Ecological aspects of early life stages of *Cotylorhiza tuberculata* (Scyphozoa: Rhizostomae) affecting its pelagic population success

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Abstract

Cotylorhiza tuberculata is a common symbiotic scyphozoan in the Mediterranean Sea. The medusae occur in extremely high abundances in enclosed coastal areas in the Mediterranean Sea. Previous laboratory experiments identified thermal control on its early life stages as the driver of medusa blooms. In the present study, new ecological aspects were tested in laboratory experiments that support the pelagic population success of this zooxanthellate jellyfish. We hypothesized that planulae larvae would have no settlement preference among substrates and that temperature would affect ephyra development, ingestion rates and daily ration. The polyp budding rate and the onset of symbiosis with zooxanthellae also were investigated. Transmission electron microscopy revealed that zooxanthella infection occurred by the polyp stage. Our results showing no substrate selectivity by planulae and high polyp budding rates in high temperatures suggest increased benthic polyp populations, which would lead to higher medusa abundances. Rates of transition from ephyrae to medusae and the feeding of early medusa stages also increased with temperature. Continuing changes in coastal ecosystems such as future climate warming and marine construction may lead to increased populations of jellyfish to the detriment of fish globally.

Introduction

The worldwide proliferation of marine jellyfish has become a crucial ecological and social issue in recent decades. Most jellyfish compete with fish for food resources and are potential predators of fish eggs and larvae (Möller, 1980). Some gelatinous species appear to be responsible for abrupt changes in the species abundance and composition of zooplankton, ichthyoplankton, and/or fish (Vinogradov & Shushkina, 1992; Pérez-Ruzafa et al., 2002; Richardson et al., 2009). Mass-occurrences of jellyfish are numerous (Hamner & Dawson, 2009) and increasingly interfere with economic and recreational activities. Jellyfish have been reported to clog fishing nets, spoil commercial catches, cause serious damage to aquaculture, clog the cooling systems of coastal power plants, and sting or even kill tourist swimmers (Arai, 1997; Mills, 2001; Uye & Ueta, 2004; Hay, 2006; Purcell et al., 2007).

Concern about gelatinous outbreaks has resulted in extensive recent scientific interest (Mills, 2001; Shiganova et al., 2001; Purcell, 2005; Purcell et al., 2007; Pitt & Purcell, 2009; Richardson et al., 2009). Several factors have been proposed to explain their occurrence including eutrophication (Arai, 2001), an increase in hard substrates for polyp attachment (Parsons & Lalli, 2002; Holst & Jarms, 2007), exotic translocations (Purcell et al., 2001), over-fishing (Pauly et al., 2002), and climate change (Purcell, 2005). The underlying causes of blooms are difficult to determine because the processes involved are not mutually exclusive and the conclusions may depend on the focus of the study (i.e., global or local-scale; Gibbons & Richardson, 2009).

Cotylorhiza tuberculata (Macri, 1778) is a common symbiotic rhizostome scyphozoan from the Mediterranean Sea. The medusae reach very high abundances in enclosed areas such as Vlyho Bay in the Ionian Island of Lefkada-Greece (Kikinger,

70 1992) and the Mar Menor coastal lagoon in the western Mediterranean Sea where
71 annual blooms have been observed since 1995 (Pérez-Ruzafa et al., 2002). Kikinger
72 (1992) described the life history of the population of *C. tuberculata* from Lefkada
73 Island. Prieto et al. (2010) parameterized the life cycle of *C. tuberculata* from the Mar
74 Menor within the context of global warming and highlighted thermal control as the
75 mechanism driving medusa blooms; low winter temperatures, which reduced polyp
76 survival, and abrupt warming, which triggered strobilation in springtime, determined the
77 abundance of medusae in summer. Thus, milder winters and hotter summers, as
78 predicted by future climate scenarios, may increase blooms of this jellyfish (Prieto et
79 al., 2010).
80 The life cycle of *C. tuberculata*, as in most scyphozoans, includes a benthic
81 asexual phase and a sexually dimorphic pelagic phase. The free-swimming planulae
82 liberated after internal fertilization is a relatively fast and resistant larval stage (Prieto et
83 al., 2010) that ends when it reaches a suitable surface for attachment and develops into a
84 polyp. The natural aggregating tendency of settling planulae (Kikinger, 1992) and the
85 asexual reproduction by lateral budding of the resulting polyps can lead to formation of
86 colonies with hundreds of individuals (Kikinger, 1992; Prieto et al., 2010). Polyps
87 produce a single bud at a time and do not reproduce asexually by podocyst formation.
88 Ephyrae originate from polyps after environmental changes trigger monodisc
89 strobilation. Budding and strobilation processes do not occur simultaneously in this
90 species (Astorga et al., unpublished data) and the rate of re-strobilation is minimal,
91 resulting in only one ephyra per polyp per year (Prieto et al., 2010).
92 One hypothesis proposed for increasing jellyfish outbreaks is increased artificial
93 hard substrates for polyp attachment in coastal areas (Parsons & Lalli, 2002). The
94 assessment of the settlement preferences of planulae may help to determine if this

95 explanation applies to *C. tuberculata* in the Mar Menor. The onset of blooms in the
96 lagoon came after a shift in benthic vegetation with an increase in *Cymodocea nodosa*
97 (Ucria) Ascherson during the 1980s (Pérez-Ruzafa et al., 2002) after the enlargement of
98 the El Estacio channel (Pérez-Ruzafa & Marcos, 1992). If planulae have higher
99 settlement and survival rates on live seagrass blades, the rise of jellyfish blooms in the
100 lagoon may be related to an increase in these natural attachment sites.
101 The presence of symbiotic dinoflagellates could also be required for polyp
102 strobilation in symbiotic scyphomedusae (Table 1). The absence of strobilation in
103 aposymbiotic polyps (Kikinger, 1992) suggests that zooxanthellae have a crucial role in
104 the transition between the benthic and pelagic phases in *C. tuberculata*. Given that one
105 polyp results in one ephyra, the proliferation capacity of this species depends directly on
106 the strobilation success of the polyp population. Therefore, the onset of zooxanthellae
107 infection could be of great importance in determining the success of the pelagic
108 population of this species.
109 Temperature regulation was found to be the physical mechanism controlling
110 polyp survival and strobilation in *C. tuberculata* (Prieto et al., 2010); however, effects
111 on ephyra development and the consequences for medusa population success were not
112 investigated. Ephyra, metaephyra, and small medusa correspond to the sequence of
113 stages during growth of the pelagic phase of scyphozoans. These stages are
114 distinguishable by the development of the central disc with respect to the total body
115 diameter, the degree of maturation of the oral system and shaping up of the umbrella
116 (Kikinger, 1992; Prieto et al., 2010; Straehler-Pohl & Jarms, 2010). The influence of
117 temperature on growth and ingestion during these three early medusa stages is
118 unknown.

In this study, we hypothesized that *C. tuberculata* planulae larvae would have no settlement preference among substrates and that temperature would affect ephyra development, ingestion rates and daily ration. Additionally, the polyp population increase by budding was investigated and transmission electron microscopy on planulae and polyps was used to identify the life phase at which zooxanthellae infection occurred. All these ecological aspects provided insights into the factors controlling the pelagic population success of this zooxanthellate jellyfish.

