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Maintenance, feeding and growth of *Carybdea marsupialis* (Cnidaria: Cubozoa) in the laboratory

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ABSTRACT

The box jellyfish *Carybdea marsupialis* has proliferated in some areas of the north-western Mediterranean Sea since July 2008. As for many species, controlled experimentation in the laboratory is needed to improve our knowledge about *C. marsupialis*, with the ultimate goal of extrapolating the knowledge gained to the marine environment. The aims of this study were to identify the optimal conditions (i.e. aquarium design, environmental parameters and prey type) for the growth and maintenance of this cubomedusa in the laboratory and, additionally, to quantify the feeding rates of the juveniles of this species. We were able to maintain healthy medusae for 140 days. During this time they reached the subadult condition (fact corroborated by observing the gonadal tissue), growing from 2 to 15 mm in diagonal bell width from June to November 2010, respectively. We observed a progressive shift in their preferred dietary composition as the individuals grew. The medusae fed on *Artemia salina* nauplii along the entire development. Other, larger, prey types (e.g. *Mysis* sp., *Acartia granii* copepods and adult *Artemia salina*) were progressively ingested at the same time as they increased their umbrella size. We also describe the clearance rates, ingestion rates, prey selectivity and digestion times of juvenile *C. marsupialis* on natural zooplankton and on the copepod *Acartia grani*. Growth and mortality rates were also calculated.

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1. Introduction

Harmful jellyfish blooms are amongst the most conspicuous events in the oceans worldwide (Mills, 2001). Even though there is a very recent publication questioning the rise of gelatinous zooplankton in the world's oceans and bringing in the question of the media-driven public perception of such increase (Condon et al., 2012), other studies have demonstrated the existence of a significant growth in jellyfish abundance in coastal systems worldwide using analytic methods designed to minimize the effect of the bias reporting (Brotz et al., 2012). The periodicity of occurrence of some jellyfish species (both native and nonindigenous) has shortened in recent decades and the recurrence of blooms has increased in a local or regional scale, probably due to food web modifications and climate change (Daly Yahia et al., 2010; Kogovšek et al., 2010; Licandro et al., 2010; Mills, 2001; Purcell et al., 2007). Human activities are thought to be contributing to increasing jellyfish abundances in coastal waters worldwide, which are in turn affecting swimmers, fisheries, aquaculture and other coastal industries (Purcell et al., 2007). Reviews have proliferated recently speculating that jellies have benefited from human-caused changes, including climate change, eutrophication, overfishing, coastal construction, and species introductions (Purcell, 2012).

The species *Carybdea marsupialis* is the only cubozoan known to inhabit the Mediterranean Sea (Linnaei, 1758); it was recorded for the first time at the Adriatic Sea in 1878 by Claus (Di Camillo et al., 2006). Since then, *C. marsupialis* has been recorded in high densities several times in the Adriatic region (Avian et al., 1997; Boero and Minelli, 1986; Corbelli et al., 2003; Di Camillo et al., 2006). It was never considered that *C. marsupialis* could form blooms in the western Mediterranean; however, since July 2008, populations in at least two localities (Denia and Santa Pola beaches; east coast of Spain) along the NW Mediterranean coast (~120 km apart) have increased reaching unusual very high densities in some beaches (Bordehore et al., 2011 for Denia; C. Bordehore unpublished data for Santa Pola).

It is particularly important to determine whether high densities of *C. marsupialis* in the NW Mediterranean will alter ecosystem function and/or biodiversity. Moreover, some details of the lifecycle of this species are unknown for the Mediterranean Sea. This cubozoan species has a metagenetic life cycle; we know that the cubomedusa stage is present from May to November in the NW Mediterranean region, but we still

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lack information on the cubopolyp stage, which is still to be found in the field or obtained *in vitro*. Considering the importance of benthic stages for jellyfish outbreak formations due to their asexual reproduction and resting capacity (Boero et al., 2008), it is vital to acquire data both on the polyp and the medusa stage of this species. The ability to maintain local *C. marsupialis* in aquaria will allow the development of *in vitro* experiments and assays, facilitating the comprehension of the role of *C. marsupialis* in coastal ecosystems and the consequences of future bloom events.

In our case, we successfully monitored the development and growth of the cubomedusa stage of *Carybdea marsupialis*; moreover, visually mediated behaviour and feeding behaviour could be studied in the laboratory in an appropriately designed aquarium. To date, there are no published evidences of cultivation of carybdeid medusa to maturity in captivity, except for *Tripedalia cystophora* (Straehler-Pohl and Jarms, 2011). The complete life cycles of different carybdeid species in La Parguera (Puerto Rico) have been described; however, no laboratory-raised *Carybdea* sp. medusa lived beyond 15 days (4 mm in umbrella height (UH)) (Studebaker, 1972; Werner, 1971, 1983). Pioneering studies have also been conducted with other cubozoan species, especially in Australia (Hamner et al., 1995), where the most venomous species are present. Yamaguchi and Hartwick (1980) described the early life history of the sea wasp *Chironex fleckeri*. The largest medusae observed were 7 weeks old and exceeded 10 mm in UH, but they did not show any signs of branching in their pedalia (Yamaguchi and Hartwick, 1980). Hamner et al. (Hamner et al., 1995) were also able to maintain one individual of *Chironex fleckeri* over a 9-month period to sub-adult condition, describing in detail the swimming, feeding, circulation and vision of this Australian cubomedusa. Some adult individuals of different carybdeid species, such as *Carybdea sivikisi* (Hartwick, 1991; Lewis and Long, 2005) and *Tripedalia cystophora* (Buskey, 2003), have been kept in aquaria for a limited time with the aim of studying their behaviour and life cycles. Nevertheless, there is still very limited data on the early life stages of carybdeids. The present study represents the first long-term maintenance and growth of a carybdeid species from recent detached cubomedusa to subadult condition in the laboratory. We focus on the diet as one of the most important factors involved in survival of juvenile cubomedusae and we describe the feeding rates of *C. marsupialis* and dietary preferences of the species. These experiments will improve our knowledge of the trophic requirements of cubomedusae and they will identify areas for further research on the predatory impact of this species.

