Catostylus tagi (Scyphozoa, Rhizostomida) life cycle and first insight into its ecology (#61520)

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Catostylus tagi (Scyphozoa, Rhizostomida) life cycle and first insight into its ecology

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As jellyfish outbreaks are conspicuous and natural events, these blooms can lead to severe environmental and anthropogenic activities. Although research during the last decade has been focused on the factors influencing the different jellyfish life stage, only a few species have its full life cycle understood. In this context, we describe for the first time the developmental stages in the life cycle of *Catostylus tagi*, from planula to young medusa, reared in the laboratory. The species displays the typical Rhizostomida metagenetic life cycle. Mature scyphistoma presents 18 tentacles and a total body length of 1.5 ± 0.2 mm. Only podocyst production was observed. Strobilation, occurring continuously in the laboratory conditions, was mainly polydisk. The eight-rayed typical ephyrae, with a total body diameter of 2.4 ± 0.4 mm at liberation, showed development typical of the Rhizostomida. As a first step in studying this species' ecology, we also present preliminary assessments of the influence of (i) different temperature and salinity regimes on the planulae's survival and polyps' development and (ii) the effect of temperature and diet on asexual reproduction. The results showed a high tolerance of the planula to the wide range of salinities, while polyp development was significantly faster at high temperature and lower salinities. Strobilation onset was significantly influenced by temperature and diet. Finally, we present *C. tagi* spatial and seasonal distribution in the Tagus estuary (Portugal) in 2019, showing its occurrence along that year (except in April), with most of the observations recorded on the northern shoreline.

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Abstract

As jellyfish outbreaks are conspicuous and natural events, these blooms can lead to severe environmental and anthropogenic activities. Although research during the last decade has been focused on the factors influencing the different jellyfish life stage, only a few species have its full life cycle understood.

In this context, we describe for the first time the developmental stages in the life cycle of *Catostylus tagi*, from planula to young medusa, reared in the laboratory. The species displays the typical Rhizostomida metagenetic life cycle. Mature scyphistoma presents 18 tentacles and a total body length of 1.5 ± 0.2 mm. Only podocyst production was observed. Strobilation, occurring continuously in the laboratory conditions, was mainly polydisk. The eight-rayed typical ephyrae, with a total body diameter of 2.4 ± 0.4 mm at liberation, showed development typical of the Rhizostomida. As a first step in studying this species' ecology, we also present preliminary assessments of the influence of (i) different temperature and salinity regimes on the planulae's survival and polyps' development and (ii) the effect of temperature and diet on asexual reproduction. The results showed a high tolerance of the planula to the wide range of salinities, while polyp development was significantly faster at high temperature and lower salinities. Strobilation onset was significantly influenced by temperature and diet. Finally, we present *C. tagi* spatial and seasonal distribution in the Tagus estuary (Portugal) in 2019, showing its occurrence along that year (except in April), with most of the observations recorded on the northern shoreline.

Keywords: Catostylidae, planula, polyp, ephyra, gastric system, salinity, Tagus estuary, temperature, diet, Tagus estuary, Atlantic Ocean

Introduction

In the last decades, jellyfish have attracted much attention due to the intense blooming events in coastal waters. The outbreaks often have severe repercussions (e.g. fisheries, aquaculture, tourism and power plants) and ecosystems (Purcell *et al*., 2007). Nonetheless, no common consensus on whether jellyfish are increasing globally (Condon *et al*., 2012, 2013) or on the implication of the different anthropogenic causes, such as pollution, overfishing, artificial structures, as triggers for these gelatinous organisms proliferations (Sanz-Martín *et al.,* 2016).

With some exceptions (e.g. *Pelagia noctiluca*), Scyphozoan species are meroplanktonic with a bipartite life cycle. The pelagic medusa stage typically reproduces sexually, producing a free-swimming planula. After the planula fixes on a substrate, it grows into a sessile polyp. The polyp reproduces asexually through different modalities (e.g. budding, swimming buds, podocysts, fission, strobilation) (Arai, 1997). Environmental factors (*e.g.* temperature, prey supply) can affect both the sessile stage (*e.g.* asexual reproduction timing and intensity (Yongze *et al*., 2016)) and the pelagic stage (*e.g.* somatic growth, sexual maturation (Pitt and Kingsford, 2000)), making both phases integral to the study of scyphozoan biology. Understanding the influence of environmental factors on each life stage is crucial to understand their dynamics better while identifying early stages is primordial to detect potential blooms. Paradoxically, the complete life cycle of scyphozoans has been described for less than 25% of known species (Tronolone *et al.*, 2002). Generally, only the adult stage of scyphozoans is known (Mills, 2001; Jarms and Morandini, 2019).

The *Catostylus* genus consists of ten valid species, occurring in predominantly temperate regions and subtropical and tropical regions (Jarms and Morandini, 2019). In the Atlantic, the genus is currently represented by three species: *C. cruciatus* (Lesson, 1830) in Brazil, *C. tagi* (Haeckel, 1869), whose distribution extends from the Atlantic coast of Europe (France, Spain and Portugal) to the West African coast (south of Congo), and *C. tripterus* (Haeckel, 1880) off Equatorial Guinea. The distribution of *Catostylus tagi* has been extended eastward since it was recorded as a non-indigenous species (NIS) in the Mediterranean Sea in June 2010 in the Sicily Channel

(Nastasi, 2010). Among the genus, only the life cycle of *C. mosaicus* has been investigated (Pitt, 2000).

Catostylus tagi is a common Scyphozoa in the Tagus estuary (Portugal), where juveniles and adults have been observed (GelAvista citizen science project). No records of ephyrae or polyps have been reported yet, and general information on this species' biology and ecology remain scarce. To date, no studies on its population dynamics, biology, and ecology have ever been published. Only the medusa stage of *Catostylus tagi* has been described, and its complete life cycle remains unknown. However, several studies on *C. tagi* biochemical properties have been conducted, finding edible species for human consumption (Raposo *et al.*, 2018; Amaral *et al.*, 2018). Its collagen and antioxidant properties showed a potential new bio-resource for the cosmetics and food sector (Calejo *et al.*, 2009; Morais *et al.*, 2009). *C. tagi* is moderately venomous in a similar manner to *R. pulmo* (Gueroun, *pers. observ*.) and is considered a harmless species in Portugal (Morais *et al.*, 2009).

In the present study, we describe for the first time the complete life cycle of *Catostylus tagi* based on fertilisation trials conducted in the laboratory. In addition, we conducted a preliminary assessment of the simultaneous influence of temperature, salinity and diet regimes on the early life stages (planula, polyp and ephyra). Finally, we present a pionner overview on its spatial and seasonal distribution along the Tagus estuary.

Materials and methods

Ethics statement

The jellyfish *Catostylus tagi* is not an endangered or protected species.

Fertilisation

In early October 2019, six specimens were collected from the Tagus estuary (Portugal) near the Oceanário de Lisboa and individually transferred to the laboratory within 30 mins in 15 L buckets. Specimens sex and gonads maturity were determined using a microscope. Of the six individuals, two were males, and four were females. Gonads were extracted from the two most mature individuals. The female and the male bell diameter were 39.5 cm and 43 cm, respectively. After extraction, gastric cirri were meticulously removed from the gonads with a scalpel. The extracted gametes were mixed and incubated for 48 h with constant aeration in 3 L containers of ultraviolet treated artificial seawater (salinity 35) at 18°C. After 48 h, the planulae were collected by filtering the medium on gradient mesh (200 µm and 55 µm). Petri dishes, previously incubated for four days in natural seawater for biofilm development, were used as substrates. Petri dishes were placed in 500 ml glass bowls, given to planula choice to fix on both substrates sides.

Culture maintenance

Planulae and polyps were incubated in artificial seawater (salinity 35) at 23°C and in natural light/dark cycle. Collected ephyrae were maintained in several 500 ml jars. Once the metaephyra stage was reached, individuals were transferred to a 60 L pseudo-Kreisel under the same conditions (i.e. temperature, salinity and feeding regime).

Polyps were fed daily with rotifers (*Brachionus plicatilis*) during the first week, then newly hatched *Artemia* nauplii were added three times a week. Ephyrae and juveniles were fed rotifers (four times a day), *Artemia* (three times a day), and mashed mussel (once a day). Nauplii of AF *Artemia* Vietnam strain (small nauplii with high HUFA content, Inve Aquaculture NV®) and enriched EG *Salt Lake Artemia fransiscana* (Inve Aquaculture NV®, Baasrode, Belgium) were used for ephyrae and juvenile, respectively. Fifty per cent water exchange was conducted every 2-3 days.

Anatomical analysis

Two different Stereomicroscopes (Leica ® SAPO and Leica ® M165C) were used to describe the various life stages, as well as to follow the development of the gastric system, manubrium, and marginal lappets of the newly released ephyra (stage 0) through to the metaephyra stage (stage 7).