Materials and Methods

Benthic stage

Cotylorhiza tuberculata planula spatial and substrate settlement preferences, the suitability of *Cymodocea nodosa* as polyp attachment surface, and the budding capacity of polyps were analysed. We tested the null hypothesis that *C. tuberculata* planulae had no preference among substrates. First, planula larvae were removed from gravid female medusae collected in the Mar Menor coastal lagoon in late September 2006 (Experiment 1) and October 2010 (Experiment 2). Planulae were gathered in a container filled with unfiltered seawater from their natural habitat (temperature: 20ºC and 22ºC, salinity: 47 and 39, for Experiments 1 and 2, respectively). Replicates of 150 ml of mixed planula-rich seawater were allocated to different cylindrical glass flasks of 6.5-cm diameter with a 5-cm water column (approximately 120 and 170 planulae per flask in Experiments 1 and 2, respectively). In addition to the container surfaces, a glass slide of 7.5 x 2.5 cm was placed diagonally to enable inverted settlement in six replicates to test for spatial preferences for planula settlement (Experiment 1).

144 A glass slide (7.5 x 2.5 cm), a small stone (~1.8 x 1.3 x 0.5 cm), and one piece
145 each of brick (~1.7 x 1.4 x 0.5 cm), wood (~0.5 x 6 cm), and shell (half of empty shell
146 of *Pholas dactylus* Linnaeus: 1 x 2 cm) were offered to planulae as hard substrates to
147 determine their substrate preferences (Experiment 2). The glass slide and the wood stick
148 were placed diagonally and other substrates were placed on the bottom in each of three
149 flasks. The inside of the shell faced down and enabled free movement of planulae on all
150 surfaces. When no planulae remained in the experimental flasks (polyp numbers
151 corresponding to ~40% of the introduced planulae; Prieto et al., 2010), the different
152 substrates were transferred individually to new containers and the number of polyps
153 attached on all exposed surfaces counted with the aid of a stereomicroscope. The polyp
154 abundance was standardized by area of exposed substrate surface.
155 The ability of polyps to settle on *Cymodocea nodosa* leaves was tested in
156 Experiment 3. Two freshly-collected plants that each included a horizontal rhizome and
157 four ramets were placed in a glass aquarium filled with 3.2 l of filtered seawater (base:
158 14 x 17 cm, water column height: 13 cm, salinity: 38) with two Petri dishes (diameter: 9
159 cm, height: 1.3 cm) each holding one rhizomes and roots on the bottom and keeping the
160 plants in a natural upward position. The plants had a total of 22 leaves (mean leaf
161 dimensions: 12 x 0.3 cm). 379 detached polyps and 69 swimming buds were introduced
162 in the aquarium right below the water surface. Four days later the number of polyps
163 attached to *C. nodosa* leaves and other available substrates were counted visually to
164 avoid polyp detachment by manipulation. Polyp abundance per unit of available
165 substrate surface was calculated.
166 Asexual reproduction of polyps by budding was evaluated in the laboratory. The
167 polyp culture was maintained at a constant temperature of 17.5ºC, salinity 38, with a
168 photoperiod of 12:12, which ensured asexual reproduction only by budding (Prieto et

al., 2010). An IBERCER F-4 incubator provided a light intensity of 360 µmol quanta m^{-2} s^{-1} by means of four Philips master TL-D 18W/840 fluorescence lamps. Polyps were kept without aeration in 6.5-cm-diameter cylindrical glass flasks with 150 ml of 45-µm-filtered seawater that contained a glass slide leaning diagonally bottom-up. Rotifers (~400 l^{-1}) were provided as prey once per week. One hour after feeding, the rearing medium was exchanged with new, aerated water. Four replicates, each with 22 polyps, were monitored with the aid of a stereomicroscope at intervals of 2-4 days for two weeks to determine the daily budding rate of polyps (Experiment 4).

Onset of zooxanthella infection

To determine the onset of zooxanthella infection during ontogeny, planulae were carefully extracted from the oral arm grooves and the brood-carrying filaments of gravid females. Additional samples of planulae naturally liberated in the medusa collection container were taken for comparison (control group). The three different planulae sets obtained (oral arms, brood-carrying filaments, and control planulae) were treated separately for their study by means of transmission electron microscopy (TEM).

Freshly collected planulae were carefully washed with sterile filtered seawater and transported to the laboratory in sterile seawater flasks. Before providing any food items, the planulae and resulting polyps were transferred by 1.5-ml Eppendorf pipettes and fixed in 2.5% glutaraldehyde in 0.1-M sodium cacodylate for 1 h at ambient temperature. Following three 10-min rinses in 0.1-M sodium cacodylate, samples were post-fixed for 1 h in 1% osmium tetroxide in 0.1-M sodium cacodylate and rinsed three more times in 0.1-M sodium cacodylate. After a sequential dehydration of 15 min in 30% and 50% ethanol, samples were left overnight in 70% ethanol. Dehydration was

194 completed through 90% and 100% (x3) ethanol. After transfer to propylene oxide,
195 samples were gradually embedded in Spurr's epoxy resin. After 48-h-polymerisation at
196 55ºC, thin sections of the resulting capsules were cut by an ultramicrotome, mounted on
197 copper grids, stained with lead acetate, and viewed on a JEOL transmission electron
198 microscope. Five planulae and three polyps per set were analysed.
199
200 Growth of early medusa stages
201
202 Laboratory experiments were conducted in order to establish the growth of ephyrae over
203 the range of temperatures typical of the Mar Menor (Pérez Ruzafa et al., 2002), with
204 20ºC as the temperature of ephyra liberation (Prieto et al., 2010). First, early medusa
205 growth was studied at a constant temperature of 20ºC, salinity 38, and photoperiod of
206 12:12 (normal culture conditions). For one month after the day of liberation from the
207 strobila, groups of 10 ephyrae were introduced into cylindrical glass flasks with 150 ml
208 of 45-µm-filtered seawater without aeration and fed daily with newly-hatched *Artemia*
209 nauplii at ~220 prey l^{-1}. The ephyrae were transferred daily to new containers with
210 aerated water and fresh prey. Once the metaephyra stage was achieved, the number of
211 animals per flask was reduced to three and the prey items changed to Selco-enriched
212 *Artemia* nauplii (220 prey l^{-1}). To establish the allometric relationships in early medusa
213 stages, 100 ephyrae, 20 metaephyrae, and 10 medusae were removed from their rearing
214 containers and measured with the aid of a stereomicroscope. Additionally, 23 of these
215 specimens (11 ephyrae, 6 metaephyrae, and 6 medusae) were individually put onto pre-
216 combusted, pre-weighed glass-fiber filters, dried at 60ºC to constant weight, weighed,
217 then ash-free dried at 450ºC for 1 day and re-weighed. The correlations of diameter, dry
218 weight (DW), and ash-free dry weight (AFDW) with age were determined.

