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ORIGINAL ARTICLE

Ephyrae and metaephyrae of *Pelagia noctiluca*: stage determination, morphometry and shrinkage

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Understanding processes occurring in the different life stages of jellyfish is key to advance knowledge on their trophic interactions and population dynamics. We describe four developmental stages of *Pelagia noctiluca* ephyrae and metaephyrae based on the progress of feeding structures and morphometric measurements on the central disc diameter and total body diameter. Size differs significantly among stages, but it can overlap substantially, suggesting that it is not always coupled with development progress due to different somatic growth. Morphological distinction of stages is biologically important because it implies different levels of food specialization and capture efficiency. We further report a 25% (± 13 SD) shrinkage of ephyrae and metaephyrae after storage in 4% formaldehyde solution. This metric can be used in ecological studies focusing on size-related traits of field observed individuals.

KEYWORDS: jellyfish; early life stage; morphology; formaldehyde shrinkage

INTRODUCTION

Increasing observations of jellyfish blooms in coastal areas have risen awareness that human activities can influence jellyfish population dynamics (Purcell *et al.*, 2007; Richardson *et al.*, 2009). However, linking global change to changes in jellyfish population remains controversial (Duarte *et al.*, 2015; Pitt *et al.*, 2018) as they remain poorly studied compared with other marine taxa (Gibbons *et al.*, 2013). To better understand how jellyfish populations respond to environmental changes, further knowledge in the biology and ecology of all their life stages is necessary. Particularly during the pelagic early life stages, as most studies focus on adult and polyp stages.

Pelagia noctiluca is a scyphozoan jellyfish of warm-temperate waters that thrives particularly well in the Mediterranean Sea (Licandro *et al.*, 2010; Canepa *et al.*, 2014), where recurrent blooms have caused damage to fisheries, aquaculture and tourist activities (Purcell *et al.*, 2007; Boero, 2013). As opposed to most other jellyfishes, *P. noctiluca* is a holoplanktonic species that completes its lifecycle in pelagic waters, skipping the benthic polyp stage (Krohn, 1856).

Pelagia noctiluca can only reproduce sexually—adult males and females gather near the surface to release their gametes and favor egg fertilization (Rottini Sandrini *et al.*, 1983; Zavodnik, 1987; Canepa *et al.*, 2014). Fertilized eggs develop into planula larvae that metamorphose to ephyrae stage within 2–7 days depending on water temperature (Avian, 1986; Rosa *et al.*, 2013). Ephyrae have a compressed shape with oral lips, rhopalia (sense organs) and eight marginal lobes that bifurcate forming lappets. Metaephyra is the subsequent stage after ephyra. In the transition from ephyrae to metaephyrae they increase the proportional size of the central disc and develop tentacles and the digestive and oral systems to feed more efficiently (Straehler-Pohl *et al.*, 2010; Kamiyama, 2018; Kienberger *et al.*, 2018; Ishii *et al.*, 2004). The juvenile stage is achieved when oral arms and marginal tentacles are well established (Russell, 1970; Ramondenc *et al.*, 2019).

Rottini Sandrini and Avian (1983) described nine stages of ontogenetic development from planula larvae to ephyrae of *P. noctiluca*. However, no further ontogenetic stages have been described from ephyrae to metaephyrae. This period is critical in their life cycle, as they start relying heavily on external sources of food for growth and survival (Lilley *et al.*, 2014; Kamiyama, 2018; Ramondenc *et al.*, 2019). Their capacity to capture prey increases with size and development stage, and prey items range from phytoplankton and ciliates in early ephyrae (Bamstedt *et al.*, 2001; Miranda *et al.*, 2016; Kamiyama, 2018), to micro- and mesozooplankton (including ichthyoplankton)

as they grow larger (Purcell *et al.*, 2007; Gordoa *et al.*, 2013; Ramondenc *et al.*, 2019).