Measurements were used for the scyphistoma following Straehler-pohl *et al.* (2011): total body length (TBL), calyx length (CL), hypostome length (HL), mouth disc diameter (MDD), and stalk length (StL) (Fig. 1A). The following standard measurements were used for the young ephyrae (Straehler-Pohl and Jarms, 2010): total body diameter (TBD), central disc diameter (CDD), total marginal lappet length (TMLL), lappet stem length (LStL) and rhopalial lappet length (RLL) (Fig. 1B). Relative body dimensions (%) were calculated for scyphistomae (measurements compared with body length, *e.g.* CL/TBL x 100, and calyx diameter, MDD/CL 9 100) and for ephyrae (measurements compared with body diameter, *e.g.* CDD/TBD 9 100 and lappet length, e.g. RLL/TMLL 9 100). A total of 11 scyphistomae and 20 ephyrae from 5 strobilae were measured.

The effect of temperature and salinity on planula development

Two orthogonal treatment sets were established with three temperature (15, 20, and 25°C) and four different salinities (20, 25, 30, and 35). Water was prepared by diluting artificial seawater (salinity: 35) with distilled water. Eighteen replicates per treatment were tested following Conley and Uye (2015) and Takao and Uye (2018) methods. Thirty-six polycarbonate culture plates, each 10-ml, six-wells, were prepared (three plates per experimental condition). Culture plates were filled four days before the incubation with natural seawater to allow for biofilm development. The planulae acclimated to lower salinity (30, 25, and 20) were done step-wise by soaking them in the water of each salinity for 5 min until the target salinity. Over six days, planulae were surveyed daily with a stereomicroscope (Zeiss ® Stemi 305). Their respective life stages were recorded in the following manner: Dead, Stage 0: fixed but no tentacles; Stage 1: 1-4 tentacles; Stage 2: 5-7 tentacles; Stage 3: 8-15 tentacles; Stage 4: 18 tentacles. None of the planulae or polyps was fed during this trial.

The effect of temperature and feeding on asexual reproduction

Two orthogonal treatment sets were established with two temperature levels (15 and 20°C) and three feed regimes (*Brachionus plicatilis*, *Artemia* sp. nauplii, and unfed). Eighteen polyps were tested in each of the six combinations. Eighteen polycarbonate culture plates, each 10-ml, six-wells, were prepared (three plates per experimental condition) with one polyp per well. A water bath was used to maintain the two temperatures. Photoperiod was maintained at 12 h light:12 h dark. After polyps had one week to reattach and acclimate to the experimental temperatures, newly hatched *Artemia* nauplii and *B. plicatilis* were fed in excess every two days. After the polyps fed for 1.5 h, the wells were cleaned with swabs; uneaten food was discarded, and seawater replaced with new water of the same temperature. This feeding protocol provided saturating prey briefly, resulting in equal feeding in all treatments by minimising enhanced feeding at warmer temperatures (Ma and Purcell, 2005). Specimens in the unfed treatment never received food. Polyps were examined daily for strobilation and ephyrae release and twice a week for podocyst production. After enumeration, new ephyrae were removed but not the new podocysts. The experiment lasted 33 days.

Several responses variable were defined for analysis: the number of podocyst produced by the polyps; the time from the beginning of the experiment to strobilation onset was the "*pre-strobilation*" period (*Pre-str*); the time from the beginning of strobilation to the release of the first ephyrae was the "*bet-strobilation"* period (*bet-str*); and the time from the first release of ephyrae to the release of the last ephyrae was the "*strobilation period*" (*Str*); the number of ephyrae produced for each strobilation event.

Community science data

This study presents data on *C. tagi* sightings from Tagus estuary for the year 2019 gathered in the GelAvista Project's scope (gelavista.ipma.pt), mainly based on the GelAvista smartphone App. The project is a citizen science program that provides information on jellyfish' presence in Portugal through volunteer contributions of jellyfish sightings via the GelAvista smartphone application, email address, and Facebook page. The collected data include GPS location, date, and hour of sighting and the approximate number of specimen spotted. Species identification is made through the examination of photographs or videos. A confidence level was assigned to all reports, taking into account the veracity and sufficiency of the information received.

Statistical analysis

Since the measurement on planula is repeated measures of binary outcomes, data on the planktonic were assigned to durations from the experiment onset to settlement time (maximum of 160 hours). The combined effect of temperature and salinity on the planktonic duration was tested by a two-way ANOVA, followed by Tukey pair-wise comparison. Data were squared root transformed to meet residuals homogeneity assumption.

The *Pre-str* data were analysed with generalised linear models for counts data using Poisson distributions (Zuur *et al.,* 2009). When data presented overdispersion, the models were fitted with a negative binomial distribution. Due to excessive zero in the *Pre-stre* data, a zero-inflated negative binomial model was used.

Since only two combinations produced ephyrae, bet-strobilation (*bet-str*), strobilation period (*Str*) and ephyra released by strobilation event were analysed with a *T* or Wilcox ranking test depending on the variance homogeneity.

Data were analysed with the free R platform (version 3.0.2; R Development Core Team 2011) using *car* (Fox and Weisberg, 2019), *pscl* (Zeileis *et al.,* 2008) and *lmtest* (Zeileis and Hothorn, 2002) packages.

Results

Life cycle

Catostylus tagi displayed a typical meta-genetic life cycle with scyphistoma and ephyra phases (Table 1). The first polyps were observed approximately 96 hours after the planulae had been added to the culture plates. The polyps were translucent-white, cone-shaped, and typically had four tentacles. Mature scyphistoma of *C. tagi* had an average of 1.5 ± 0.2 mm in total body length (TBD) and 0.6 ± 0.1 mm in mouth disc diameter. They had 18 tentacles in a single whorl around a slightly sunken mouth disc. The four-lipped hypostome was short, club-shaped, and 22 % of the total body length (TBD). The calyx had an elongated cup shape, which was distinctly longer than wide (Table 1). Scyphistoma colour varied from white to pale orange depending on the feeding. The scyphistoma proliferated asexually via podocysts (Table 1). This proliferation was observed starting as a periderm-enclosed podocyst, formed based on the stolon on the substrate. The podocysts were typically yellow or brown. A finger-shaped stolon developed from the lower part of the stalk and attached to the substrate allowing the scyphistoma to move over.

No other reproduction modalities, such as lateral budding by stolon, lateral scyphistoma budding, or pedalocysts were observed.

Both monodisk (one ephyra production) and polydisk (multiple ephyrae production) strobilation were observed. Monodisk strobilation was only observed once. The polydisk strobiles produced up to 15 ephyrae per strobilation (average of 6.8 ± 3.6 ephyrae per strobilation). At the strobilation first stage, the calyx elongated, and the first marginal lobe formed by the calyx upper part constriction. The ephyra lappet elongated, and rhopalia appeared. Scyphistoma tentacle resorbed progressively until complete resorption. Once the last ephyra was released, the residuum developed new tentacles (n = 18) and hypostome. Some scyphistomae were able to perform new strobilation between 10 to 16 days after ending the first strobilation. The number of ephyrae produced by those scyphistomae did not change between the first (4 ± 1.7) and the second strobilation (3.7 ± 0.6).

The newly released ephyrae had a total body diameter (TBD) of 2.5 ± 0.4 mm and a central disc diameter (CDD) of 0.9 mm ± 0.2 mm, about 39% of the TBD. Ephyrae typically had eight marginal arms with a pair of antler palm-like rhopalial lappets and a single rhopalium *per* lappet (Fig. 2). The rhopalial lappets represented about 51% of the total marginal lappet length (TMLL). The eight rhopalial canals were slightly forked, with rounded points. The tips of the rhopalial canals end at the red-coloured rhopalium base; however, the eight velar canals were either not developed or distinguishable (Table 3). There were 1-2 gastric filaments *per* quadrant. The manubrium presented a four-lipped shape (Fig. 3A). Ephyrae exhibited colours from dark pink to dark red.

Stage 1: The TBD doubled in size 4.8 ± 0.4 mm. Eight rhombical velar canals appear. The first oral tentacles develop on the distal ends of the manubrium. The base of the rhopalial canal thickens (Table 2). The tips of the rhopalial canals become rounded. The first oral tentacles develop around the manubrium opening (Fig. 3B).

Stage 2: Lappet bulb grows between the marginal lappets. The velar canals form a pair of branches midway (Table 2). The manubrium starts to split into four oral arms (Fig. 3C).

Stage 3: The lappet bulbs develop into serrated velar lappets. The velar canals lengthen centrifugally, and the rhopalial canals form a pair of side branches that grow centrifugally toward the velar canals (Table 2). The four oral arms divide, forming eight arms (Fig. 3D).