The aims of the present study were (1) to determine the optimum conditions for the maintenance and growth *C. marsupialis* in the laboratory, and (2) to assess the clearance, ingestion and digestion rates of juveniles of *C. marsupialis* by incubation experiments. In order to achieve these aims, medusae were collected from the field and an experimental culture of *C. marsupialis* was established.

2. Material and methods

2.1. Medusae collection

We collected juveniles of *Carybdea marsupialis* from Rasset Beach (Alicante, Spain) on 16th June 2010. The medusae were caught very close to the beach (~2 m depth) by superficial trawling with a 200-μm plankton net (40 cm diameter) for 5 min at a speed of ~2 knots. The medusae collected were immediately transported in 25 L plastic containers filled with ambient seawater and without air spaces (to avoid damage) to the Institut de Ciències del Mar – Consejo Superior de Investigaciones Científicas (ICM-CSIC) in Barcelona, where they were kept in an especially designed aquarium.

2.2. Aquarium design and maintenance

The recent detached medusae were placed in a 140 L, black sided, aquarium especially designed with low, horizontal flow in a continuous flow-through seawater system. This type of aquaria prevents the attachment of the tentacles to the glass, which could cause death by starvation (Straehler-Pohl and Jarms, 2011). We generated a vertical (i.e. parallel to the lateral walls of the aquarium), laminar flow by coupling a vertical pipe with holes in the aquaria to the input stream and four buffer plates across at each corner of the main chamber of the aquarium. This mechanism prevented the medusae for getting stuck to the corners. The input stream was adjusted to achieve adequate water renewal and velocity so that the cubomedusae were kept in the water column away from the walls of the aquarium, and also allowing them to swim freely against the current. The output of the overflow water was in a chamber behind a 300-μm mesh screen, which allowed the passage of food debris but not medusae (Fig. 1A). Cubomedusae use to swim near to the bottom, especially during the ingestion of food. Therefore, it was necessary to clean the bottom of the aquarium every day in order to avoid the accumulation of leftover food. During summer 2012 we put on trial a new version of the aquarium (Fig. 1B) specially designed for the adult stages, with some changes and adjustments, which allowed us to maintain adult cubomedusae for more than 50 days. This 180 L aquarium has two separate circulation systems. The circular and laminar flow of the water in the main chamber, where the animals are kept, is also generated by coupling a vertical pipe with holes in the aquaria to the input stream and four buffer plates across at each corner of the main chamber of the aquarium. The secondary circulation system is located in the lower part of the aquarium, within a space separated from the main chamber using an acrylic plate of 5 mm thickness, with perforations of 50 mm which were covered with 500 μm mesh. This constant flow generates homogenization of the water, which prevents anoxia.

Lighting is also crucial for the maintenance of cubomedusae because they have a well-developed sense of vision with 24 eyes, including simple and complex eyes that allow them to distinguish colours, form images (Coates, 2003), and even avoid objects (Hamner et al., 1995). For the smallest aquarium (Fig. 1A) we used three-beam central lightning (white light), controlled with a photoperiod of 12:12 h light:dark. The central vertical light shaft and the black sides and bottom of the aquarium kept cubomedusae away from any surfaces that could damage them. For the maintenance of the adult stages in the larger aquarium (Fig. 1B) we used also central lightning but with a blue coloured filter.

Both aquaria were kept with running seawater at the natural temperature and salinity of the NW Mediterranean Sea during throughout the period of the study. Consequently, we were able to monitor the growth and development of the cubomedusae under simulated natural conditions. Temperatures ranged from a maximum of 25.1 °C during summer, to a minimum of 17.2 °C in November when the cubomedusae died.

The number of live individuals and measurements of the diagonal bell width (DBW) and UH of the medusae were recorded every week. We decided to rely on these sorts of non-destructive biomass estimates to obtain growth rates, instead of weight, to maximize the final number of individuals. All measurements were conducted under a calibrated stereoscopic microscope until the juveniles reached 10 mm wide, when they could be easily manipulated and measured using callipers (Medid, 1/20 mm; ±0.05 mm).

2.3. Prey preference and feeding experiments

We maintained cultures of different prey to meet the feeding requirements of *C. marsupialis*. *Artemia salina* nauplii (~400 μm) were provided *ad libitum* every day as the main diet item to maintain the medusae. During the early days of maintenance we also tested to feed the recent detached medusae with cultured rotifers. The diet was supplemented every 2 days, either with natural plankton when available, or with adult copepods (the calanoid *Acartia grani*). Copepods were maintained in a 20 L transparent, acrylic cylinder at 20 °C in 5-μm filtered seawater and fed *ad libitum* with *Rhodomonas salina*. As the juvenile cubomedusae increased in DBW we added to the diet live *Mysis* sp. and adults of *Artemia salina*. *Mysis* sp. were kept in 5 L transparent beakers and fed