Temperature effects on ephyra growth rates also were tested (Experiment 5). We tested the null hypothesis that ephyra development was unaffected by different temperatures. Fifteen ephyrae individually identified by micro-photograph were randomly assigned by groups of five specimens to growth temperatures of 20, 25, and 30ºC. Each individual was maintained in a 3.3-cm diameter cylindrical glass flask with 40 ml of 45-µm-filtered seawater (salinity: 38, water column height: 4.3 cm) without aeration. Photoperiod was kept at 12:12 and ephyrae were fed daily with *Artemia* at ~220 nauplii l^{-1}. Every day, before adding fresh prey, the rearing medium was exchanged with new, aerated water. Once per week for four weeks, every ephyra was removed, allowed to relax, photographed under the stereomicroscope, and returned to the rearing medium. The mean diameter between opposite lappet tips (usually 8 measurements per ephyra) was measured from the photographs. At the end of the experiment, ephyrae were put individually in pre-combusted and pre-weighed glass-fiber filters and their DW and AFDW measured as above.

Ingestion by early medusa stages

The ingestion rate and daily ration of early medusa stages were studied at 20, 25, and 30ºC (Experiment 6). We tested the null hypothesis that ingestion rates of *Artemia* nauplii by ephyrae were unaffected by different temperatures. Cylindrical glass flasks (3.3-cm diameter) filled with 40 ml of 45-µm-filtered seawater (salinity: 38, water column height: 4.3 cm) were used as incubation containers providing recently hatched *Artemia* nauplii at a concentration of 400 prey l^{-1} without aeration. Twenty seven ephyrae (mean: 3.6 ± 0.4 mm) previously unfed for 24 h were randomly placed in 3 temperature treatments (3 per flask, 3 flasks per treatment). In order to compare

between ephyrae and medusae in the same experiment, six unfed medusae (mean: 7.1 ± 0.4 mm) were individually placed in the experimental flasks and randomly distributed among 3 replicates in each of the lowest and intermediate temperature treatments. An additional container without predators served as a treatment prey control. After 6 hours of incubation, the remaining nauplii in each glass container were counted under a stereomicroscope. The respective ingestion rate and daily ration of ephyrae and small medusae were determined according to the equations in Båmstedt et al. (2001 and 1999, respectively). Specimens were measured from digital images before the experiment to ensure size homogeneity.

Statistical Analyses

Statistical analyses of data were performed using SPSS Statistical Software. Assumptions of analysis of variance (ANOVA) were tested on data sets before statistical testing. If data failed normality and equality of variances and homogeneity could not be met by transformations, non-parametric Kruskal-Wallis analysis of variance was applied. When looking for between-subjects and within-subjects effects as in the case of subjects measured over time, repeated ANOVA tests were applied once sphericity and homogeneity of dependent variables were confirmed. If significant differences were found between treatments, multiple comparisons were made using the Tukey test or its non-parametric homologue.

Results

Benthic stage

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270 *Cotylorhiza tuberculata* planulae had attached and developed into polyps on the glass
271 slides and surfaces of the glass container in Experiment 1 after twenty days (Fig. 1a,
272 Table 2). Greater relative densities of polyps on the bottom of the flasks clearly
273 indicated their spatial settlement preference (χ^2_3=19.815, p<0.01). Planulae attached to
274 the underside of the water-air interface in relative densities similar to those on the glass
275 slides (upper and underside combined), but in significantly higher relative densities than
276 on the sides of the glass containers (Fig. 1a). Settlement on the lower and upper surfaces
277 of the glass slides was similar ($F_{1,10}$ = 1.429, p > 0.05).

278 *C. tuberculata* planulae also attached to all substrates provided in Experiment 2:
279 glass slide, brick, wood, stone, shell, and the surfaces of the glass container (Table 2,
280 Fig. 1b). Therefore, the null hypothesis tested was accepted as the relative densities did
281 not differ significantly among substrates (Fig. 1b) in spite of the apparent preferences
282 for wood, brick, shell, or the water-air interface versus the other surfaces (χ^2_7=14.021,
283 p>0.05). No settlement preferences were detected between organic and inorganic
284 substrates or natural and artificial substrates (Fig. 1b). As occurred in Experiment 1,
285 polyp settlement on the underside of glass slides was similar to that of the upper side
286 ($F_{1,4}$=0.571, p>0.05) and no polyps settled upside-down on the shells internal surface.
287 Polyps were observed after settlement at densities of up to 4 cm^{-2}, but densities were
288 reduced to 1.3 cm^{-2} within a month.

289 Experiment 3 showed that *Cymodocea nodosa* seagrasses were a suitable surface
290 for polyp attachment. Four days after introduction to the *C. nodosa* aquarium, polyps
291 attached along the upper and underside of the leaves at a density of 0.54 polyps cm^{-2}.
292 They also settled on the few exposed areas of the rhizomes at a density of 3.33 polyps
293 cm^{-2}. The *C. nodosa* plants sheltered an overall polyp density of 0.79 cm^{-2}. Polyp

densities on the other substrates available were: glass bottom (Petri dishes, 0.34 polyps cm^{-2} and aquarium, 0.13 polyps cm^{-2}) and sides of the aquarium (0.04 polyps cm^{-2}).

Polyps maintained at 17.5ºC and 12:12 photoperiod reproduced exponentially by budding during the first 13 days (Experiment 4, Fig.3) at a rate of 3.9 ± 1.27 % per day (polyp number = 22.17 $e^{0.04\ day}$, $R^2 = 0.94$).

Onset of zooxanthellae infection

The infection with zooxanthellae occurred early in the *C. tuberculata* life cycle (Fig. 3). Zooxanthellae were not observed directly on planulae (n = 15); however, TEM revealed the presence of algae inside the polyps from all sets of planulae (n = 9): (1) naturally liberated by gravid females and (2) extracted in aseptic conditions from medusa brood-carrying filaments and oral arm grooves.

Growth of early medusa stages

The diameter of *C. tuberculata* ephyrae ranged between 2.1 and 2.8 mm on the day of liberation from the strobila (n = 60, 2.46 ± 0.34). When maintained at a constant temperature of 20ºC, the diameter of early medusae (Φ) increased 0.08 mm per day (n = 130, $\Phi = 0.081day + 2.049$, $R^2 = 0.90$, Fig. 4). Linear correlations were found between individual size, DW, and AFDW. An increase of 1 mm in diameter represented ~213 µg DW and ~90 µg AFDW (n = 23; $DW = 212.850\Phi - 496.750$, $R_{DW}^2 = 0.82$; $AFDW = 89.677\Phi - 173.560$, $R_{AFDW}^2 = 0.67$).

Different rearing temperatures had no effect on ephyra growth in diameter increase; however, ephyrae grown at 20, 25, and 30ºC differed in the time to attain the

medusa stage (Experiment 5: temperature: $F_{2,12} = 3.075$, $p > 0.05$; week: $F_{3,36} = 15.629$, $p < 0.01$; interaction: $F_{6,36} = 7.863$, $p < 0.01$, Fig. 5). By day 21, none of the ephyrae reared at 20ºC had reached the metaephyra stage, but 80% of those incubated at 25ºC and 100% at 30ºC had already developed into medusae. Thus, the null hypothesis tested was rejected as ephyra development was affected by temperature.