Stage 4: The velar's side branches and the rhopalial canal fuse forming the primary ring canal. The velar lappets extend outward, and their serrations retract (Table 2).

Stage 5: The velar lappets continue their extension outwards. The serrations on the antler palm-like rhopalial lappets retract. Two new canals grow on the velar canal developing centripetally (tertiary canals) parallel to the radial canals, and two other canals develop horizontally toward the rhopalial canal (Table 2).

Stage 6: The midway-side branches of the velar canals fuse with the radial velar forming the secondary ring canal. Below this new ring canal, the velar and rhopalial canals develop the last set of side branches to form the last ring canal. The velar lappet extremities extended until the rhopalial lappets complete the umbrella (Table 2).

Stage 7: The final ring canal forms. The space between the canals extends, forming a meshed network of anastomosing canals (Table 2).

Five months after the ephyrae are released from strobilae, juvenile *C. tagi* are fully developed (Fig. 4).

Temperature and salinity effect on planulae development

Planulae of *C. tagi* showed relatively low mortality of ≤ 20 %, predominantely at 15 °C. No mortality was observed at 30 salinity (Fig. 5). The first planulae settlement occurred within four to six hours in all treatment combinations. The final settlement proportion varied from 53% (15°C and 25 salinity) to 100 % (25°C and 25 - 35 salinities). The planktonic duration (Fig. 6, Table 3) was significantly influenced by temperature ($p < 0.001$) and salinity ($p < 0.01$), but no interaction was detected ($p = 0.31$). Tukey post hoc test revealed that planula settlement was faster at high temperature (25°C) while the planktonic stage duration was significantly prolonged at 30 salinity compared with 35 salinity.

No polyps with tentacles were observed at 15°C (all salinities considered). Polyp development was enhanced at higher temperature with an optimum of 25°C and all salinities. Up to 54.2% of the planulae developed into polyps at 25°C and 20 salinity. During the experiment, polyps developed a maximum of eight tentacles at the highest temperature (25°C) and lower salinities (20 and 25). Morphological deformities were not detected.

Temperature and food effect on asexual reproduction

The podocyst production, varying between 0.8 ± 0.9 and 1.7 ± 1.5 podocyst.scyphisotma^{-1}, was not influenced by temperature ($p = 0.21$) neither by feeding regime ($p = 0.3$), and no significant interaction was detected ($p = 0.17$) (Fig. 7A, Table 3).

Strobilation occurred only for three groups: 20°C-Rotifer & -Artemia (61.1%) and 15°C-*Artemia* (28%). *Pre-strobilation* period, significantly influenced by temperature and diet, was shorter in the 20°C-rotifers (11 ± 4 days) and 20°C-*Artemia* (15 ± 7 days) groups than in the 15°C-*Artemia* (32 ± 3 days) (Fig. 7B, Table 3).

Ephyrae release were only observed at 20°C. The *bet-strobilation* periods (rotifers: 5.7 ± 1.4 days; *Artemia*: 5.6 ± 2.6 days) and the number of ephyrae produced by strobilation (rotifer: 6.3 ± 3.5; *Artemia*: 6.9 ± 3.9) were not significantly affected by the diet (Fig. 7C-E, Table 3). The strobilation period was significantly shorter in the rotifer group (2.5 ± 2.3 days) than in the Artemia (26.4 ± 3.9 days) (Fig. 7D, Table 3).

Seasonal distribution of Catostylus tagi *adults in the Tagus estuary*

During 2019, *C. tagi* adult specimens' sightings were reported via GelAvista smartphone App, along both margins of the Tagus estuary, from the inner bay, and «*Cala do Norte*» to the estuary's mouth opening to the Atlantic Ocean (Fig. 8). Sightings were recorded in all months of the year except April. Many sightings (>5) were recorded from September to February, a proxy for a higher abundance of this species.

Discussion

The *jellyfish Catostylus tagi* displays a typical meta-genetic life cycle observed in Rhizostomatidae: a benthic scyphistoma phase that reproduces asexually via strobilation releases ephyrae that grow into a pelagic medusa, which reproduce sexually. Our observations of adult gonads from individuals collected in the Tagus estuary (Portugal) showed the absence of planulae, supposing that *C. tagi,* unlike *C. mosaicus* (Pitt, 2000) and other Rhizostomtida (Table

4), might be a non-brooding medusa. It is also plausible that *C. tagi* is brooding, and the sexual reproduction occurs later in the year than the present sampling period. This hypothesis requires a more extended sampling program to validate one or the other possibility.

Catostylus tagi and *C. mosaicus* adult medusae present clear morphological distinctions (Jarms and Morandini, 2019), such as the presence of a terminal portion of tapering filaments on the oral mouth and a purple margin on the umbrella in *C. tagi*. On the other hand, differentiation between earlier stages is more challenging (Table 3). Comparison between polyps and ephyrae reared in laboratory conditions showed few differences between the two species.

The ephyrae of *C. tagi* develop into an 8-rayed medusa, typical of the Scyphozoan taxa (Straehler-Pohl and Jarms, 2010), with 16 antler palm-like rhopalia lappets, also exhibited by *C. mosaicus*. The main contrast between these two species lies in the velar canal shape, which is rhombical in *C. tagi* and spade-like in *C. mosaicus* (Straehler-Pohl and Jarms, 2010). This anatomic characteristic of *C. tagi* distinguishes the species from the other two Rhizostomatidae, *Rhizostoma luteum* (Kienberger *et al.*, 2018) and *Rhizostoma octopus* (Holst *et al.*, 2007), occurring in the same geographical area.

Scyphozoan species exhibit several propagation strategies, including various budding modes (*e.g.* lateral budding, budding from stolon, motile bud-like tissue particles, etc.) and podocysts (Arai, 1997). These propagation strategies are species-specific. Some species adopt a mono-mode, such as *Phyllorhiza punctata* free-swimming planuloids (Rippingale and Kelly, 1995) or *Rhopilema nomadica* and *Rhizostoma lutem* podocysts (Lotan *et al.*, 1992; Kienberger *et al.*, 2018). In contrast, some species combine two or more strategies, as seen in *Aurelia* spp. (i.e. lateral budding, lateral budding through stolons, reproduction from parts of stolons/stalks, motile bud-like tissue particles, podocysts) (Schiariti *et al.*, 2014). Unlike *C. mosaicus,* which combines various strategies, including lateral polyp buds, podocysts, pedalocysts, and longitudinal fission (Pitt, 2000; Straehler-pohl, 2009), *C. tagi* propagation seems to be limited to podocyst (under the used conditions). Moreover, the podocysts production was not influenced by temperature (15 and 20°C) nor feed regime (unfed, rotifer and *Artemia*). The reproductive strategy adopted by the respective scyphozoan plays a significant role in the polyp reproduction rate (Schiariti *et al.*, 2014) and, consequently, in potential medusae outbreaks. Among the various propagation modes, podocysts present the lowest reproduction rate. Podocysts contain stored nutritional reserves in carbohydrates, lipids, and proteins (Black, 1981; Chapman, 1968), which can remain dormant for an extended period. Thein *et al.* (2012) found that *Aurelia aurita* s.l. podocysts were able to survive for up to 3.2 years. Consequently, early studies speculated that podocysts represented an asexual reproduction strategy induced by poor environmental conditions and protection against predators (Cargo and Schultz, 1967). However, recent studies show that, like the other propagation modes, podocyst production is enhanced by food supply (Schiariti *et al.*, 2014) and temperature increase (Thein *et al.*, 2012).

Falling in line with other Rhizostomida species, except for *Rhizostoma luteum*, the predominant strobilation type observed in *C. tagi* was polydisk (Table 4). The first *C. tagi* scyphistomae were observed three days after fertilisation and strobilated approximately ten days later (18C°, 35 salinity). Strobilation onset, duration, and the number of ephyrae produced by polydisk scyphistoma are influenced by environmental factors, such as temperature (Purcell *et al.*, 2012) and food supply (Wang *et al.*, 2015). Other factors, such as age and size (Russell, 1970; Holst, 2012), are intrinsic to scyphistoma. Under the various conditions used to rear *Catostylus tagi*, strobilation onset was faster at the higher temperature. Surprisingly, while diet appeared as a primordial trigger for strobilation induction (no strobilation in unfed scyphistoma) and strobilation duration, the number of produced ephyrae was not influenced by the diet. Under the divers' condition (temperature and diet) used in the present study (broodstock and experiment), *C. tagi* scyphistomae were repeatedly strobilated twice a month (≥18°C), producing up to up to 15 ephyrae (6.8 ± 3.6), exceeding *C. mosaicus* ephyra production (up to 5 ephyrae per strobilation, Pitt, 2000). However, it is unknown whether the culture conditions of previous *C. mosaicus* studies were optimum for strobilation. *C. tagi* scyphistoma strobilated through a wide temperature range varying from 15°C to 25°C (Gueroun, *unpublished data*). This temperature range (15-25°C) has been recorded in the Tagus (Gameiro *et al.*, 2007). It might explain the continuous occurrence of *C. tagi* medusa stage in the estuary from June to March.