Only recently have scientists started to realize how important *P. noctiluca* ephyrae and metaephyrae are as predators of fish eggs and larvae. Purcell *et al.* (2014) estimated that they can consume up to 13.4% of the available fish larvae per day in the front zone of a shelf slope; Tilves *et al.* (2016) estimated that they can consume 1.5–2.7% of the ichthyoplankton community per hour and Ottmann *et al.* (2021) estimated that occasional high densities of metaephyrae (>10 metaephyrae m^{-3}) can reduce survival chances of tuna early life stages by 99% in specific locations. Considering their voracious killing potential and the ontogenetic changes of prey items during this period, differentiating finer stages of development from ephyrae and metaephyrae can facilitate further studies of trophic ecology.

Outbreaks of *P. noctiluca* in the Mediterranean Sea appear to have increased in recent decades (Kogovšek *et al.*, 2010; Bernard *et al.*, 2011), but how it relates to climate change remains uncertain (Daly Yahia *et al.*, 2010). Increasing evidence points that mild winters and productive springs favor reproductive activity and survival of early life stages (Malej *et al.*, 2004; Milisenda *et al.*, 2018; Ottmann *et al.*, 2021). However, we still struggle to understand how climate change affects early survival during the summer months, as warm water and low food supply may drive ephyrae and metaephyrae to starve. Thus, further deterministic studies are necessary to link climate variation with stage-specific survival and population dynamics. Classifying early life stages based on ecological traits can facilitate studies linking climate change to early survival and population outbreaks.

Here we describe four developmental stages of ephyrae and metaephyrae and assess size-at-stage based on morphometric measurements. Our goal is to set a stage classification that is useful for further ecological studies. We also estimate size shrinkage of specimens stored in formaldehyde solution—a widespread procedure used to preserve plankton samples.

METHODS

Field sampling

We used ephyrae and metaephyrae from plankton samples collected with bongo nets during daytime in four annual surveys around the Balearic Islands, western Mediterranean Sea, between June 21 and July 9. The nets (90-cm diameter and 500- μm mesh size) were towed obliquely down to 30-m depth for 8–12 minutes at 2 knots cruising speed. For the morphological and

morphometrical description, we preserved the plankton samples directly in 4% formaldehyde solution buffered with borax for further processing in the lab (14 stations in 2014, 13 stations in 2015 and 12 stations in 2016). For the shrinkage experiment, we sorted and photographed 108 live individuals immediately after sampling in 2019. Images were taken under a dissecting microscope with a camera attached, and specimens were preserved in individual vials for further processing in the lab. They were selected to cover different sizes and morphologies.

Laboratory processing

We sorted 2576 individuals ($n = 892$ from 2014), ($n = 624$ from 2015) and ($n = 1\,060$ from 2016) under a dissecting microscope with a camera attached and classified them in four stages upon the level of development of their body shape, gastric system (digestive chamber and filaments), oral system (manubrium and oral arms), marginal system (marginal lobes, lappets, and tentacles) and nematocysts (Fig. 1). We then photographed each individual and measured the central disc diameter (CDD; Strahler-Pohl and Jarms, 2010) to the nearest 0.01 mm with ImageJ analysis program (Schneider *et al.*, 2012). For the shrinkage experiment, we measured the CDD (Strahler-Pohl *et al.*, 2010) on live individuals and repeated the measurements 6 months after preservation in formaldehyde using Image J. Previous research has shown that gelatinous plankton stored in 4% formaldehyde solution typically stops shrinking 1–3 months after preservation (Thibault-Botha *et al.*, 2004; Lafontaine *et al.*, 1989; Möller, 1980). A subset of 46 live individuals with good conditions (without broken or bended lappets and marginal lobes) was used to measure total body diameter (TBD; Strahler-Pohl *et al.*, 2010). TBD was not measured in preserved individuals due to the poor condition of most samples (e.g. Fig. S1).