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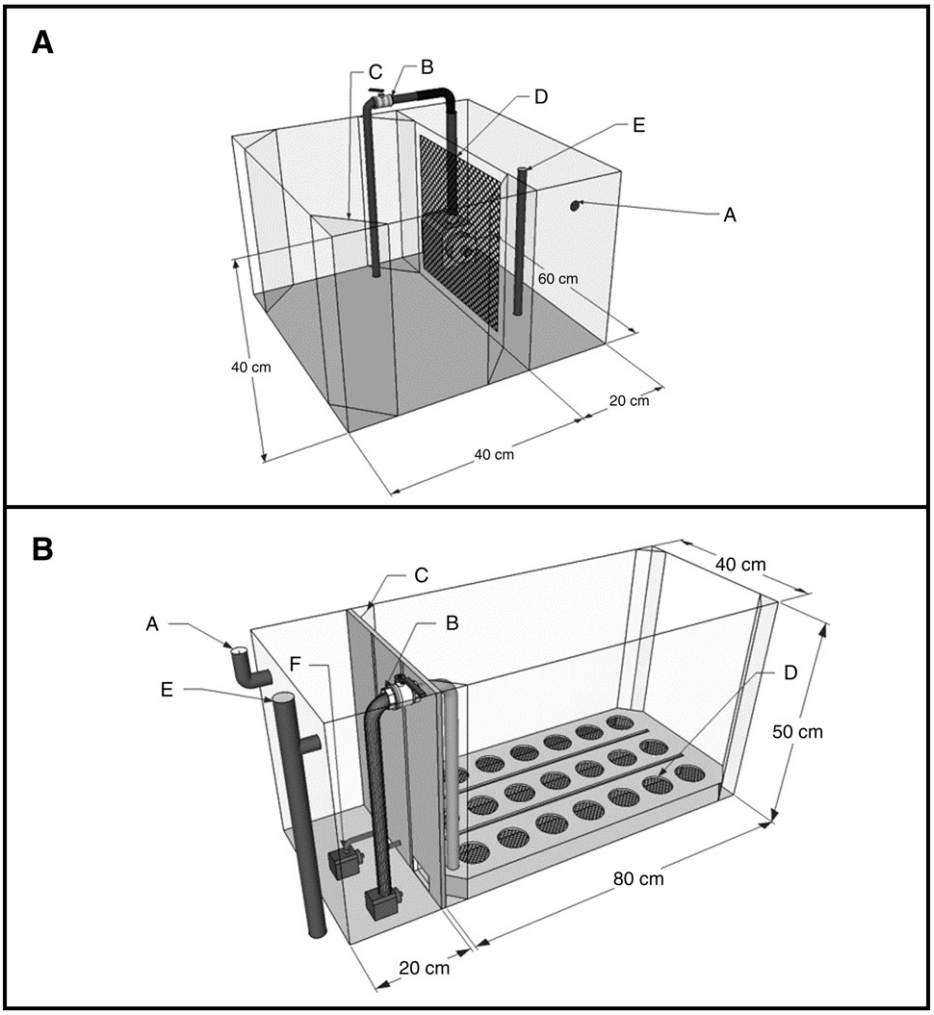

Fig. 1. Especially designed aquariums used to keep *Carybdea marsupialis* under controlled conditions. (A) Aquarium for the early stages: A = water inflow; B = overflow outlet water; C = buffer plate; D = vertical pipe with holes that creates horizontal laminar flow; E = 300-μm mesh. (B) Aquarium for the adult stages (>1.5 cm diagonal bell width (DBW)): A = water inflow; B = main circulation system composed by a pump coupled to a vertical pipe with holes that creates horizontal laminar flow; C = buffer plate; D = acrylic plate with perforations covered with 500-μm mesh; E = overflow outlet water; F = secondary circulation system.

with rotifers and pellet food. We also feed *C. marsupialis* with frozen *Mysis* sp. and *Artemia salina*.

Prey consumption by jellyfish has been estimated for several species using different approaches: (1) clearance rate experiments (incubations); (2) gut content analysis of field-collected specimens to measure digestion times; and (3) respiration rate experiments (Purcell et al., 2010). Here, three types of incubation experiments were conducted with juveniles of *C. marsupialis* to assess prey consumption: (1) incubations with different concentrations of natural zooplankton to investigate prey selectivity and to calculate clearance and ingestion rates; (2) incubations with different concentrations of *Acartia grani* to calculate clearance and ingestion rates; and (3) individual observations of gut contents over time to calculate digestion time. In addition, we present information on prey selectivity from visual gut content analysis of field-caught medusae.

For the natural plankton incubations, zooplankton were collected close to the coast of Barcelona (Spain), by vertical trawls, using a 300-μm Nansen net with a plastic bag as cod-end to avoid damage to the plankton. In the laboratory, different concentrations of the natural plankton were prepared by diluting the sample with 5-μm filtered seawater. Thirteen glass Nalgene bottles (1200 mL) were initially filled with 5-μm filtered seawater: two replicates for each control and three replicates for experimental treatment. In order to achieve the final concentration desired at each treatment, we calculated the volume of the corresponding plankton dilution necessary to add into the experimental bottles (i.e. 8 mL). The three concentrations used were 27, 58 or 90 organisms bottle^{-1} (22.5, 48.33 and 74.58 organisms L^{-1}, respectively). For the control bottles, only 8 mL of the corresponding plankton suspension was added. An 8 mL aliquot from the initial sample was fixed with 4% formalin in seawater at time 0 h (t_0) to determine the start prey concentrations. At time 0 one medusa was placed in each of the nine experimental bottles. The bottles were incubated for 24 h at 24.5 ± 0.5 °C on a plankton wheel (0.2 rpm) to keep both medusae and prey suspended. At the beginning and at the end of the experiment, we measured the DBW of the cubomedusae, and filtered and preserved the zooplankton of all bottles for later quantification and identification. The zooplankton components analysed to determine prey selectivity were cladocerans, copepods and icthyoplankton (fish eggs and larvae). The final prey concentrations from each experimental treatment were compared to the final prey concentrations of the control treatments. If there was a significant difference (*t*-test $p<0.05$) in the prey concentrations between the experimental and the control treatments then the individual clearance and ingestion rates were calculated. The clearance rate (CR: the volume of water filtered in L medusa^{-1} day^{-1}) for each incubation was calculated as:

$$CR = ([V/(n \times t)] \times \ln(C_0/C_t)) \times 24 \quad (1)$$

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where CR = clearance rate; V = volume of the container (L); n = number of cubomedusae; t = incubation time (hours); C_0 and C_t = initial and final number of prey (Purcell and Arai, 2001). The individual ingestion rates (IR) were then calculated as:

$$IR = CR \times C_m \quad (2)$$

where CR = clearance rate calculated; C_m = mean prey concentration calculated as:

$$C_m = \exp[\ln(C_0 \times C_t)/2] \quad (3)$$

A similar experimental design to that described above was used with the cultured copepod *Acartia grani* as the only available prey. This species of copepod was selected due to the fact that calanoid copepods were the principal prey item detected (23.52%) in the gut contents of 102 *C. marsupialis* captured during previous work (M. J. Acevedo, unpublished data). The experimental design was equal to that previously described; however, the three different prey concentrations prepared were 10, 20 or 40 copepods bottle^{-1} (8.3, 16.6 and 33.3 copepods L^{-1}, respectively). Similarly, at the beginning and at the end of the experiment, the DBW of the cubomedusae from the experimental bottles was measured. The copepods were filtered and fixed from all bottles for later quantification and the CR and IR for each treatment was calculated.

Because *C. marsupialis* are often observed in the field with empty gastrovascular cavities (M. J. Acevedo, unpublished data), we prepared an experiment with the aim of calculating the digestion time of this cubomedusan species. Three juvenile medusae (~10 mm in DBW) were chosen and placed in individual aquaria. When their gastrovascular cavities were empty, we gave one *Mysis* sp. shrimp to each of them. Then, we recorded the state of the prey item every 15 min until complete digestion was achieved. Digestion time was the mean time taken to reach complete digestion for the three medusae. The end point for digestion was easy to determine due to the transparency of the bell of *C. marsupialis*.

2.4. Statistical analyses

In order to test the correlation between DBW and UH-based growth rates and between temperature and mortality rate, we used model II regression analysis, as the independent and dependent variables were estimated with comparable error (Laws and Archie, 1981). Model II regression analysis was performed using the Major Axis (MA) method, and confidence intervals were obtained with a total of 99 permutations. The analysis was conducted using the R package "lmodel2" (Legendre, 2011).

Multiple ANOVA analyses were conducted in order to test differences among ingestion rates on different prey type (i.e. copepods, cladocerans and icthyoplankton). The differences between controls and treatment bottles were proved using t-test in both feeding experiments (i.e., incubations with natural mesozooplankton and *Acartia granii* copepods).

3. Results

3.1. Sampling and medusae maintenance

During the sampling to collect *Carybdea marsupialis*, a total of 38.76 m^3 of water were filtered as calculated by the flowmeter readings. A total of 288 cubomedusae were collected (size: 2–3 mm in DBW), giving a density of 7.45 medusa m^{-3}. When collected, the cubomedusae had a mean DBW of 2 mm (n = 288). A total of 30 individuals reached a mean DBW of 15 mm (Fig. 2A), ~60% of the maximum natural size observed in the sea; the mean DBW of the wild adults captured for gastric content analysis (n = 102) in 2009 was 25 mm. There was a significant linear relationship (R^2 = 0.87; P = 0.01; slope = 0.99) between DBW and UH growth ratios (Fig. 2B). Therefore, all further comparisons presented are based on DBW.

We were able to maintain 200 healthy medusae in the aquarium for ~60 days (Fig. 3A). We observed the higher mortality rates (i.e. 4% day^{-1}) during the first week of maintenance and acclimatization to the aquarium. After this period, the mortality rate decreased to 0.52% until day 65th of the culture (21th of August 2010) when gradually increased from 0.95 to 2.1% day^{-1}, and survival percentage was 24% of the initial population. Then, during September we observed a month of stability either in the number of cubomedusae (n = 70), temperature (~22 °C) and mortality rate (1.87 ± 0.25% day^{-1}). Later, from the 107th day of the culture, temperature decreased to 19.1 °C, and at that moment only 10.5% of the initial population remained alive. After 137 days (4.5 months) 18 healthy medusae remained; at this point they had reached the pre-adult stage growing from 2 to 15 mm in DBW. In November 2010 the temperature of the seawater in the flow-through system decreased to 17.8 °C, and after this decrease in water temperature, only two cubomedusae survived. We maintained them in another tank at 21 °C, where they were able to live for 3 more weeks. We renewed the water in this tank every 2 days. We could detect an association between survival rate and water temperature, where the decreasing trend in the number of live medusae were linearly related to the decrease in temperature (Fig. 3B; R^2 = 0.91; P = 0.01; slope = −0.0018).

3.2. Food supply

Different prey items were tested as a potential food source during the growth and maintenance of the medusae. We observed a progressive shift in dietary composition as individuals increased in DBW. *Artemia salina* nauplii were eaten by juvenile medusae of all sizes and progressively complemented with other prey types. After a few attempts with a new prey type, they fed the new items provided to them, and finally they ingested frozen food. Rotifers were not ingested by medusae of any stage (Table 1).

When we fed individuals with prey items of similar size than the cubomedusae (i.e., *Mysis* sp.), at first they did not ingest them; however, after a few days the cubomedusae were capable to catch and immobilise these prey with their tentacles and introduce them into their gastrovascular cavity by flexing their tentacles towards manubrium. In addition, fish larvae (*Dicentrarchus labrax*) were offered several times to the adult cubomedusae kept in aquaria. The preys were cached and killed, but the jellyfish later refused to ingest either alive or dead fish.

3.3. Feeding experiments

When the medusae were offered natural assemblages of zooplankton they only significantly ingested copepods (MANOVA; p<0.01) (i.e., no significant ingestion of cladocerans and icthyoplankton). Therefore, the actual prey concentrations for the three experimental treatments with natural zooplankton were 23, 53 and 88 copepods L^{-1}. The clearance rate of *C. marsupialis* feeding on naturally occurring species of copepods ranged from 0.89 to 2.1 L medusa^{-1} day^{-1} (Fig. 4A). The ingestion rate on natural zooplankton increased with increasing prey (copepod) concentration, ranging from 8 to 31.6 copepods medusa^{-1} day^{-1} (Fig. 4B).