C. tagi is common in the Tagus estuary (Portugal), where salinity (1-37) and temperature (6.4-33.5°C) display large spatial and seasonal variations (Gameiro *et al.*, 2007; Rodrigues *et al.*, 2017). This suggests the euryhaline and eurythermal character of the medusa stage like *C. mosaicus* (Loveridge et al., 2021). Several scyphozoans species occur in river estuaries and brackish waters. For example, *Aurelia aurita*, *Cyanea capillata* and *Cyanea lamarckii* are found in the Baltic Sea (Holst and Jarms, 2010) and *Chrysaora quinquecirrha* is found in Chesapeake Bay (Purcell *et al.*, 1999). Similarly, *Rhopilema esculentum* is found in the Yangtze estuary (Xian *et al.*, 2005), and *Nemopilema nomurai* is found in the East China Sea and Yellow Sea (Zhang *et al.*, 2012; Wang *et al.*, 2013). Temperature and salinity play a considerable role in planulae survival, settling rate, settling timing, and somatic growth (Takao and Uye, 2018; Webster and Lucas, 2012). Given the survival responses observed at different temperature (15-25°C) and salinity (15-35) regimes during this study, *C. tagi* planulae exhibited euryhaline and eurytherm character at the adult stage. Planulae with a tolerance for a wide range of salinities have been recorded for several other estuarine and brackish water scyphozoan medusa (Takao and Uye, 2018; Conley and Uye, 2015; Dong *et al.*, 2015; Holst and Jarms, 2010).

Conclusion

Knowledge of *C. tagi* ecology is still scarce. However, the present observations (planula survival and polyp development in different salinity and temperature regimes, strobilation from 15 to 25°C) indicates its high tolerance and plasticity, contributing therefore to a better understing of the biology and ecology of this species. The euryhaline and eurytherm characteristics of the species at multiple life stages are advantageous for future *C. tagi* aquaculture, research, and production. Further studies must be conducted on the polyp and ephyra stages to determine the critical environmental factors on asexual reproduction and growth. Finally, studies such as the one we present here are essential to evaluate *C. tagi* response to climate change and predicting any temporal and geographic spreading of the species.

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Table 1(on next page)

Morphology and body proportions of the polyp and ephyrae of *Catostylus tagi*

ml: marginal lobe; *ed*: ephyrae disc; *ep*: ephyrae; *te*: tentacle; *pd:* podocysts. *Scale bars*: phase 1 to 4: 1 mm; ephyrae: 500µm (Photo credit Sonia KM Gueroun)

Table 1: Morphology and body proportions of the polyp and ephyrae of *Catostylus tagi*. *ml*: marginal lobe; *ed*: ephyrae disc; *ep*: ephyrae; *te*: tentacle; *pd:* podocysts. *Scale bars*: phase 1 to 4: 1 mm; ephyrae: 500µm (Photo credit Sonia KM Gueroun)

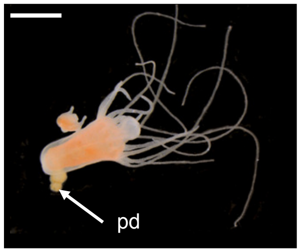
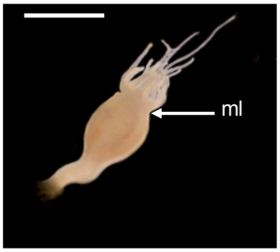
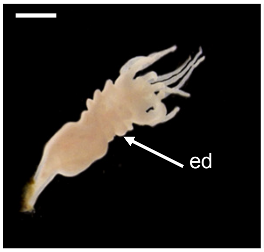
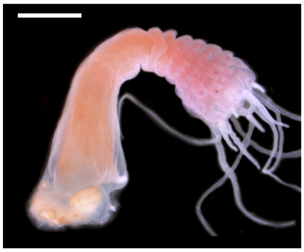
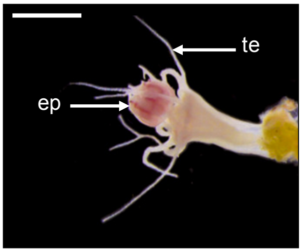
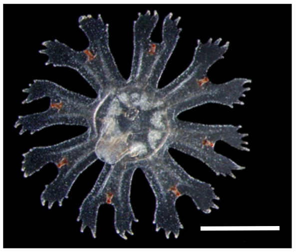
Polyp	Strobilation				Ephyrae
Phase 1	Phase 2	Phase 3		Phase 4	
					
• 18 filiform tentacles • Long, club-shape and flexible • Hypostome: club-shaped, four lipped • Calyx shape: elongated cup-shaped *Body proportions* • HL ≈ 22 % of the TBL • CL ≈ 78 % of the TBL • StL ≈ 22 % of the TBL • MDD ≈ 44 % of the TBL	• Formation of the 1st marginal lobe of ephyrae by forming a constriction directly below the tentacles	• Absorption of tentacles • Formation of other marginal lobes below the first one • Development of the ephyrae and appearance of the lappets and rhopalii		• Detachment of the ephyrae • Formation of new tentacles on the residuum	• 8 lappets stems • 16 antler palm-like rhopalia lappets, , with 2 to 7 finger-like appendages • 1-2 gastric filaments per quadrant • No marginal tentacles or tentacles bulbs *Body proportions* • RLL ≈ 17 % of the TBD • LStL ≈ 17 % of the TBD • CDD ≈ 39 % of the TBD • TMLL ≈ 85 % of the CDD • RLL ≈ 51 % of the TMLL LStL ≈ 51% of the TMLL

Table 2(on next page)

Development stages of gastric system of the ephyrae of *Catostylus tagi.*

rc: rhopalial canal, *rl*: rhopalar lappet, *ma*: manubrium, *rh*: rhopalium, *vc*: velar canal, *lb*: lappet bud, *vl*: velar lappet, *pr*: primary ring canal, *sr*: secondary ring canal. All *scale bars* 1 mm. (Photo credit Sonia KM Gueroun)

Table 2:

Development stages of gastric system of the ephyrae of *Catostylus tagi. rc*: rhopalial canal, *rl*: rhopalar lappet, *ma*: manubrium, *rh*: rhopalium, *vc*: velar canal, *lb*: lappet bud, *vl*: velar lappet, *pr*: primary ring canal, *sr*: secondary ring canal. All *scale bars* 1 mm. (Photo credit Sonia KM Gueroun)

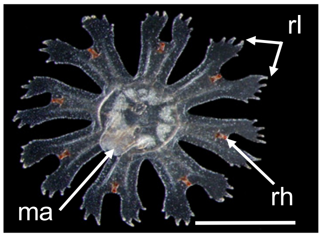
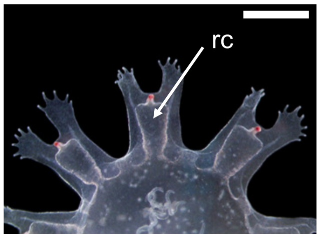
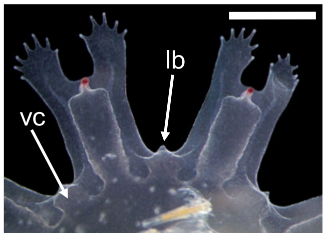
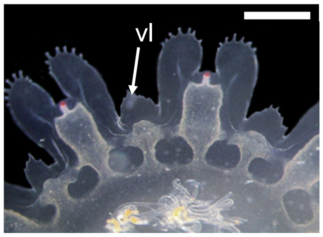
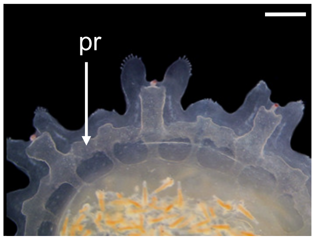
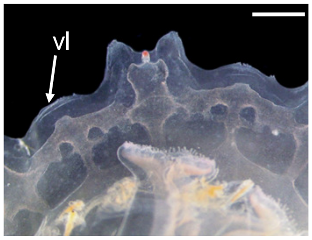
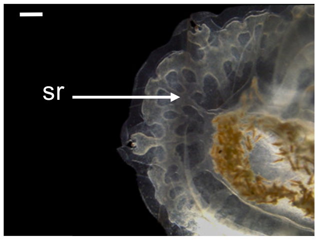
	Development of the gastric systems		
Stage 0 (-24h)	Stage 1	Stage 2	Stage 3
			
• antler palm-like rhopalial lappets • Rhopalia canals are s forked, rounded points • • rhopalial canals slightly forked with rounded points • velar canals were not developed	• rhombical velar canal • the **velar canal** thicken, and the tips round-up	• **lappet bulb** grow between the marginal lappet • the velar canals form midway pair of side branches	• the velar canals grow **centrifugally,** and the pair of side branches are spreading • the rhopalial canals form midway a pair of side branches • **serrated velar lappets** grow between the marginal lappets
Development of the gastric systems			
Stage 4	Stage 5	Stage 6	Stage 7
			
• the side branches of the velar canals fuse with the pair of side branches of the rhopalial canals to form **a primary ring canal**	• on velar canal grow two new canals developing **centripetally** (tertiary canals) parallel to the radial canals, and two other canals are developing horizontally toward the rhopalial canal.	• the horizontally midway-side branches of the velar canal fuse with the radial velar to form a **secondary ring canal** • the centripetally canal fuse with the second ring and continue growing centripetally • below the second ring, the radial canal develop another set of the side branch to form the **last canal ring**	• the tips of the centripetally growing canals stay clear of the fusion and protrude into space between the last ring canal and the stomach • the bottom and the roof of the canals fuse, forming «*Inseln*» that woven constituting a mesh network of anastomosing canal

Table 3(on next page)

Statistical results on the effects of temperature, salinity and diet on *C. tagi* different life stages.