Data analysis

Size at a given stage can vary upon temperature and food availability affecting growth (Avian, 1986; Kamiyama, 2018). Because these variables differ among years (e.g. Ottmann *et al.*, 2021), annual differences in size-at-stage should be considered in this analysis. Thus, we apply a two-way analysis of variance on CDD measurements of each individual i considering development stage and sampling year;

$$\begin{aligned} CDD_i = & \beta_0 + \beta_1(Stage_i) + \beta_2(Year_i) \\ & + \beta_3(Stage_i \times Year_i) + \varepsilon_i \quad (\text{Model 1}) \end{aligned}$$

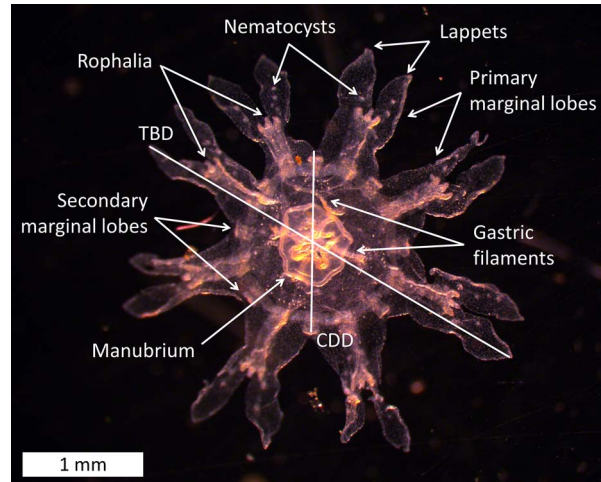


Fig. 1. Morphological structures of a *P. noctiluca* ephyrae.

where β_0 is the intercept, β_{1-3} are the parameter slopes and ε_i is the model error. Model diagnostics indicate the data are heterogeneous (residuals are greater at greater stages of development). However, repeating the analysis using log-transformed values of CDD does not violate any statistical assumption and yields similar values of significance as with the original data. Thus, we include the model diagnostics of both tests in the supplementary materials and report the results of the untransformed measurements below. CDD measurements from 2019 were not included in this analysis because the sample size was 6–10 times smaller than in 2014–2016.

Shrinkage is calculated as

$$\text{Shrinkage} = \frac{\text{Live CDD} - \text{Preserved CDD}}{\text{Live CDD}} \quad (1)$$

and we apply a linear regression to test if shrinkage differs across different CDD sizes.

$$\text{Shrinkage}_i = \beta_0 + \beta_1(\text{live CDD}_i) + \varepsilon_i \quad (\text{Model 2})$$

Model diagnostics are shown in the supplementary materials and all test results are considered statistically significant at a P -value < 0.05 . The data and R code are available on https://github.com/dottmann/pelagia_ephyrae_stage_determination.

RESULTS

Morphological stages of development

We differentiate four stages of development from newly developed ephyra (stage I) to fully developed metaephyrae (stage IV).

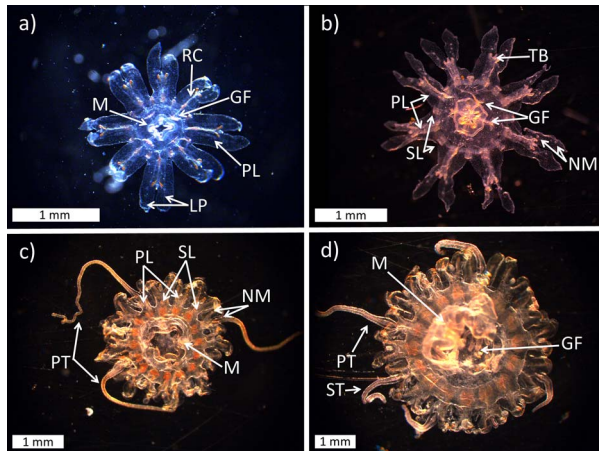


Fig. 2. Oral view of four development stages of ephyrae and metaephyrae. (a) Stage I, (b) stage II, (c) stage III, (d) stage IV. GF = gastric filament; LP = lappet; M = manubrium; NM = nematocyst; PL = primary lobe; PT = primary tentacle; RC = rhopalial canal; SL = secondary lobe; ST = secondary tentacle; TB = tentacle bud. Some tentacles in (c) and (d) are retracted.