Ingestion by early medusa stages

The ingestion rate and daily ration of early medusa stages depended on the incubation temperature, rejecting the null hypothesis tested (Experiment 6; Table 3). Ephyrae maintained at 20ºC ingested a similar amount of prey daily as those incubated at 30ºC, but significantly less than those at 25ºC. Ephyra daily ration varied among treatments with the highest DW digestion of prey at 25ºC.

Differences between the ingestion rates of ephyrae and medusae were highly significant, with medusae consuming more prey than ephyrae in all incubation temperatures (Table 3). Medusae as well as ephyrae ingested more prey at 25ºC than at 20 ºC. The small medusae in Experiment 6 were twice as wide as the ephyrae (2.0 ± 0.3) and consumed 2-3-times more *Artemia* nauplii daily (2.8 ± 1.2 medusa^{-1}); however, the respective daily rations were determined by the incubation temperature and were independent of stage. The AFDW-specific percentage ingested was higher at 25ºC than at 20ºC, but similar for ephyrae and medusae (Table 3).

Discussion

Benthic stage

344

345 The settlement preferences of planulae confirmed that these ciliated larvae represent a
346 highly versatile stage of *C. tuberculata* development (Prieto et al., 2010). Planulae
347 attached to all surfaces available, with a clear spatial preference for the bottom but no
348 preference between organic or inorganic substrates and/or natural or artificial substrates.
349 Settlement of planulae at the water-air interface also has been observed in other
350 scyphozoan species like *Lychnorhiza lucerna* Haeckel (Schiriati et al., 2008) and
351 *Aurelia aurita* Linnaeus (Holst & Jarms, 2007), and probably is an artifact related to the
352 motionless water within laboratory containers. Although Kroiher & Berking (1999)
353 suggested that planula settlement on the water surface is normal in natural conditions,
354 evidence is missing for *C. tuberculata* given that few polyps have been observed *in situ*
355 (only three polyps). A stable air-water interface is unlikely in almost all natural
356 environments.
357 Planula settlement on the water-air interface could reflect the preference of
358 scyphozan polyps to attach to the underside of surfaces (Pitt, 2000; Holst & Jarms,
359 2007). Although polyps of *Cyanea* sp. discriminated among different textures and
360 preferred rough substrates (Brewer, 1976), settlement did not differ significantly for the
361 upper and lower surfaces of diverse substrates, including shell, glass, and seagrass
362 leaves, for *C. tuberculata* planulae. Indeed, planulae were not particularly selective,
363 even though artificial surfaces are often preferred by scyphopolyps (Pitt, 2000; Holst &
364 Jarms, 2007).
365 The lack of substrate preference suggests that the increase in *Cymodocea nodosa*
366 was unlikely to be a factor responsible for the onset of blooms in the Mar Menor,
367 because similar surface areas of submerged macrophytes were present in the lagoon
368 before *C. nodosa* dominance (Pérez-Ruzafa et al., 1991). There is evidence that other

369 macrophyte species such as *Zostera marina* Linnaeus, *Zostera noltii* Horneman (Pérez-
370 Ruzafa et al., 1987; Calvín et al., 1999), and the green alga *Caulerpa prolifera*
371 (Forsskål) Lamouroux (Pérez-Ruzafa et al., 1991; Calvín et al., 1999) may be suitable
372 for colonization by *C. tuberculata* polyps. Two polyps on *Zostera* sp. were observed by
373 Kikinger in 1981 (Kikinger, 1992) and 2010 (personal communication) in Vlyhho Bay
374 and a third polyp was found attached to a *C. prolifera* leaf in Mar Menor (personal
375 observation). This is in contrast with the scyphozoan *Catostylus mosaicus* Quoy &
376 Gaimard, which avoided seagrasses when offered glass, shell, wood, or sandstone (Pitt,
377 2000).
378 Nevertheless, the suitability of *C. nodosa* as attachment surface for polyps is
379 important, given that without this seagrass, sandy and muddy sediments, which
380 predominate in the Mar Menor, do not allow successful settlement (Holst & Jarms,
381 2007). Rocky substrates in the lagoon are limited to the islands and El Estacio channel
382 and compact red clay sediments with *Pholas dactylus* shells and *C. prolifera* are
383 exclusively located on the central eastern shore (Pérez-Ruzafa et al., 1987, 2008). The
384 increase in total available artificial surface resulting from the growing anthropogenic
385 activities in the littoral zone is difficult to assess (24 km of the 58-km shoreline is
386 affected) (Pagès, 2001). Retaining walls, sport harbors, bypasses, artificial channels,
387 jetties, docks, and boats around the Mar Menor shore greatly increase the suitable
388 settling surface for planula and polyp attachment. Given the absence of substrate
389 selectivity and the high asexual reproduction by budding compared to other
390 scyphozoans (reviewed in Purcell et al., submitted), this extension of available surface
391 in the lagoon potentially could have increased the benthic population of *C. tuberculata*,
392 which combined with the appropriate environmental conditions (Prieto et al., 2010) may
393 have contributed to increase of medusa blooms (Parsons & Lalli, 2002; Holst & Jarms,

2007). Worldwide substrate additions by human modification of shorelines are considered to favor the sessile stages of scyphozoans (Purcell, 2012), and the Mediterranean coastline is a very antropogenic modified area (Halpern et al., 2008). Among all the Mediterranean sub-basins, *C. tuberculata* has been reported in the Catalan Sea (Fuentes et al., 2010), Ligurian Sea (Carli et al., 1991), Strait of Sicily (Daly et al., 2003), Adriatic Sea (Kogovsek et al., 2010), Ioanian Sea (Kikinger, 1992), Aegean Sea (Gülsahin & Tarkan, 2011) and in the Levantine Basin (Lakkis, 1991). This ubiquity of *C. tuberculata* joined with the plasticity to attach to any type of substrate allow to extrapolate the implications of the present study to the whole Mediterranean. Indeed, *C. tuberculata* is the most common rhizostomae in the Mediterranean Sea (Kikinger, 1992).

Onset of zooxanthellae infection

Among the six species of rhizostomae jellyfish occurring in the Mediterranean Sea, only three of them are symbiotic: *Cassiopeia andromeda* Eschscholtz, *C. tuberculata* and *Phyllorhiza punctata* von Lendelfeld. Of these three species, the symbiotic zooxanthellae are not always essential for the phase transition between the benthic and the pelagic stages (Table 1). *Cassiopeia andromeda* aposymbiotic polyps can strobilate and aposymbiotic ephyrae can be obtain (Rahat & Adar, 1980). The same occurred with *Phyllorhiza punctata* (Galil et al., 2009), a Pacific jellyfish observed in several regions in the Mediterranean recently (Cevik et al., 2011). The crucial role of zooxanthellae in medusa formation in *C. tuberculata* was discovered by Kikinger (1992) when he observed no strobilation in hundreds of laboratory aposymbiotic polyps during a two-year period. We believe that planulae are infected by *Symbiodinium* sp. while still