Table 3:

Statistical results on the effects of temperature, salinity and diet on *C. tagi* different life stages

Variable tested	Planktonic duration (ANOVA)	Podocyst (ANOVA)	Bet-str (Wilcox)	Str (*t*-test)	Ephyra (*t*-test)	Pre-str (ZINB)	Estimate	Std. Error	z value	p value
Temperature	$F(2, 18) = 25.8$	$F(1, 18) = 1.62$	-	-	-	*Count model*				
	$p < 0.001$	$p = 0.21$				Intercept	4.92	0.34	14.53	< 0.001
Salinity	$F(3, 18) = 4.1$	-	-	-	-	Temperature	-0.76	0.16	-4.71	< 0.001
	$p < 0.01$					Feed	-0.34	0.14	-2.39	0.02
Feed	-	$F(2, 18) = 1.23$	$p = 0.8$	$p < 0.01$	$p = 0.7$	Log(theta)	3.03	0.67	4.51	< 0.001
		$p = 0.3$				*Zero-inflated model*				
Temperature X salinity	$F(6, 18) = 1.21$	-	-	-	-	Intercept	11.79	2.49	4.74	< 0.001
	p = 0.3.1					Temperature	-0.51	0.12	-4.15	< 0.001
Temperature X feed	-	$F(1, 18) = 1.89$	-	-	-	Feed	-1.37	0.37	-3.71	< 0.001
		$p = 0.17$				Theta: 20.67 ; Log-likelihood: -135.9 (Df =7)				

Table 4(on next page)

Polyp and ephyra morphology of Rhizostomida species. *rc*: rhopalial canal, *vc*: velar canal, *: brooding species.

Source: 1: Pitt (2000); 2: Straehler-pohl (2009); 3: Straehler-Pohl and Jarms (2010); 4: Kienberger et al. (2018); 5: Fuentes et al. (2011); 6: Purcell et al. (2012); 7: Schiariti et al. (2014); 8: Holst et al. (2007), 9: Holst and Jarms (2007); 10: You et al. (2007); 11: Lotan et al. (1992); 12: Cargo (1971); 13: Calder (1973); 14: Schiariti et al. (2008); 15: Kawahara et al. (2006); 16: Calder (1982); 17: Sugiura (1966); 18: Kikinger (1992); 19: Prieto et al. (2010); 20: Sugiura (1963); 21: Sugiura (1964); 22: Sugiura (1965); 23: Rippingale and Kelly (1995); 24: Gohar and Eisawy (1960a); 25: Gohar and Eisawy (1960b); 26: Bigelow (1900)

Table 4: Polyp and ephyra morphology of Rhizostomida species. *rc*: rhopalial canal, *vc*: velar canal, *: brooding species. Source: 1: Pitt (2000); 2: Straehler-pohl (2009); 3: Straehler-Pohl and Jarms (2010); 4: Kienberger et al. (2018); 5: Fuentes et al. (2011); 6: Purcell et al. (2012); 7: Schiariti et al. (2014); 8: Holst et al. (2007), 9: Holst and Jarms (2007); 10: You et al. (2007); 11: Lotan et al. (1992); 12: Cargo (1971); 13: Calder (1973); 14: Schiariti et al. (2008); 15: Kawahara et al. (2006); 16: Calder (1982); 17: Sugiura (1966); 18: Kikinger (1992); 19: Prieto et al. (2010); 20: Sugiura (1963); 21: Sugiura (1964); 22: Sugiura (1965); 23: Rippingale and Kelly (1995); 24: Gohar and Eisawy (1960a); 25: Gohar and Eisawy (1960b); 26: Bigelow (1900)

Species	Culture conditions (°C)	Polyp					Ephyrae						Source
		Polyp size range (mm)	MDD (mm)	Nb. of tentacles	Scyphistome hypostome	Asexual reproduction	Strobilation (ephyrae per strobilation /strobilation type)	Ephyrae size after release (mm)	Nb. of marginal lappets	Shape of rhopalial lappet	Shape of velar canal / rhopalar canal	Colour of ephyrae	
Catostylus tagi	15 - 25	1.08-1.83	0.44 - 0.86	18	Long, club-shaped	Podocysts	1, monodisk (rare) Up to 15, polydisk	1.5 - 3.1	8	Antler palm-like, with 2 to 7 finger-like appendages	Rhombical / slightly forked, rounded points	Dark pink to dark red, red statocysts	Present study
*Catostylus mosaicus**	15 - 25	1.57-1.90	0.69-0.81	12-20	Long, club-shaped	Lateral polyp buds, podocysts, pedalocysts, longitudinal fission	1, monodisk 2-5, polydisk	1.9 – 2.26	8	Antler palm-like, with 3 to 5 finger-like appendages	Spade-like *vc* / slightly forked *rc*	na	1, 2, 3
*Rhizotoma luteun**	17-17.5	1.34-2.5	1.02, mean	14-16	Conspicuous and flexible in all stage	Podocysts	1, monodisk	3.41-4.52	Typical 8, 11	Bread knife shaped	Rhombical *vc* / slightly forked *rc*	Light yellow to light brown	4
Rhizostoma pulmo	5-28	0.96-2.15	0.53-1.16	14-16	Long, club-shape and flexible	Lateral polyp buds, podocysts, lateral buds, stolonial polyp buds, pedalocysts	8-13.5, polydisk, oligodisk	2.28-3.93	Typical 8, 5-9	Spade like to lancet shaped	Rhombical or absent *vc* / slightly forked *rc*	Milk transparent to opaque white	2, 3, 5, 6, 7
Rhizostoma octopus	5-20	1.9-2.3	1.25	16-24	Long and flexible	Podocysts,, lateral buds, longitudinal fission	1, monodisk Up to 15, polydisk	2 7-5.96	8	Bread knife-like	flat rhombical *vc* / slightly forked *rc*	Milky transparent, light yellow to light brown	2, 8, 9

Table 4 continued

Species	Culture conditions (°C)	Polyp					Ephyrae						Source
		Polyp size range (mm)	MDD (mm)	Nb. of tentacles	Scyphistome hypostome	Asexual reproduction	Strobilation (ephyrae per strobilation /strobilation type)	Ephyrae size after release (mm)	Nb. of marginal lappets	Shape of rhopalial lappet	Shape of velar canal / rhopalar canal	Colour of ephyrae	
Rhopilema esculentum	5-22	1.00-3.50	1.6	16	Moderately long	Podocysts	7-17, polydisk	1.5-4.0	8	Talon-shaped with 4-6 branches ; Hand saped with 4-6 finger-like appendages	arrow tip-like *vc* / spatula-like *rc*	Milky to transparent	2, 3, 10
Rhopilema nomadica	20	1.8-2.0	na	16	Large clavate shape, third of polyp length	Podocysts	5-6, polydisk	1.5-2.0	8	Single or twin-typed, lancet-shaped	Convex with arched corners	na	11
*Rhopilema verrilli**	9-20	2.5	0.35	8- 20	Large, flexible, quadrate, irregular in outline	Podocysts, pedalocysts	1, monodisk Up to 3, polydisk	3.0	8	Rounded, slender, pointed distally	Rhopalar pouches with prominent «horns»	Peach, orange-red to rose coloured ; birefringent, bright yellow gold statocysts	12, 13
Lychnorhiza lucerna	16-22	1.5	0.55-0.8	18-22	Prominent dome-shaped	Podocysts	3, polydisk	1.4	8	Hand shaped with 2 to 9 tips	na / square-shaped ends with slight lateral horns *rc*	Translucent	14
Nemopilema nomadica	13-23	2.6	0.8-1.1	16	Dome-shaped, one third of scyphistome height	Podocysts	3-7, polydisk	2.2-3.8	8	Hand shaped with 2 to 6 pointed tips	Unforked triangular *vc* / unforked, spatula shaped *rc*	Translucent	15