Stage I—Planula larvae develop into ephyrae when the bell has flattened, and the manubrium has a clear cross-shape attached to the central cavity (Fig. 2a). They have eight primary (first to appear) marginal lobes, each with a pair of pointed spoon-like marginal lappets and a single rhopalium (sensory structure) at the extreme of a rhopalial canal that ends in a slightly forked and rounded tip. The gastric system has a central stomach with four gastric chambers that are difficult to see. Some specimens have developed one to three gastric filaments.

Stage II—The bell is still flat, eight secondary marginal lobes (appear after the primary ones) are developing and tentacle buds are apparent (Fig. 2b). All four gastric filaments, one per chamber, are fully formed and the manubrium is thickening. Nematocysts start to appear on the aboral part of the ephyrae.

Stage III—Ephyra has transitioned to metaephyrae. Mesoglea in the umbrella starts to thicken, and four primary tentacles have emerged between the lappets of primary lobes (Fig. 2c). Secondary marginal lobes have grown substantially, but they are still shorter than the primary lobes. Four tentacle buds have appeared in alternate positions regarding the primary tentacles. Each gastric chamber has up to three filaments and the manubrium has elongated. More nematocysts have appeared in the aboral side, covering the bell.

Stage IV—Mesoglea keeps thickening in the umbrella, primary tentacles are well developed and the secondary tentacles have appeared (Fig. 2d). The secondary marginal arms are getting close to overlap with the primary ones, and the space between lappets (lappet

stem) has almost disappeared. More gastric filaments appear in each chamber, and the manubrium continues to elongate and starts to differentiate primordial oral arms. The juvenile stage is achieved when the marginal lobes are equally long, the lappets of the primary and secondary lobes overlap, the lappet stem disappears and the oral arms are formed. The shape of the juvenile stage resembles the typical umbrella shape of adults.

Stage morphometry

CDD and TBD of live specimens range 0.53–5.85 mm and 1.29–6.92 mm, respectively, with increasing CDD:TBD ratio with size (Table I). On average, CDD of ephyrae and metaephyrae shrinks 24.6% (± 12.8 SD) after 6 months preserved in formaldehyde (Fig. 3a), and shrinkage is the same across different sizes (*F*-test, $P = 0.16$). CDD of individuals preserved in formaldehyde ranges 0.43–4.43 mm and differs significantly across stages (Fig. 3b; *F*-test, $P < 0.05$). For each stage CDD varies among years (*F*-test, $P < 0.05$).

DISCUSSION

We have distinguished four morphological stages of development of ephyrae and metaephyrae, along with their respective metrics of CDD and TBD in live and CDD in preserved individuals. Despite interannual variations in size-at-stage, size becomes consistently larger and more variable toward advanced stages of development. The increasing variability of size at stage (Table I) is unsurprising as it is influenced by feeding condition—ephyrae and metaephyrae can reduce somatic growth and shrink under poor feeding conditions (Miranda *et al.*, 2016; Kamiyama, 2018; Ramondenc *et al.*, 2019). Thus, we emphasize that morphological classification is a biologically meaningful approach to determine early development stages (Ishii *et al.*, 2004), particularly in ecological studies where the development of feeding structures matters. Furthermore, this approach enables a faster stage determination of large plankton collections and the inclusion of partially damaged individuals.