The clearance rate of *C. marsupialis* feeding on *Acartia grani* averaged 1.7 L medusa^{-1} day^{-1} (from 0.78 to 2.4 L medusa^{-1} day^{-1}) at prey concentrations ranging from 8.33 to 33.3 ind L^{-1} (Fig. 5A). The ingestion rates increased with prey concentration from 6.5 to 19.0 copepods medusa^{-1} day^{-1} (Fig. 5B). There was no evidence of feeding saturation at the prey concentrations tested. Given prey concentration decreased considerably along the incubations (from 40 to 100% in 1 case; mean decrease 73.67 ± 15.94%), we present the CR and IR based on average prey concentrations. Because final prey concentration decreased more than 50%, and that fact may alter both the CR and IR (Båmstedt et al.,

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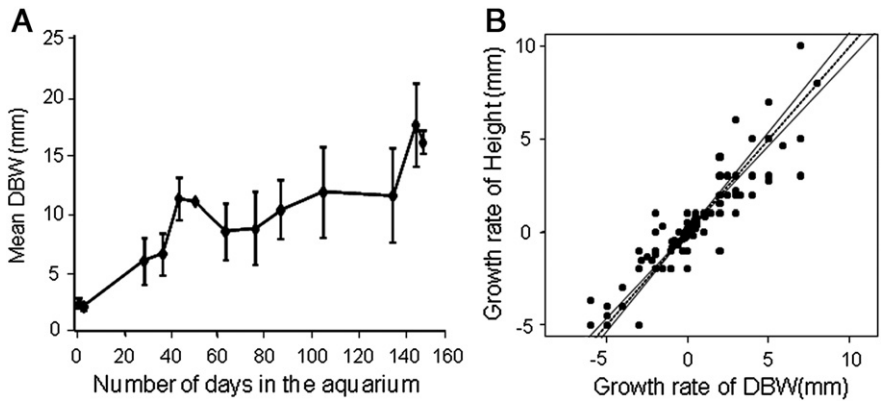

Fig. 2. Evolution of cultured *Carybdea marsupialis*. (A) Size evolution of *C. marsupialis* medusae DBW over the course of the study. (B) Linear regression of the diagonal bell width (DBW) and umbrella height (UH)-based growth rates ($R^2 = 0.87$; $P = 0.01$; slope = 0.99).

2000), we have to consider that the CR and IR we obtained from our incubations could be underestimated.

The digestion time of *C. marsupialis* feeding on *Mysis* sp. at 23 °C was 2.24 h ± 0.63 (mean ± SD) for complete digestion to be achieved. During that time some indigestible wastes (i.e. exoskeleton) were expelled. The head and the eyes of the preys were the last part to be digested.

4. Discussion

4.1. Cultivation of Carybdea marsupialis

Previous attempts to cultivate carybdeids did not succeed in obtaining individuals larger than 5–6 mm in DBW (Straehler-Pohl and Jarms, 2011). Aquarium design, water quality, diet and feeding regimen are key factors for a successful maintenance of jellyfish species, and also box jellies. As it can be inferred from our experiments, horizontal flow of the water, central lightning and black sides are necessary in order to prevent the attachment of the tentacles to the aquarium, which could cause death by starvation. The size and the stage must be considered when feeding the cubomedusae in order to provide them the right range of prey size. It is also very important to feed box jellies with different types of prey, since feeding only on *Artemia salina* seems not to be nutritionally enough for supporting the development. We previously observed high mortalities of adult medusae caught from field that were fed with *Mysis* sp. (either alive or frozen) and juvenile fish (M. J. Acevedo, personal observation). This suggests that copepods could be a necessary component on the diet of large and small medusae. Moreover, a percentage of 23.5% of adult cubomedusae (~25 mm in DBW) caught from the field on previous occasions had copepods in their gastric cavities (M. J. Acevedo, unpublished data). Also Larson (1976) described the diet of cubomedusae consisting mostly of crustaceans and fish; crustaceans (mostly *Acartia* sp.) were eaten by all sizes of *Carybdea* sp.; however, small medusae seem to be more dependent on them than larger ones because their difficulty of capturing fish (Larson, 1976). Other carybdeid species, such as *Tripedalia cystophora*, has been registered to prey on dense swarms of the copepod *Dioithona oculata* in the mangrove prop-root habitat of Puerto Rico (Buskey, 2003). By completing the diet with natural mesozooplankton, or cultured copepods, the cubomedusae in our experiments were able to progress in their development. We also observed that an inadequate food ration resulted in bell deformation, but when feeding was increased the bell shape returned to normal. Feeding the adult cubomedusae was more difficult than the recent detached medusae, since they have a more complex feeding behaviour. Although adult individuals of this species are known to occasionally ingest fish in the field (Larson, 1976), cultured specimens refused the fish larvae (*Dicentrarchus labrax*) provided, either alive or dead. It was probably not the adequate fish species to feed this type of jellyfish and most of the adults died after 10 days due to starvation. During summer 2012, we maintained adult cubomedusae for more than 50 days feeding them with a culture of *Mysis* sp. which was kept in the same aquaria were the box jellies were maintained. A detailed analysis of the gastric contents in wild specimens will determine the key prey species for this medusa.