Table 4 continued

Species	Culture conditions (°C)	Polyp					Ephyrae						Source
		Polyp size range (mm)	MDD (mm)	Nb. of tentacles	Scyphistome hypostome	Asexual reproduction	Strobilation (ephyrae per strobilation /strobilation type)	Ephyrae size after release (mm)	Nb. of marginal lappets	Shape of rhopalial lappet	Shape of velar canal / rhopalar canal	Colour of ephyrae	
Stomolophus meleagris	20-27	2.0	na	16	Large, flexible, and dome-or knob-shaped	Podocysts	1, monodisk 2-3, polydisk	1.5-2.0	8	Slender, distally pointed	Adradial bulges *vc* / blunt-ended *rc*	Pale straw coloured	16
*Cephea cephea**	20-30	1.4-2.9	0.44-0.6	14-17	Short, club-shaped	Lateral budding, swimming buds	1, monodisk	1.6-3.24	8	Round spoon shaped	Rhombical *vc* / slightly forked *rc*	Pale yellow to yellowish brown	2, 3, 17
*Cotylorhiza tuberculata**	16-25	3.23-5.0	0.82-0.86	16-17	Short, cylindrical	Lateral budding, swimming buds	1, monodisk	1.5-3.25	8	Rounded to rounded spoon shaped	spade-like to slightly rhombical *vc* / slightly forked *rc*	Transparent with yellow hemmed gastric system	2, 3, 18 , 19
*Mastigias papua**	15-25	1.0-10.22	0.4-0.92	15-18	Very short,cylindrical	Planuloids	1, monodisk	1.5-3.91	8	Rounded; Trapered, broad spoon shaped	spade-like to slightly rhombical *vc* / slightly forked *rc*	Brown to orange brown	2, 3, 20, 21, 22
*Phyllorhiza punctata**	25	na	na	16	na	Ciliated buds	1, monodisk	0.46-2.5	8	Pointed spoon shaped	spade-like to slightly rhombical *vc* / slightly forked *rc*	Yellowish brown to ochre	3, 23
*Cassiopea andromeda**	19-25	4.72-10.0	1.95	32	Conspicuous long, tetragonal	Swimming buds, planuloids	1, monodisk	3.69-3.95	12-23	Spatula-like	tongue-like and reach the tips of the velar lappets, tips are rounded *vc* / long forked *rc*	Yellowish green	2, 3, 24, 25
Cassiopea xamachana	na	na	1.5-2.0	Up to 42	na	Planula-like larvae	1, monodisk	na	na	na	na	na	26

Figure 1

Measuring points and measurements defined and taken in a polyp (A) and newly released ephyra (B).

TBL: total body length, CL: calyx length, HL: hypostome length, MDD: mouth disc diameter, StL: stalk length, TBD: total body diameter, CDD: central disc diameter, TMLL: total marginal lappet length, LStL: lappet stem length, RLL: rhopalial lappet length.

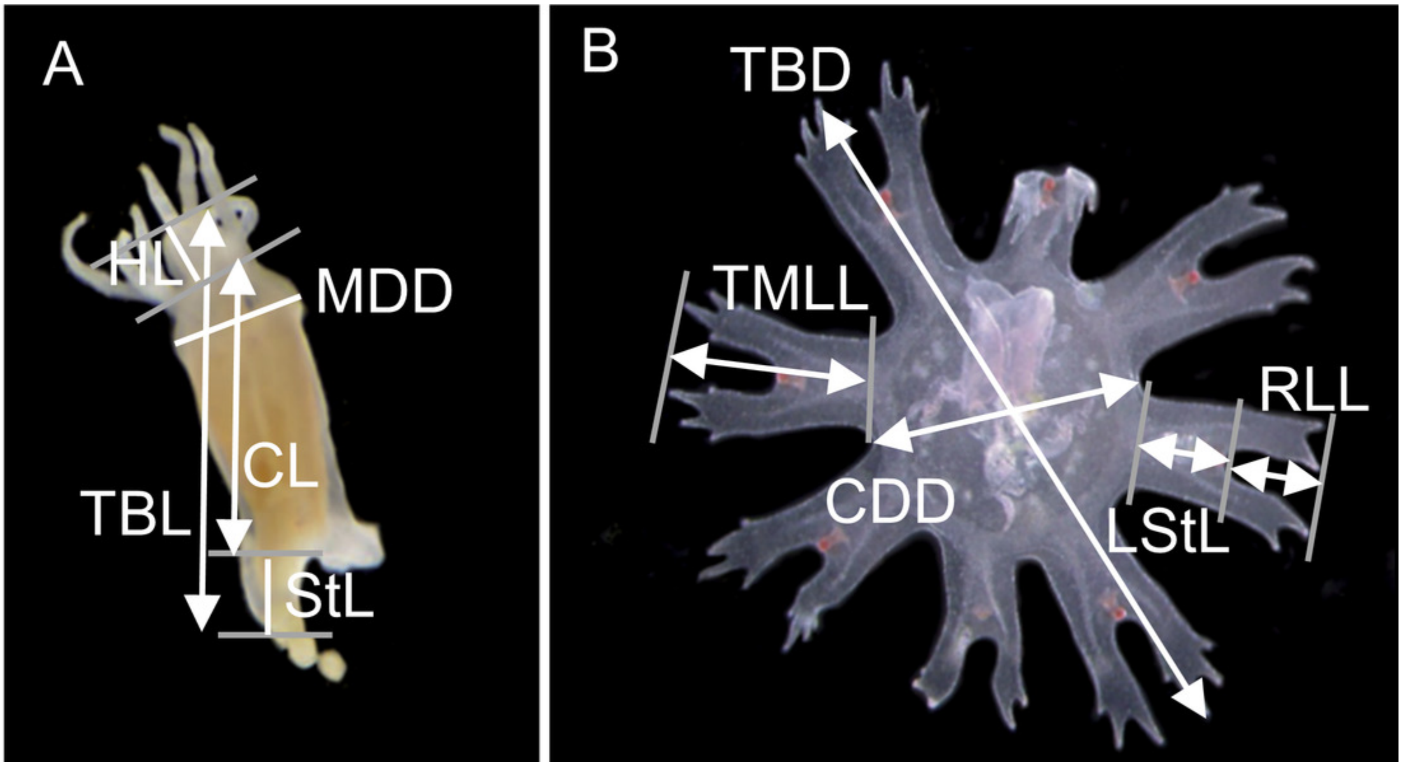


Figure 2

Enlargement of lappet and rhopalium of *Catostylus tagi*.

rh: rhopalium, *rc*: rhopalar canal; *rl*: rhopaliar lappets (Photo credit Sonia KM Gueroun)

