Recently, Milisenda *et al.* (2018) estimated its trophic position through the stable isotope approach by assuming an average increase of $\partial^{15}N$ and $\partial^{13}C$ of 3.2‰ and 2‰, respectively, between successive trophic levels. However, the trophic fractionation of jellyfish is still largely debated in the literature due to several methodological uncertainties (D'ambra *et al.*, 2014). Thus, the enclosed system presented in our study could be used to develop cultures of *P. noctiluca* by considering several types of food sources to derive the corresponding enrichment ratios during the development of the jellyfish. Then, the resulting stable isotopes ratios could be used to identify the specific preys of *P. noctiluca* from gut contents sampled *in situ*. Interestingly, *P. noctiluca* can withstand long starvation periods, suggesting it can feed on unexplored or underestimated food sources such as small plankton.

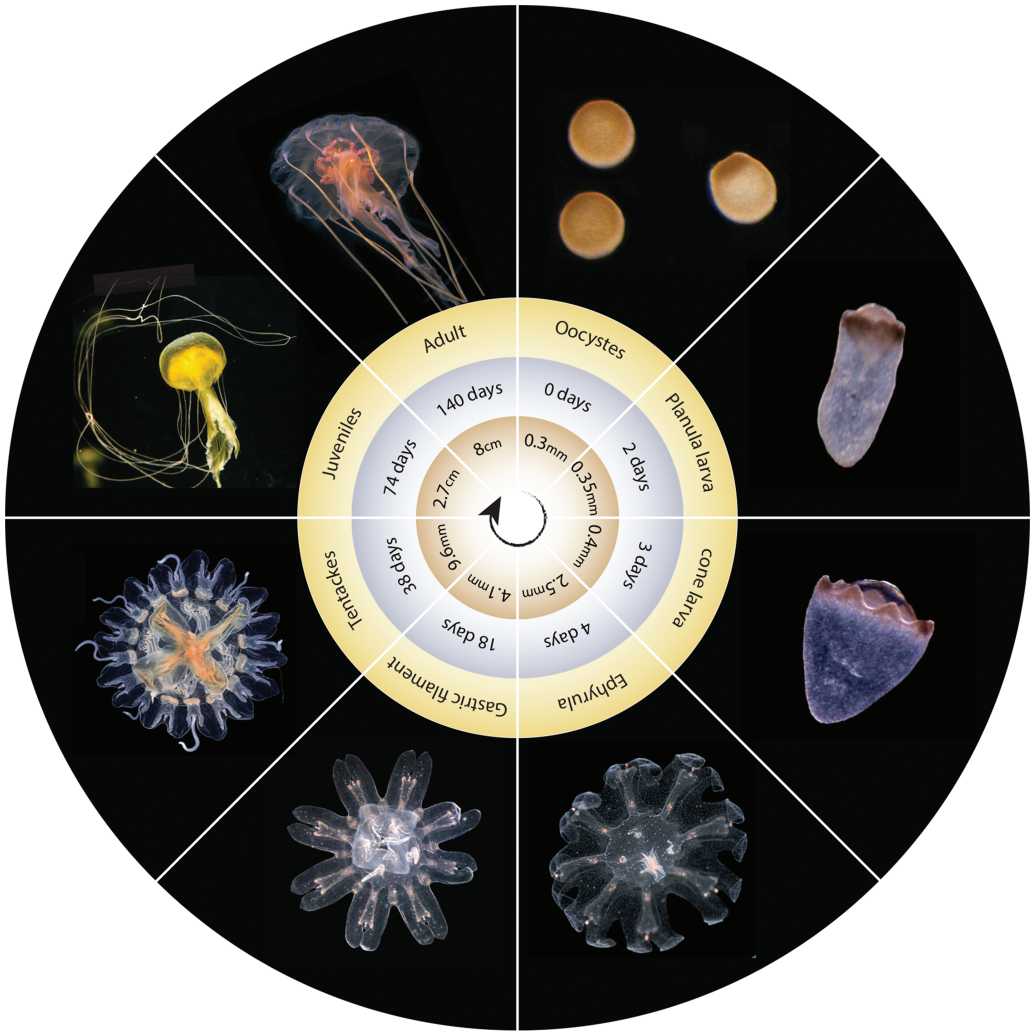

Fig. 4. Overview of the *Pelagia noctiluca* life cycle at 18 C following to the body size and the post-fecundation time. The arrow indicates the morphological stage, days post-fecundation and size order from the fertilized eggs to the adults. The *Pelagia noctiluca* pictures were released © by Dr. Christian Sardet.

From an ecophysiological point of view processes such as respiration or reproduction are relatively well known, but many uncertainties remain concerning the rates of ingestion, assimilation, excretion or egestion of jellyfish (Lilley *et al.*, 2014a). These mechanisms are very difficult to estimate because they are strongly controlled by environmental conditions and jellyfish size (Lilley *et al.*, 2014a). The present culturing system could help to understand their variation as a function of jellyfish growth and temperature. In addition, the gelatinous zooplankton are poorly represented in ecosystem models due to many uncertainties and lack of information on this particular group (Laufkötter *et al.*, 2015). Thus, these ecophysiological measures could also be used to calibrate numerical models and predict their growth, distribution and temporal variability. Consequently, these research topics (trophic position in the food web, mechanistic modeling) can help to explain and predict the spatio-temporal variability of *P. noctiluca* distribution in the oceans, as well as to identify the processes underlying its blooms dynamics. Finally, this new rearing system will help to carry on reconstructions of the jellyfish life cycle, providing support to morphological and molecular tools to clarify phylogenetic relationships and related systematic assignments.

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