Subsequent ephyrae and metaephyrae stages develop structures that enable them to capture and digest different prey items. Research conducted on newly developed ephyrae of other scyphozoan shows that they feed primarily on particulate organic matter and microzooplankton (Bamstedt *et al.*, 2001; Miranda *et al.*, 2016; Kamiyama, 2018), and it is likely that stage I *P. noctiluca* do so too. Stage II ephyrae, with improved swimming capacity, more gastric filaments and the appearance of nematocysts, can already feed on small mesozooplankton,

Table I: Morphometric measurements of *P. noctiluca* early life stages alive and preserved in 4% formaldehyde solution

Stage	Condition	n	CDD (mm)				n	TBD (mm)				CDD/TBD
			Min	Max	Mean	SD		Min	Max	Mean	SD	
I	Live	20	0.53	1.24	0.82	0.20	11	1.29	3.05	1.71	0.51	0.48
II	Live	21	0.88	1.62	1.17	0.19	11	1.68	3.93	2.36	0.68	0.50
III	Live	34	1.23	3.52	2.00	0.60	13	2.20	4.46	3.15	0.81	0.64
IV	Live	33	2.78	5.85	4.16	0.81	11	3.29	6.92	4.88	1.16	0.85
I	Preserved	172	0.43	1.00	0.77	0.13						
II	Preserved	768	0.76	1.87	1.13	0.13						
III	Preserved	1 500	1.01	2.98	1.69	0.28						
IV	Preserved	136	1.98	4.43	2.87	0.54						

SD, standard deviation.