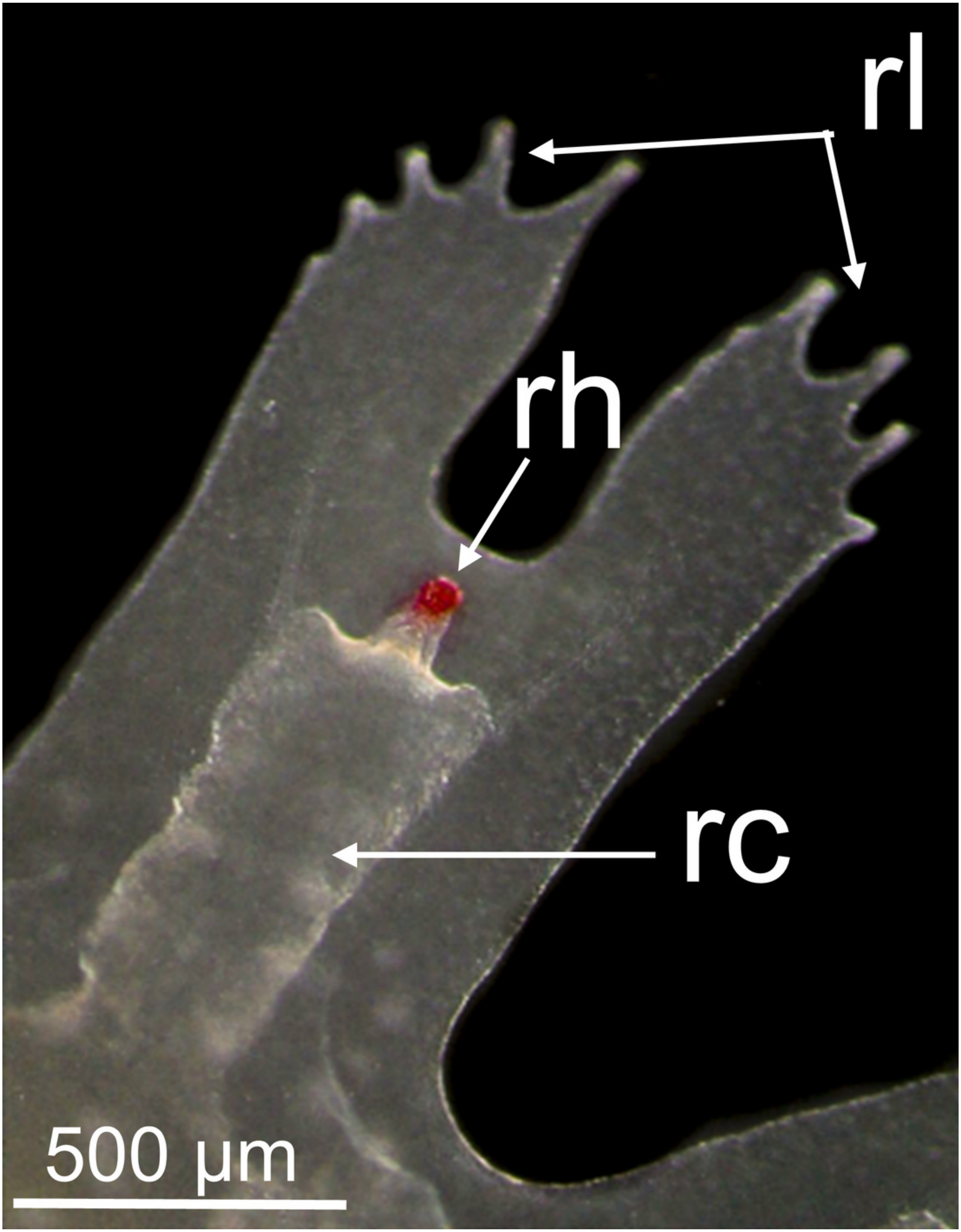

rl
rh
rc
500 µm

Figure 3

Enlargement of the mouth development of *Catostylus tagi.*

A Cross-shaped mouth without oral tentacles of a stage 0 ephyrae. **B** Appearance of tiny oral tentacles at the lips mouth in (stage 1). **C** Oral lips distally divided to eight oral arms (stage 2). **D** two oral arms in stage 6. *oa*: oral arm; *ot*; oral tentacles. (Photo credit Sonia KM Gueroun)

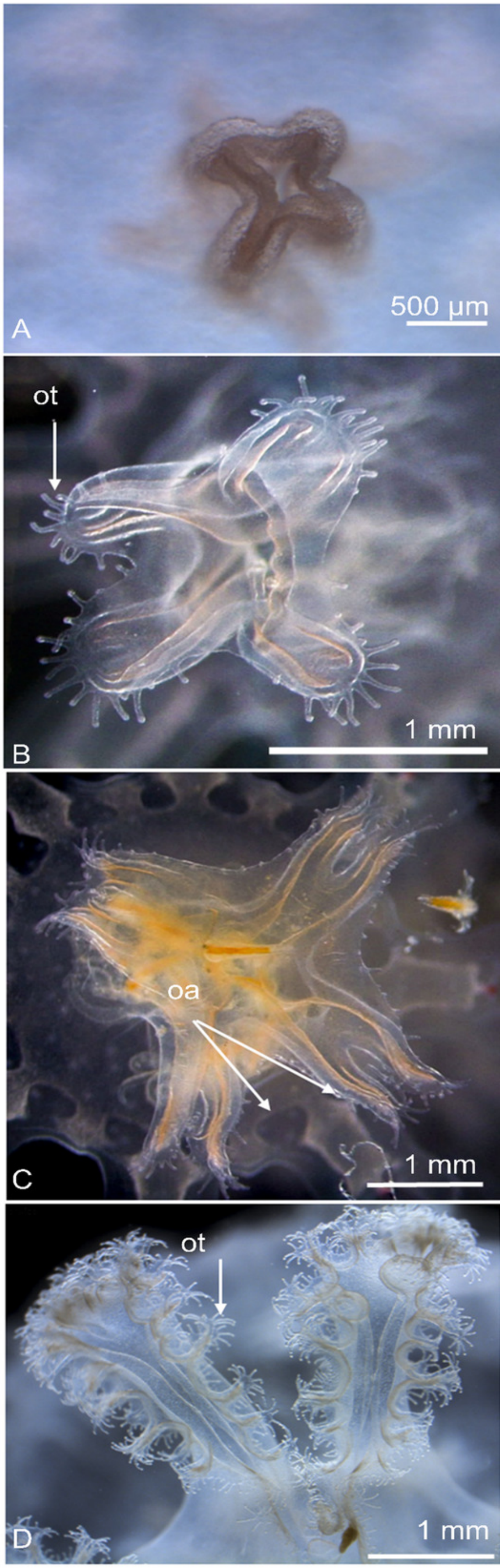

500 µm
A
ot
1 mm
B
oa
1 mm
C
ot
1 mm
D

Figure 4

Photography of a fully developed *Catostylus tagi* medusae reared in the Lisbon Oceanário (Photo credit Raul Gouveia)

Figure 5

Catostylus tagi planula survival and development (percentage) in different temperature and salinity regimes.

Stage 0: fixed; Stage 1: 1-3 tentacles; Stage 2: 5-7 tentacles; Stage 3: 8-15 tentacles; Stage 4: 18 tentacles

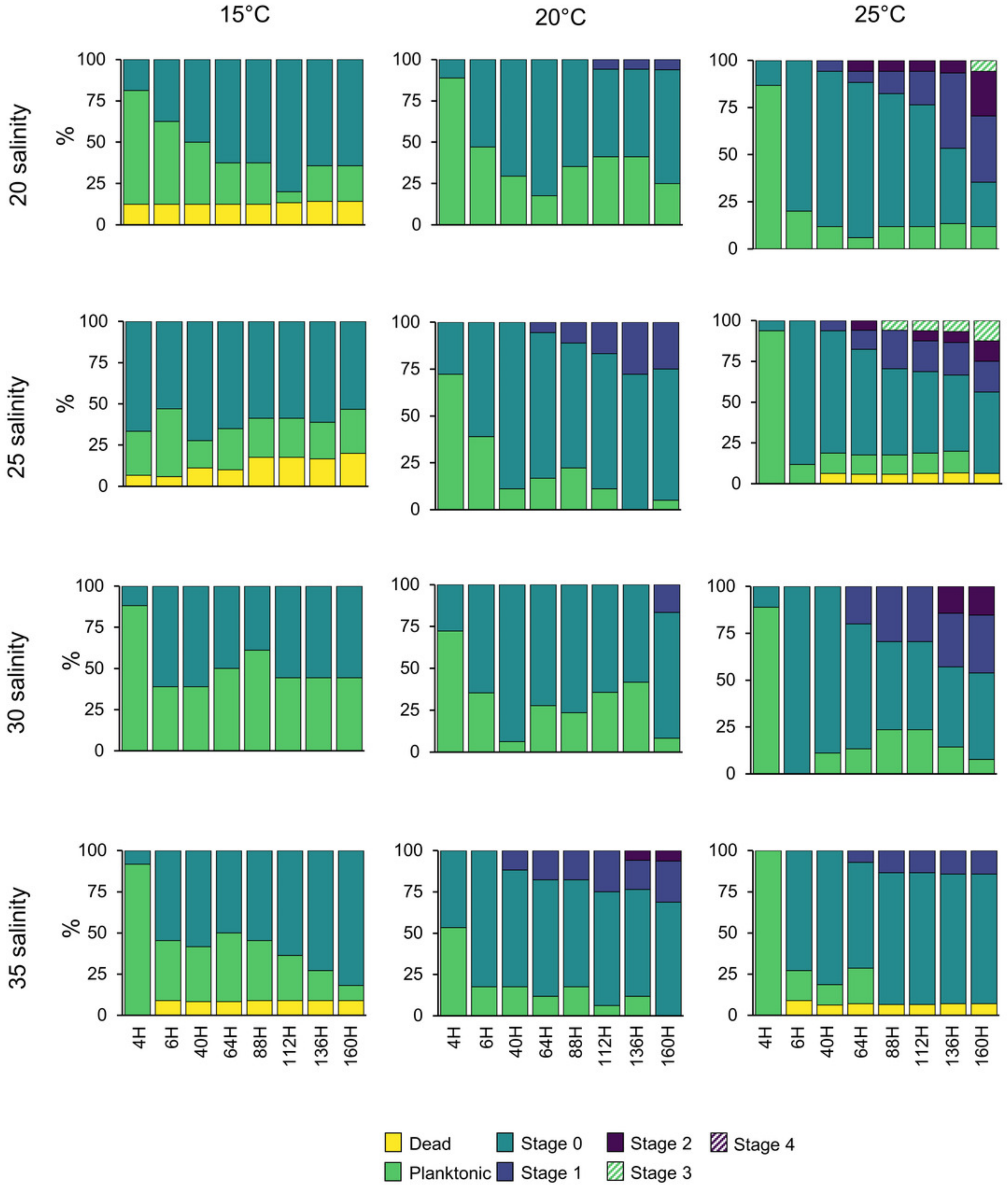

15°C
20°C
25°C
20 salinity
25 salinity
30 salinity
35 salinity
%
0
25
50
75
100
4H
6H
40H
64H
88H
112H
136H
160H
Dead
Planktonic
Stage 0
Stage 1
Stage 2
Stage 3
Stage 4