419 within the mother medusa, because the polyps we obtained in aseptic conditions had
420 zooxanthellae that could only have been transmitted previously.
421 Zooxanthellae generally are absent in the eggs and planulae in most symbiotic
422 scyphozoans (Table 1) and must be acquired from the environment during the
423 scyphistoma stage (Arai, 1997; Thornhill et al., 2006). In contrast, the coronate *Linuche*
424 *unguiculata* Swartz releases eggs in mucus strands containing zooxanthellae that infect
425 embryos and planulae in the 24 hours after fertilization (Montgomery & Kremer, 1995).
426 Algal infection at the planula stage is also known for other symbiotic marine
427 invertebrates, such as the octocoral *Xenia macrospiculata* Gohar (Achituv et al., 1992)
428 and the scleractinian coral *Fungia scutaria* Lamarck (Schwarz et al., 1999).
429 Infection of *C. tuberculata* planulae from the brood carrying filaments was
430 possible given the high zooxanthella content of the surrounding mucus (Kikinger,
431 1992). By contrast, planulae from the oral arm canals were expected not to harbor any
432 zooxanthellae (Kikinger, 1992); however, algal infection also could have occurred
433 during the embryonic development, given the abundance of zooxanthellae in the ovarial
434 mesoglea (Kikinger, 1992). The likelihood of zooxanthella infection at the planula stage
435 of *C. tuberculata* would decrease the possibility of aposymbiotic polyps in nature and
436 increase the strobilation success of the population in the appropriate environmental
437 conditions (Prieto et al., 2010). Nevertheless, further studies should be conducted to
438 find zooxanthellae in the embryos or planulae, as found in *Linuche unguiculata*
439 (Montgomery & Kremer, 1995).
440
441 Growth of early medusa stages
442

443 Field observations of a natural population of *C. tuberculata* from the Ionian Island of
444 Lefkada estimated 8 -10 weeks for newly-liberated ephyrae to reach 3 cm diameter at
445 temperatures above 24ºC (Kikinger, 1992). Live *C. tuberculata* ephyrae measured since
446 production at a constant temperature of 20ºC would need 2-3-times longer to attain that
447 size. Development of *Rhizostoma octopus* Macri ephyrae in laboratory cultures also was
448 very slow compared to natural growth (Holst et al., 2007). They thought the differences
449 were due to measurements made on living versus preserved specimens and/or to
450 disparities between natural and laboratory conditions. In fact, the youngest ephyra
451 stages in our study (2.1 - 2.8 mm in diameter) were larger than the preserved samples
452 previously described for Vlyho Bay by Kikinger (1.5 - 2 mm). Feeding of ephyrae in
453 small culture flasks often relies on *Artemia* sp. nauplii and omits any natural prey (Issel,
454 1922; Pérez-Ruzafa et al., 2002); the highest daily rations of *Aurelia aurita* ephyrae
455 occurred only when mixed zooplankton was available (Båmstedt, 1990), which would
456 promote rapid growth. A constant rearing temperature in the laboratory also may have
457 contributed to low ephyra growth rates. The beneficial consequences of natural
458 temperature fluctuations for ephyra growth are clearly shown for *Cyanea capillata*
459 Linnaeus in Gullmar Fjord (Gröndahl & Hernroth, 1987) and *A. aurita* in Tapong Bay
460 (Lo & Chen, 2008).
461 Growth of scyphomedusae is generally enhanced at higher temperatures when
462 food is not a limiting factor (Lucas, 2001; Widmer, 2005); however, the growth rates of
463 early medusa stages of *C. tuberculata* were similar at 20, 25, and 30ºC. Nevertheless,
464 the rate of transition between ephyra, metaephyra, and small medusa was strongly
465 controlled by temperature. The medusa stage was rapidly attained at the highest
466 temperature, resulting in small, completely developed medusae. Similar results were
467 obtained for *Aurelia aurita* juveniles; equal-sized individuals with no obvious

468 behavioural differences were classified as ephyrae or as medusae depending on their
469 rearing temperature (13 or 21ºC, respectively; Nawroth et al., 2010). This phenotypic
470 plasticity in ephyra development could be beneficial for scyphozoan species that
471 encounter large temperature fluctuations as those induced by climate and ocean
472 circulation changes (Nawroth et al., 2010). Mediterranean Sea water temperature
473 already has increased 0.67-0.89ºC from 1982 to 2006 (Belkin, 2009). Predicted
474 warming scenarios for the end of the 21st century are between 1.8ºC to 6ºC (best
475 estimates of temperature change at 2090-2099 relative to 1980-1999; IPCC, 2007). For
476 *C. tuberculata*, higher temperatures would enable faster medusa development, with the
477 potential to accelerate sexual maturity and spawning.
478
479 Ingestion by early medusa stages
480
481 The ingestion rate and daily ration of *C. tuberculata* ephyrae depended on the
482 incubation temperature as previously shown for the semaestome *Aurelia aurita*
483 (Båmstedt et al., 1999, 2001) and the hydrozoan *Moerisia lyonsi* Boulenger (Ma &
484 Purcell, 2005). *C. tuberculata* ephyrae consumed between 290 to 850% of their DW
485 daily at suitable growth temperatures (20-25ºC), which, as observed also for *Aurelia*
486 *aurita* (Båmstedt et al., 1999), implies that these ephyrae may be of significance
487 relevancy in decreasing dense patches of zooplankton prey in their natural environment.
488 Although the size reached by ephyrae after 21 days in culture at 30ºC was similar to
489 their sizes at 20 and 25ºC, high mortality occurred at 30ºC that could not be attributed to
490 prey limitation, given that prey concentrations corresponded to saturated feeding
491 conditions in similar experiments with *Aurelia aurita* ephyrae (Båmstedt et al., 1999,
492 2001) and never decreased below 100 Artemia sp. nauplii l^{-1}. Therefore, we conclude

493 that 30ºC is not appropriate for successful growth of *C. tuberculata* ephyrae. It is
494 unlikely that they experience 30ºC in the Mediterranean Sea during spring when
495 strobilation occurs and ephyrae are present (Prieto et al., 2010) because the temperature
496 range within the Mar Menor, much more confined and land thermal affected, is 16-
497 25ºC.

498 The ingestion rates and daily rations of small *C. tuberculata* medusae also
499 depended on their size. Larger specimens consumed more prey than ephyrae, but the
500 DW-specific daily ration of prey they ingested was relatively lower, suggesting an
501 increasing importance of zooxanthellae for the nutrition and development of the pelagic
502 stage. In fact, despite the large number of ingested prey predicted for *C. tuberculata*
503 medusae of 35 cm diameter, zooplankton feeding may not be adequate to support
504 medusa abundances of up to 0.9 individuals m^{-3} in the Mar Menor (Mas, 1999), <1
505 individuals m^{-3} in the Gulf of Tunis (Daly et al, 2003) and up to 4000 individuals in the
506 small Bay of Vlyho (Kikinger, 1992). Also, in the Aegean Sea was reported *C.*
507 *tuberculata* in large densities as 4-5 individuals m^{-2} in the Güllük Bay, 3 individuals m^{-2}
508 in the Gökova Bay and 2 individuals m^{-2} in the coast of Milas (Gülsahin & Tarkan,
509 2011). This suggests that zooxanthellae must have a substantial contribution to the
510 nutrition of this species (Pagès, 2001). Further studies are necessary to clarify the
511 importance of zooxanthellae symbiosis during the pelagic stage of *C. tuberculata*.