Despite the difficulties in maintaining juvenile cubomedusae in aquaria, we were able to sustain 18 individuals of *C. marsupialis* over a 4-month period (137 days), although only at ~60% of the mean maximum natural size observed in the sea (M. J. Acevedo, unpublished data). This percentage of reduction in the cultivated adult size is similar to the ones obtained by Hamner et al. (1995) for *Chironex fleckeri*. Their

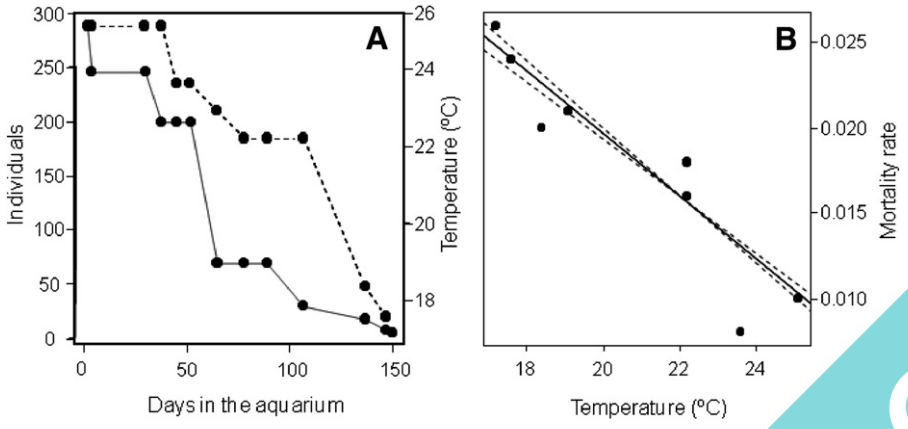

Fig. 3. Number of live *Carybdea marsupialis* medusae in relation to temperature over the course of the study. (A) Number of medusae and temperature variation over the study. Solid line: number of individuals, dashed line: temperature (°C). (B) Linear regression of mortality rates vs. temperature ($R^2 = 0.91$; $P = 0.01$; slope = −0.0018).

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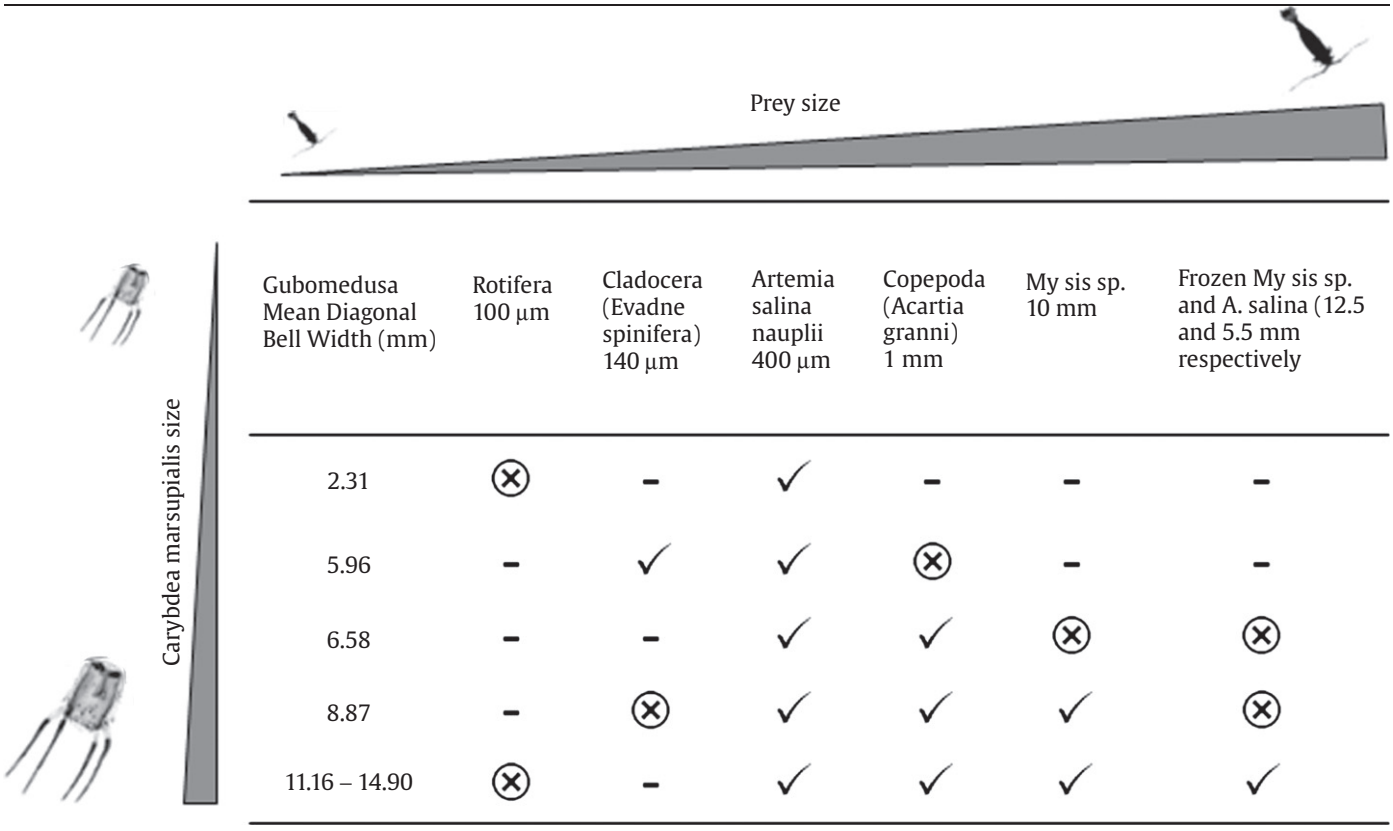
Table 1
Progressive shift in prey size and dietary composition of *Carybdea marsupialis*.