Figure 6

Planktonic duration of *Catostylus tagi* planula exposed to different temperature and salinity conditions

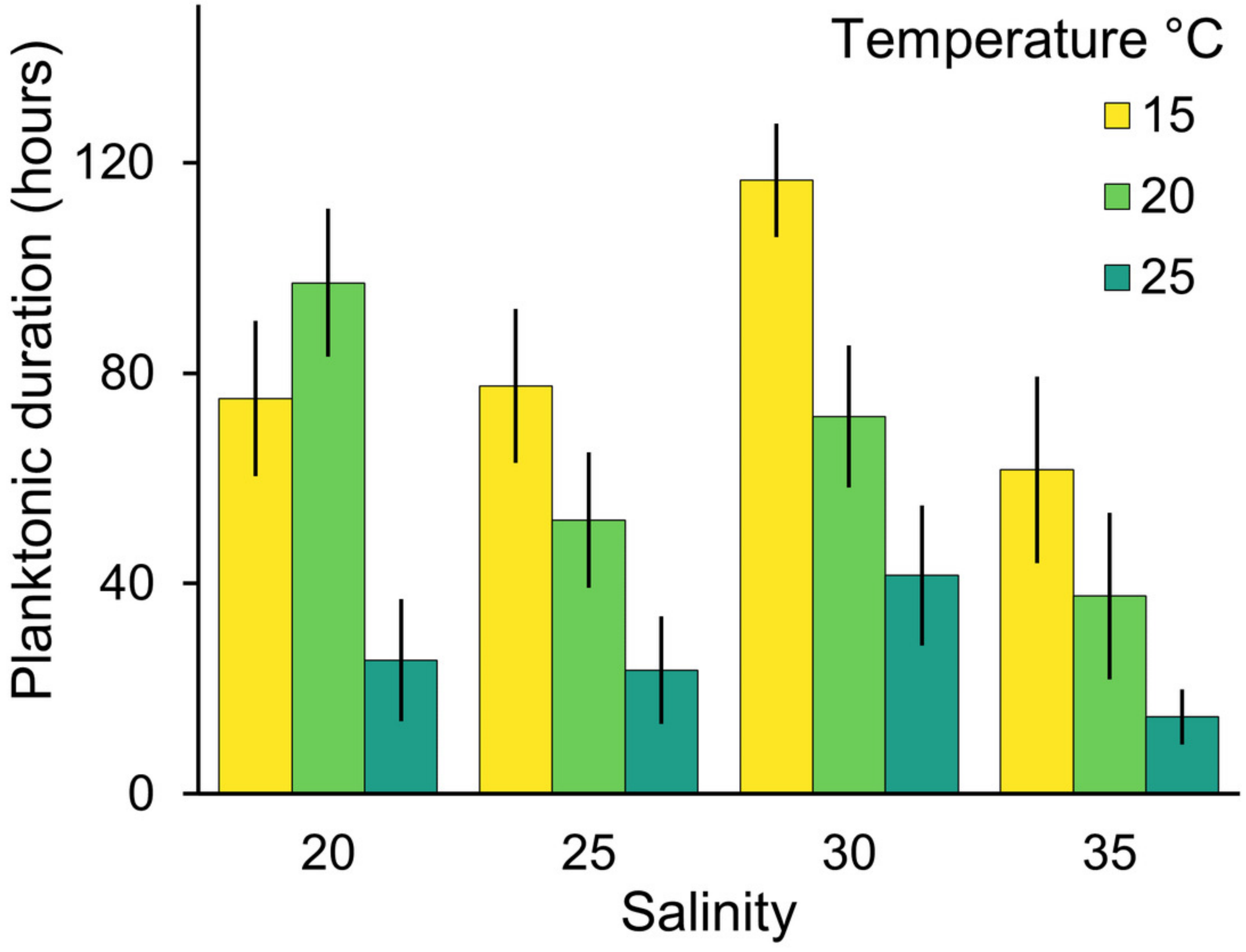


Figure 7

C. tagi asexual reproduction under temperature and diet regimes.

A. Podocyst production.scyphostome^{-1}; **B.** *Pre-str*; **C.** *bet-str*; **D.** *str*; **E.** Ephyra.strobilation^{-1}

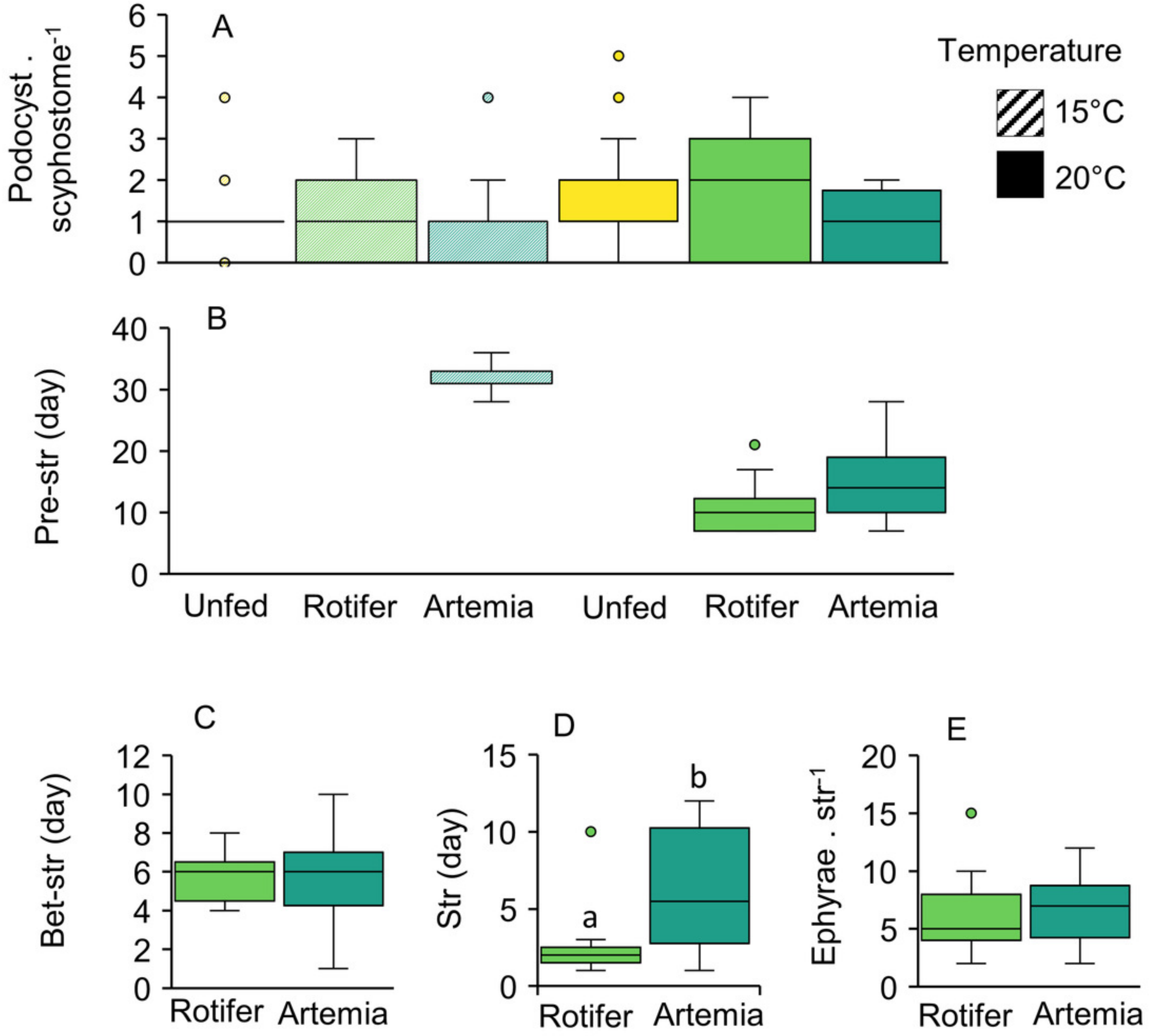


Figure 8

Spatial (A) and seasonal (B) occurrence of adult *Catostylus tagi* along the Tagus estuary in 2019 (GelAvista database)