512 Planktivorous gelatinous species are known to be important consumers in both
513 low and high productivity marine ecosystems (Mills, 1995). *C. tuberculata* effectively
514 exert a strong top-down control on the food web by selective grazing on large diatoms,
515 ciliates, veliger larvae, and copepods (Pérez-Ruzafa et al., 2002). The diet overlap
516 between zooplanktivorous jellyfish and pelagic fish (Purcell & Sturdevant, 2001,
517 Hiromi et al., 2005; Brodeur et al., 2008), combined with predictions of increased

518 jellyfish populations (Lynam et al., 2005; Purcell, 2005; Attrill et al., 2007; Richardson
519 et al., 2009), suggests potential changes in future pelagic communities (e.g., Vinogradov
520 & Shushkina, 1992; Richardson et al., 2009) that may have detrimental consequences
521 for fisheries and economies worldwide.
522
523 In summary, part of the success of the *C. tuberculata* benthic phase may be due
524 to the lack of preference among substrates for planulae settlement and the high rate of
525 asexual reproduction by budding at mild winter temperatures (17.5ºC). These aspects
526 combined with the proliferation of artificial substrates and the recovery of seagrass beds
527 may increase both the availability of suitable surfaces for the development of polyps
528 and the viable benthic population, leading to a rise of medusa abundances. Moreover,
529 the proximity of zooxanthellae in mother medusae facilitates infection early in the
530 developmental of *C. tuberculata*. Hence zooxanthellae infection is unlikely to constitute
531 a limiting factor for the proliferation of this species. Finally, warmer temperatures
532 accelerated the transition from ephyrae to medusae, which occurred at a smaller size,
533 and increased their food ingestion. The high feeding rates measured in early medusa
534 stages at 25ºC suggest the potential for changes in the pelagic communities of coastal
535 anthropogenically-altered ecosystems, especially considering predicted warming
536 scenarios (IPCC 2007) that may benefit these jellyfish.
537
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778

778 **Figure Legends**

779

780 **Fig. 1** Settlement preferences of *Cotylorhiza tuberculata* (Macri, 1778) planulae in
781 natural seawater. Mean density of polyps per substrate (bars represent standard
782 deviation) for: **a** spatial preferences (Experiment 1): n=6 replicates with 50 polyps each,
783 and **b** substrate preferences (Experiment 2): n=3 replicates with 70 polyps each. AWI =
784 air-water interface, LAT = sides of the glass container, GS = glass slides, BOTT =
785 bottom of the glass container. Different letters indicate significant differences at
786 α =0.05

787

788 **Fig.2** *Cotylorhiza tuberculata* polyp budding rate at a constant temperature of 17.5ºC,
789 salinity of 38, and photoperiod of 12:12. Mean increase of polyp number by budding in
790 13 days (Experiment 4): n=4, bars represent standard deviation

791

792 **Fig. 3** Transmission electron microscope photograph of a zooxanthella in an a priori
793 aposymbiotic *Cotylorhiza tuberculata* polyp (scale bar: 5 μm). *a* accumulation body, *ch*
794 chloroplast, *s* starch body

795

796 **Fig. 4** Allometric relationships in early medusa stages of *Cotylorhiza tuberculata*
797 incubated at 20ºC. Each dot represents an individual (n=130). Open, gray, and black
798 circles represent ephyrae, metaephyrae, and small medusae (up to 113 days),
799 respectively

800

801 **Fig. 5** *Cotylorhiza tuberculata* ephyra growth at three temperatures (20, 25, 30ºC;
802 Experiment 5). Bars represent standard deviations (n=5)

803

Table 1 Strobilation requirements of some symbiotic rhizostome scyphomedusae. Strobilation type, role of zooxanthellae, temperature change, preconditioning factors, special inducers, and number of strobilations (N) in life cycle are detailed

Species	Strobilation type	Zooxanthellae	Temperature	Preconditioning	Special inducers	N
Cassiopea andromeda	Monodiscous[a]	Not essential* (aposymbiotic planulae, symbiotic/aposymbiotic polyps) [a-c] Morphogenic effect: lower temperatures for strobilation[c]	Increase from 20 to 24°C[a] Increase from 18 to 20-30°C[c]		Accumulation of polyp factor, facilitated by zooxanthella metabolite, enables strobilation in aposymbiotic polyps[c] Iodine[d]	>1
Catostylus mosaicus	Monodiscous and polydiscous[e]	Not essential (may be absent in the whole life cycle) [e, f]	Temperature variation does not initiate strobilation[e]	Polyps need to be hanging in an inverted position[e] Food abundance[e]	Strobilation not attributed to variation in photoperiod or salinity[e]	1[e]
Cotylorhiza tuberculata	Monodiscous[g, h]	Indispensable (aposymbiotic polyps do not strobilate)[i]	Increase from 20 to 24°C[i] Increase from 17.5 to 20[j]	Zooxantellae infection[i] Food availability[i]	Potassium iodide[j]	>1
Mastigias papua	Monodiscous [k]	Indispensable* (absent in eggs and planulae, aposymbiotic polyps obtained in laboratory)[k]	Increase from 20 to 25, 28-29°C[k] 20°C critical [l]	Precooling: 1 month at 20°C [l]		>1
Phyllorhiza punctata	Monodiscous [m]	Not essential (symbiotic and aposymbiotic medusae) [n]	Increase from 16 to 24ºC[o]		Special interaction between salinity and temperature [o]	>1

*Fast multiplication of algae related to beginning of strobilation (colour of strobilae)
[a]Hofmann et al. (1978); [b]Ludwig (1969); [c]Rahat & Adar (1980); [d]Pierce (2005); [e]Pitt (2000); [f]Pitt et al. (2005); [g]Clauss (1890); [h]Clauss (1893); [i]Kikinger (1992); [j]Prieto et al. (2010); [k]Sugiura (1964); [l]Sugiura (1965); [m]Hofmann & Crow (2002); [n]Galil et al. (2009); [o]Rippingale & Kelly (1995).