Gubomedusa Mean Diagonal Bell Width (mm)	Rotifera 100 μm	Cladocera (Evadne spinifera) 140 μm	Artemia salina nauplii 400 μm	Copepoda (Acartia granni) 1 mm	My sis sp. 10 mm	Frozen My sis sp. and A. salina (12.5 and 5.5 mm respectively
2.31	⊗	-	✓	-	-	-
5.96	-	✓	✓	⊗	-	-
6.58	-	-	✓	✓	⊗	⊗
8.87	-	⊗	✓	✓	✓	⊗
11.16 – 14.90	⊗	-	✓	✓	✓	✓

Non-digested; ✓ ingested; - Non-tested at this size.

C. fleckeri achieved 75% of their natural size, growing from 40 up to 120 mm in DBW, in comparison to the 160 mm in DBW attained in the sea. In our study, although the cubomedusae grew, they did not reach full sexual maturity; however, gonads started to develop as a thin line of gonadal tissue on either side of the septa. These individuals lived the same period of time than the assumed life span of *C. marsupialis* medusae (i.e. from late May to early November), but their development was much slower than the expected at sea. We suggest that captivity affected their growth and correct development. Among many possible causes, we think that they probably needed a higher energy intake (food) to reach full gonadal maturity.

The temperature affected the survival of our cubomedusae during the period of maintenance in the aquarium, but individuals from the sea developed mature gonads during August at similar temperature. The slower growth rate of the cultured cubomedusae compared with wild individuals, in addition to the decrease in temperature under ~18 °C in November, could be the reason why they did not reach sexual maturity in the aquaria. Field observations have shown a similar trend in decreasing

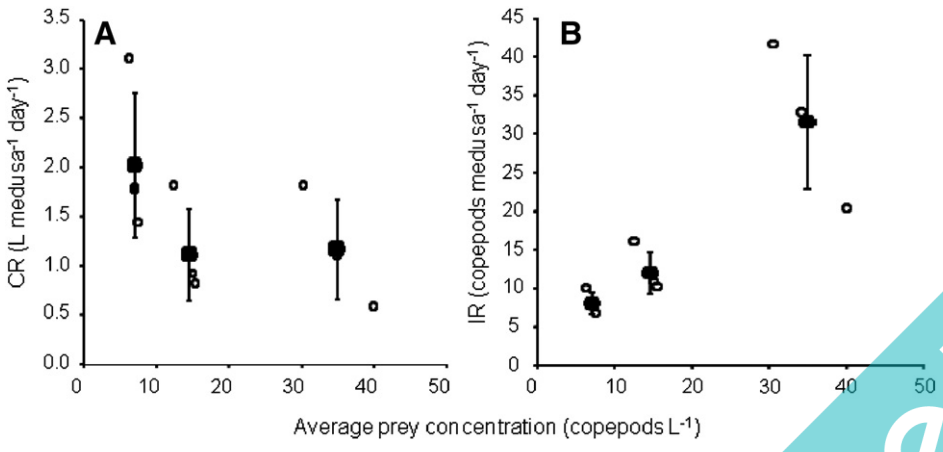


Fig. 4. Clearance (CR) and ingestion rates (IR) of *Carybdea marsupialis* feeding on natural mesozooplankton at different prey concentrations. (A) Clearance rate with natural prey (calanoid copepods). (B) Ingestion rate with natural prey (calanoid copepods). Grey-filled circles are individual values; black-filled squares are mean values ± SD for each prey concentration.

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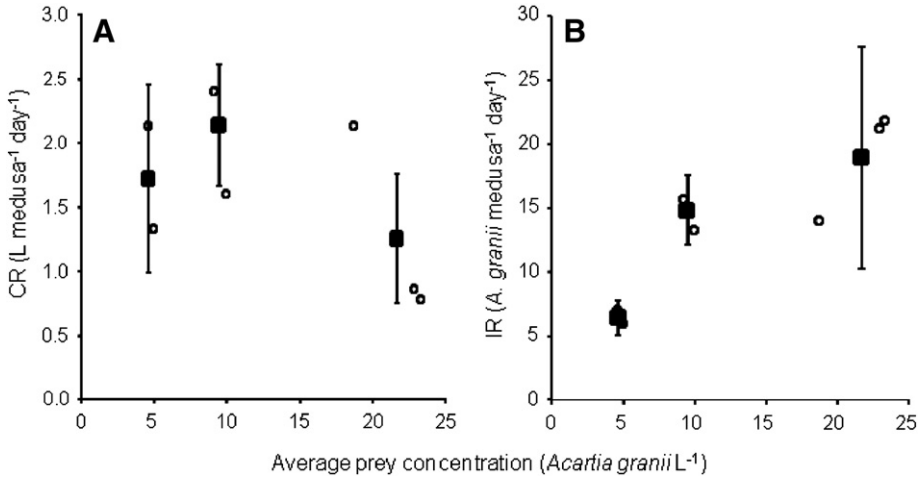

Fig. 5. Clearance and ingestion rates of *Carybdea marsupialis* feeding on *Acartia granii* at different prey concentrations. (A) Clearance rate with *A. grani*. (B) Ingestion rate with *A. grani*. Grey-filled circles are individual values; black-filled squares are mean values ± SD for each prey concentration.

densities of *C. marsupialis* with decreasing seawater temperatures (Bordehore et al., 2011). Sexton et al. (2010) have also indicated that low temperatures cause *Chrysaora quinquecirrha* to sink to the bottom and decreased their pulsation rate until they reach the limit of their temperature tolerance, at which point they would die. We observed a similar behaviour in individuals of *C. marsupialis* maintained in the aquaria, when the temperature of the seawater flow-through in the aquarium decreased they sank to the bottom and died.

4.2. Feeding behaviour

The present study has also improved our knowledge of the trophic requirements of cubomedusae. Given the few data available, it is not possible to determine the type of functional response of the IR; however, we decided it was relevant to the study because it provides an approximated vision on the amount of food these organisms require for growth.