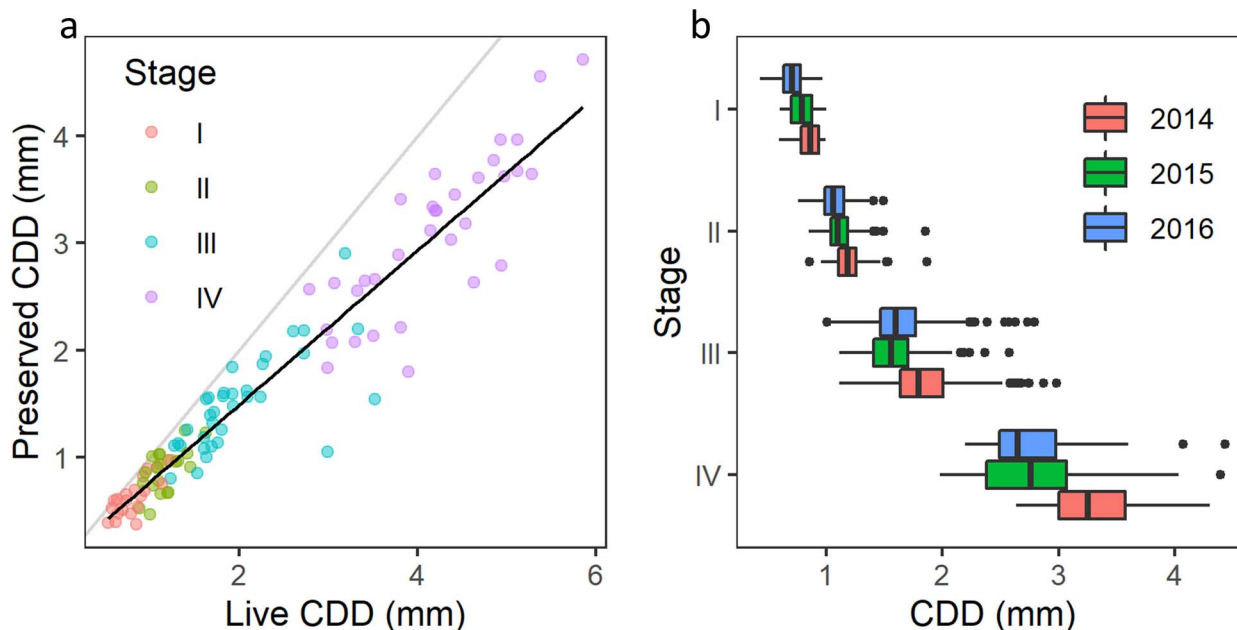


Fig. 3. Shrinkage and size-differentiation of *P. noctiluca* ephyrae and metaephyrae. **(a)** Shrinkage of the CDD of ephyrae and metaephyrae of *P. noctiluca* after 6-month preservation in 4% formaldehyde solution. Gray line: 1:1 ratio; black line: linear regression where preserved CDD = $0.05 + 0.72 \times \text{live CDD}$, $P < 0.05$ and $R^2 = 0.92$. **(b)** Boxplots of CDD measurements of 2576 ephyrae and metaephyrae of *P. noctiluca* preserved in 4% formaldehyde solution. Line in the box: median; border of left and right boxes: first (Q1) and third (Q3), respectively; left and right whiskers: minimum (Q1–1.5 × interquartile range) and maximum (Q3 + 1.5 × interquartile range), respectively; dots: outliers.

but it is likely they have a low feeding success compared with subsequent stages as they still use the lappets to capture prey (Sullivan *et al.*, 1997). The development of tentacles in stage III is a major advance enabling them to improve capture efficiency of larger prey (Lilley *et al.*, 2014), including small fish larvae (personal observation). Reaching this stage is considered a key step to improve early survival of jellyfish (Ishii *et al.*, 2004). Stage IV metaephyrae have twice as many tentacles as stage III metaephyrae, a well-developed gastric chamber with numerous filaments and abundant nematocysts. Although we have not tested capture efficiencies directly, other studies show that they are effective predators of

mesozooplankton and ichthyoplankton (Purcell *et al.*, 2014; Lilley *et al.*, 2014; Tilves *et al.*, 2016; Sabatés *et al.*, 2010).

Size shrinkage of gelatinous plankton varies between 7% and 77% upon taxon, size of the specimen and formaldehyde concentration (e.g. Lafontaine *et al.*, 1989; Nishikawa *et al.*, 1996; Thibault-Botha *et al.*, 2004). Even within a same taxonomic group, shrinkage may differ upon structural features. For instance, Nishikawa and Terazaki (1996) found that the size of two thaliaceans shrinks 7% and 24% at the same formaldehyde concentration, likely reflecting differences in the thickness of their test wall. Ephyrae and metaephyrae of *P. noctiluca*

shrink almost 25% after preservation in 4% formaldehyde solution, and we found no relation between shrinkage and size of the live specimen. Möller (1980) estimated that ephyrae and metaephyrae (1.3–8.0 mm CDD) of *Aurelia aurita* preserved in 4% formaldehyde solution shrink 15%, whereas larger medusae can shrink up to 30%. This trend contrasts with shrinkage of other gelatinous taxa like ctenophores, where larger individuals of the ctenophore *Pleurobrachia bachei* shrink less than smaller individuals (Thibault-Botha *et al.*, 2004).

It is possible that the range of sizes evaluated in this study (0.53–5.85-mm CDD) is too narrow to appreciate significant size differences in shrinkage. Having measures of shrinkage are necessary to identify the original size of specimens collected in plankton surveys. This is important because, while most field collections preserve plankton samples with formaldehyde or other fixatives, experiments on early survival and trophic ecology use live measurements. Thus, with the caveat that our metric applies to only samples stored in 4% formaldehyde, converting sizes of preserved samples to their live equivalents can help improve the accuracy of ecological models of *P. noctiluca* early life stages on field observations.

CONCLUSIONS

Ephyrae and metaephyrae of *P. noctiluca* are two early life stages that are often vaguely described and fail to summarize important body transformations during this period that affect prey capture and feeding processes. Here, we have covered this information gap describing four stages of development based on morphological and morphometrical traits that influence their feeding capacity. We further report that ephyrae and metaephyrae shrink 24.6% in size after preservation in 4% formaldehyde solution. These findings set a baseline defining finer stages of ephyrae and metaephyrae that can contribute to further ecological studies of *P. noctiluca*.

SUPPLEMENTARY DATA

Supplementary data is available at *Journal of Plankton Research* online.

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