Table 2 *Cotylorhiza tuberculata* planula settlement preferences. Total polyp number per substrate, available surface per substrate and mean settlement percentages (standard deviation, S.D.) after 20 days

	Studied variable		AWI	LAT	GS	BOTT	Brick	Wood	Stone	Shell	Statistics
Exp. 1	Polyp number	mean	13.67	1.83	7.50	29.83					
		S.D.	6.77	2.23	3.94	14.99					
		total	82	11	45	179					
	Available surface (cm^2)		33.18	102.10	37.50	33.183					
	Attachment %	mean	28.12	3.61	14.94	53.33					$F_{3,20}$=29.205**
		S.D.	13.59	3.15	5.88	12.51					
Exp. 2	Polyp number	mean	26.67	6.0	5.67	9.67	6.67	11.33	1.33	2.00	
		S.D.	21.38	2.00	3.21	6.81	3.05	11.93	2.31	2.00	
		total	80	18	17	29	20	34	4	6	
	Available surface (cm^2)		33.18	102.10	37.50	25.89	5.48	5.44	6.25	1.88	
	Attachment %	mean	35.91	10.57	8.58	13.42	14.00	14.21	1.17	2.14	$F_{7,16}$=9.516**
		S.D.	6.88	4.78	2.77	7.31	10.25	6.89	2.03	1.87	

AWI: air-water interface, LAT: sides, GS: glass slide, BOTT: bottom,
**p<0.01

Table 3 Ingestion rate and daily ration of early medusa stages incubated at 20, 25, and 30ºC. Mean and standard deviation (S.D.) of specimen diameter, ash-free dry weight (AFDW), number of prey items ingested per day, and daily ration

Variable		Temperature			Statistics	
		20ºC	25ºC	30ºC	Factor	$F_{1,28}$
Diameter (mm)						
Ephyra	mean	3.50	3.68	3.72	temp	0.576^{NS}
	S.D.	0.33	0.45	0.46	stage	354.732**
Medusa	mean	7.40	6.95		temp x stage	2.667^{NS}
	S.D.	0.35	0.26			
AFDW (µg)						
Ephyra	mean	140.18	156.29	160.41	temp	0.577^{NS}
	S.D.	29.64	40.27	41.45	stage	354.537**
Medusa	mean	489.58	449.92		temp x stage	2.667^{NS}
	S.D.	30.95	23.18			
Ingestion (prey d^{-1})						
Ephyra	mean	121.67	392.00	230.67	temp	82.491**
	S.D.	24.29	55.43	50.60	stage	192.492**
Medusa	mean	419.00	783.67		temp x stage	3.608^{NS}
	S.D.	83.47	83.58			
Daily Ration (AFDW%)						
Ephyra	mean	286.48	855.79	471.85	temp	29.503**
	S.D.	75.26	276.60	101.61	stage	3.100^{NS}
Medusa	mean	272.36	556.41		temp x stage	2.356^{NS}
	S.D.	38.33	34.16			

temp: temperature, **$p<0.01$, ^{NS} $p>0.05$

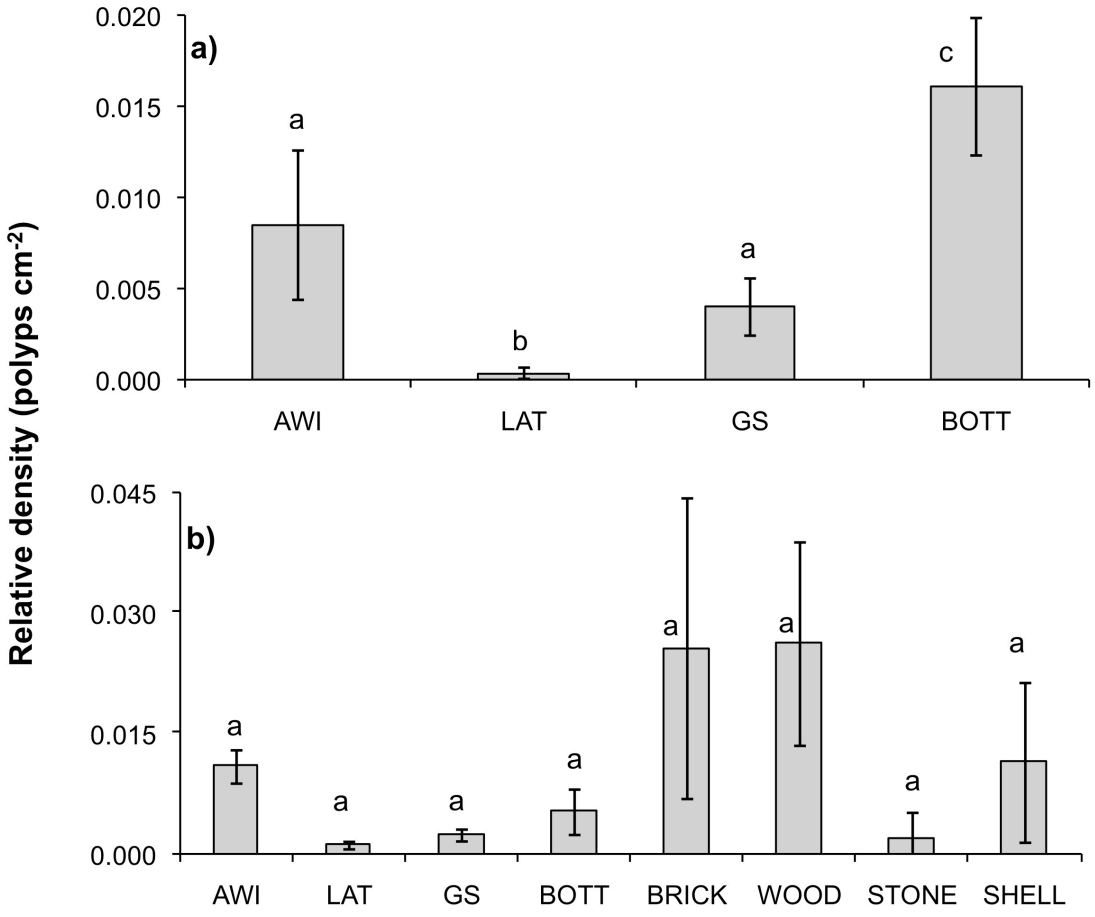
Relative density (polyps cm-2)
a)
0.020
0.015
0.010
0.005
0.000
a
b
a
c
AWI
LAT
GS
BOTT
b)
0.045
0.030
0.015
0.000
a
a
a
a
a
a
a
a
AWI
LAT
GS
BOTT
BRICK
WOOD
STONE
SHELL