It is difficult to compare clearance rates from the present work with other similar studies, since feeding experiments with cubomedusae are scarce. Because of this, we compare our results with the feeding rates of other gelatinous organisms, but must be taken into consideration that experimental conditions and swimming behaviour of the different medusae are not identical, and this may be an integrated part of the hunting technique which could produce differences between the CR of the species. As stated before, given the severe prey reduction during some incubations, it may well be our maximum rates are underestimated. Individuals of *C. marsupialis* of the size class we used (i.e., mean DBW of 10.5 mm; 0.76 g of WW) showed a mean CR of 1.57 L medusa day^{-1} in our experiments of feeding on natural zooplankton and cultured *Acartia grani*. The estimated rates in this study are similar to the ones obtained for *Mnemiopsis leidyi* in the same WW range (i.e., individuals of 0.93 g of WW) had CR about 1.94 L medusa day^{-1} (Acuña et al., 2011), although they are one order of magnitude lower than the ones obtained for other scyphomedusae species, such as *Pelagia noctiluca*, also with the same WW (i.e., individuals of 0.66 g of WW had CR of 1.94 L medusa day^{-1}) (Acuña et al., 2011; Hansson et al., 2005; Madsen and Riisgård, 2010; Purcell, 1985).

Large-sized gelatinous organisms are a problematic group in incubation experiments, particularly those whose hunting mode is 'cruising entangling', as *C. marsupialis*. Experiments performed on such organisms without prior knowledge of their feeding behaviour are unreliable (Båmstedt et al., 2000). *Carybdea marsupialis* even if moderated in size requires high incubation volumes because has tentacles at least eight times longer than its diameter. This should be considered when trying to incubate the species.

4.3. Advantages and disadvantages of being transparent

Cubomedusae are transparent; therefore, holding any opaque object in their gastric cavities for long periods of time could make them more visible and vulnerable to their natural predators. Thus, the rapid digestion of prey items might favour them; indeed, cubozoans have very active digestive extracellular proteases that are produced by cirri and stomach wall (Larson, 1976) which enable them to digest prey quickly. There are other factors influencing digestion time, such as prey type and size, number of prey in the gut content, temperature, and species of predator (Ishii and Tanaka, 2001; Martinussen and Båmstedt, 2001).

In the present study, *C. marsupialis* were able to fully digest one *Mysis* sp. in 2.24 h at 23 °C. Puerto Rican *Carybdea marsupialis* digested one 20 mm fish in 3–4 h, continuously expelling wastes of prey during digestion (Larson, 1976). The Australian cubomedusa *C. fleckeri* has a digestion time ranging from 3.5 to 4.5 h at 30 °C (Hamner et al., 1995). Other gelatinous organisms, about the same size of the cubomedusae we used for incubation experiments, showed a wide range of DT from 2.16 h in the case of *Mnemiopsis leidyi* (feeding on copepods at 21 °C), 4.62 h for *Aurelia aurita* (feeding on *Calanus* sp. at 20 °C), to 6.55 h for *Cyanea* sp. (feeding also on *Calanus* sp. at 20 °C) (Martinussen and Båmstedt, 2001). The shortest DTs correspond to the most transparent species: *M. leidyi* and *C. marsupialis* (2.16 and 2.24 h respectively).

Their aforementioned transparent bell may not only aid predator avoidance, but may also benefit them as predators, combined with a developed sense of vision and very long extensile tentacles. Cubomedusae differ from scyphomedusae and hydromedusae, in that their rhopalia (sensory structures) contain simple eyes and complex eyes (which have an apparent cornea, a spherical lens and retinal cells connected to nerve fibres) (Yamasu and Yoshida, 1976). Several investigators have speculated that complex eyes of cubozoan medusae and large numbers of simple pigment-cup ocelli should make these organisms capable of detecting changes in the distribution of light and perhaps even a crude form of vision (Pearse and Pearse, 1978). The sophisticated photosensitive behaviour of these predatory medusae may assist them in locating and remaining in copepods swarms where their prey is plentiful. We observed a pronounced positive phototropism of the medusae that were kept in the aquarium, as it is stated for other cubozoan species; we selected blue light for maintaining the cubomedusae since this colour is supposed to produce changes in the behaviour of some boxjelly species that were interpreted as feeding behaviour (Gershwin and Dawes 2008). *C. marsupialis* are ambush predators, relying on the prey to swim into contact with their nematocyst-laden tentacles for prey capture. However, it may be more of a challenge for ambush predators to locate prey patches (Buskey, 2003). Other gelatinous zooplankton

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predators that are known to exhibit behavioural responses to the presence of prey are the scyphomedusa *Aurelia aurita*, which directs their movements according to odours that are associated with prey (Arai, 1997). In the present study, juvenile *C. marsupialis* were able to capture *Artemia salina* and copepods by extending their long tentacles; however, larger prey, such as *Mysis* sp., had to be placed manually on the manubrium. Also some form of active selection for copepods is indicated when feeding on natural zooplankton, but more detailed experiments have to be conducted to prove that.

As it can be inferred from our results and observations, horizontal flow of the water, central lightning and black sides are necessary in order to prevent the attachment of the tentacles to the aquarium, which could cause death by starvation. The size and the stage of the cubomedusae must be considered in order to feed them with the right range of prey size. Moreover it is also very important to provide the box jellies different types of prey (i.e. *Artemia*, copepods and *Mysis*), since feeding only on *Artemia salina* seems not to be nutritionally enough for supporting the development.

Future studies are needed to determine the energetic requirements of the different stages of *C. marsupialis* in detail. Such studies could provide more information about trophic connections between *C. marsupialis* and other zooplankton, and therefore the potential predatory impact of *C. marsupialis* in the NW Mediterranean and other areas. Moreover, due to the importance of the polyp stage for jellyfish outbreak formations, it is vital to solve some key questions regarding to the polyp biology, ecology and location. This information will be vital if the ecological role of *C. marsupialis* and environmental issues arising from more frequent blooms of this species are to be understood.

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