Fig. 1

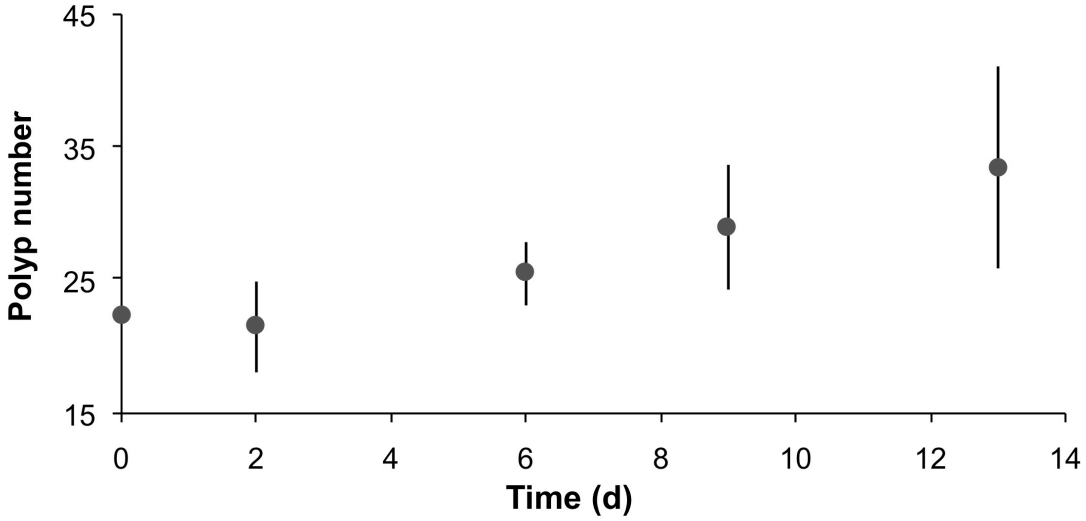

Fig.2

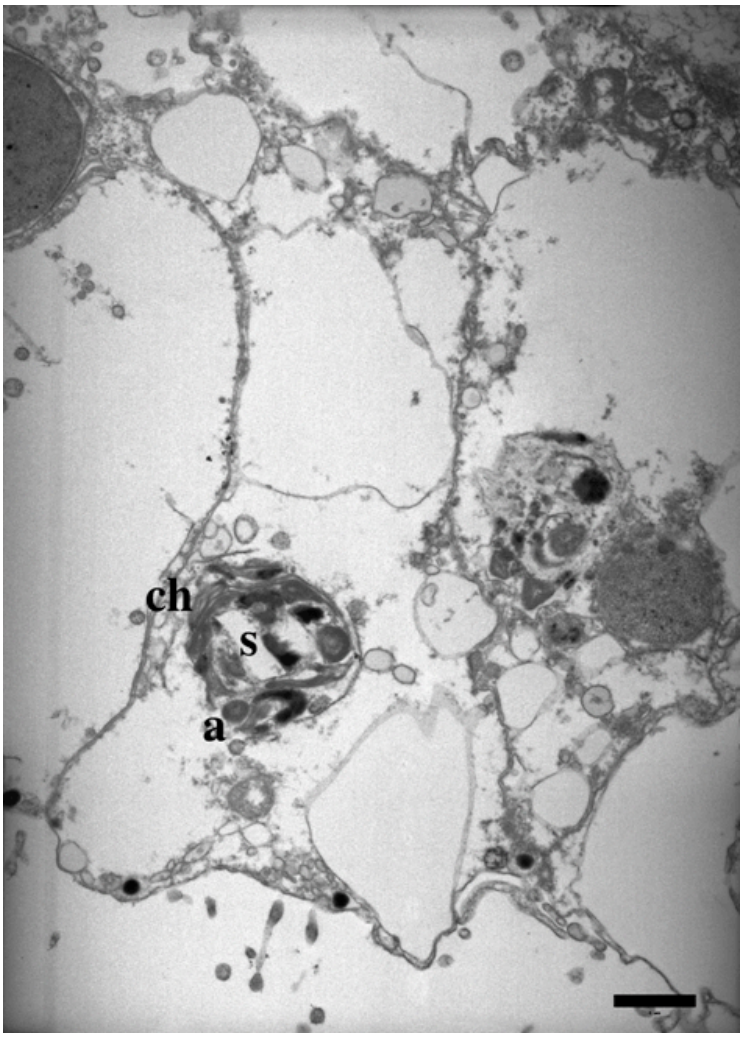

Fig. 3

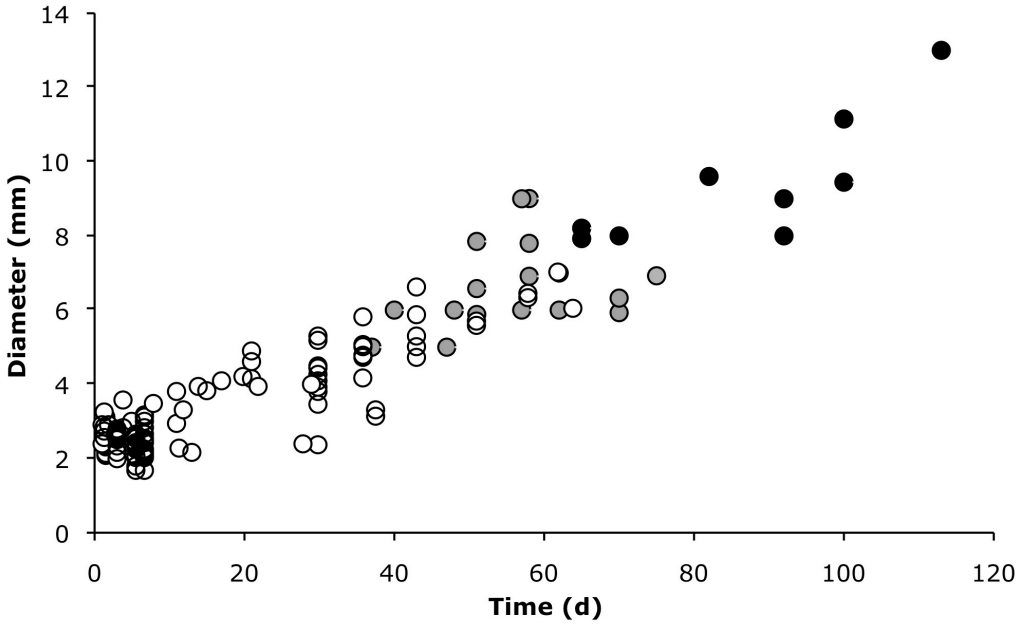

Fig. 4

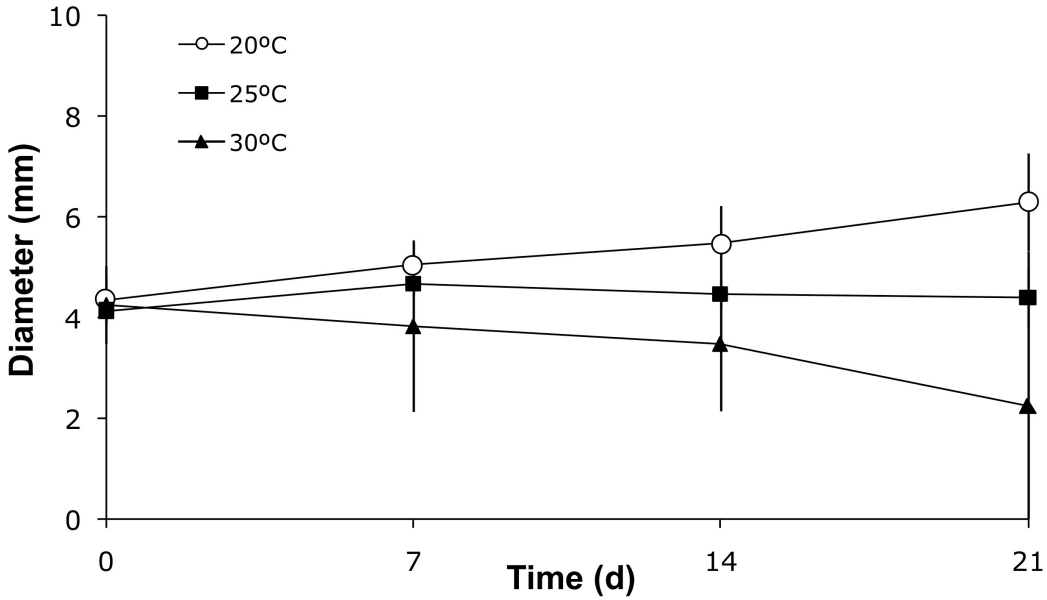

Fig. 5