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## SHORT COMMUNICATION

# Salinity effects on asexual reproduction of *Carybdea* sp. (Cnidaria: Cubozoa)

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Medusa production in cubozoans may be associated with reduced-salinity waters. Asexual reproduction of *Carybdea* sp. differed at different salinities. The metamorphosis of *Carybdea* sp. polyps into medusae was accelerated at low salinity (32), while more polyps were budded from the original polyp at the highest salinity (38), probably due to prolonged metamorphosis. High-nutrient river discharge may provide favorable conditions for *Carybdea* sp. blooms.

**KEYWORDS:** box jellyfish; budding; jellyfish bloom; metamorphosis; mixed models; survival analysis

Jellyfish blooms have been increasingly reported in recent decades (Brotz *et al.*, 2012; Condon *et al.*, 2012; Purcell, 2012). The main concerns about blooms are due to their ecosystem and socio-economic impacts (reviewed in Purcell *et al.*, 2007) and possible long-term increases of such blooms (Brotz *et al.*, 2012; Condon *et al.*, 2012; Purcell, 2012; Gibbons and Richardson, 2013). Several

authors report the appearance or increased abundance of cubomedusae after seasonal rains (Gordon *et al.*, 2004; Bordehore *et al.*, 2011). Among jellyfish, the Class Cubozoa is particularly interesting due to its visual ecology, mating behavior and toxicity (Bentlage *et al.*, 2010), but the ecology of cubozoans is poorly known and understanding their life cycle and seasonal dynamics of



all life history stages with controlled experiments is urgently needed (Kingsford *et al.*, 2012). Cubozoans have a metagenetic life cycle, characterized by a benthic polyp that can asexually produce more polyps by budding, each of which then can metamorphose into a single medusa (Werner *et al.*, 1971). Most cubozoan species inhabit coastal habitats in tropical to subtropical and even temperate waters of many parts of the world (Kraeuter and Setzler, 1975; Bentlage *et al.*, 2010; Kingsford *et al.*, 2012).

The early life cycle of *Carybdea marsupialis* (von Linné, 1758) was described from polyps found on shells of dead bivalves on the bottom of mangrove channels in Puerto Rico (Studebaker, 1972; Cutress and Studebaker, 1973; Stangl *et al.*, 2002; Fischer and Hofmann, 2004; Straehler-Pohl and Jarms, 2005). Nevertheless, some differences were found between medusae of *C. marsupialis*, from the Mediterranean, and those from Puerto Rico (Acevedo *et al.*, unpublished data), supporting the hypothesis based on molecular data that *Carybdea* in Caribbean waters might not be *C. marsupialis* (Bentlage *et al.*, 2010). The identity of the polyps herein is uncertain and currently under review (Acevedo *et al.*, unpublished data); therefore, we refer to it as *Carybdea* sp.

In the present study, we tested the null hypothesis that different salinity levels do not affect the asexual reproduction of the polyps of *Carybdea* sp. As far as we know, no previous data exist on the effects of salinity on the asexual reproduction of *Carybdea* sp.

Stock cultures of polyps established by Werner in 1971 from south western Puerto Rico (La Parguera) were kindly provided by the laboratory of Ass. Prof. Dr Anders Garm (University of Copenhagen). The polyps were maintained at the Marine Science Institute (Barcelona, Spain) in glass dishes containing 500 mL of 5- $\mu$ m-filtered ambient seawater at 23°C and salinity of 35.

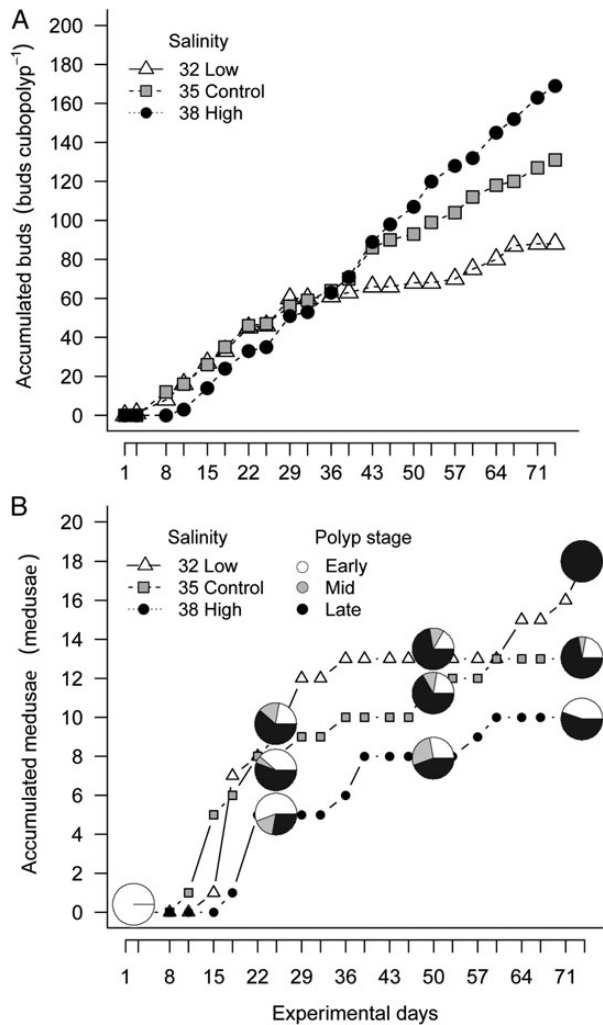
The salinity treatment simulated seawater conditions in Puerto Rico: (i) low rainfall (high salinity level = 38), (ii) the annual average salinity throughout the known polyp area (35) (Otero, 2009) and (iii) high rainfall (low salinity = 32), following Hertler (Hertler, 2002). Before the experiment, polyps were transferred and acclimated to salinity changes by increases or decreases of 15% per day, resulting in a “Rapid Dilution” scheme, following Hartwick (Hartwick, 1991).

At each salinity level, 18 polyps were kept individually in 3 6-well polycarbonate culture plates with 10 mL of 5- $\mu$ m-filtered seawater. The polyps were fed *ad libitum* twice weekly with newly hatched *Artemia salina* nauplii, resulting in equal feeding in all levels of the treatment and were kept in dark conditions and at a constant temperature of 23°C. After the polyps were fed for 1.5 h, the water was replaced with clean filtered sea water of the appropriate salinity and temperature.

Every second day each individual was checked for bud production and metamorphosis state following Straehler-Pohl and Jarms (2005). Detached buds and medusae produced were counted and removed from each well. Parameters evaluated to quantify the asexual reproduction of *Carybdea* sp. were budding index = BI (buds cubopolyp<sup>-1</sup> day<sup>-1</sup>); accumulated buds produced during the experiment = AB (buds salinity<sup>-1</sup>); accumulated medusae produced during the experiment = AM (medusa salinity<sup>-1</sup>); days to metamorphosis = DTM (days) and metamorphosis development = MD. For our analyses, the metamorphosis stages given in Straehler-Pohl and Jarms (Straehler-Pohl and Jarms, 2005) were simplified to: metamorphosis stages a–c = early, stages d–f = mid, and stages g and medusa = late. The experiment ran for 74 days.

Comparisons of the BI among salinities were made using one-way ANOVA. For AB and AM, the effects of repeated measurements on the same individuals were assessed using random slope for each polyp through the generalized linear mixed models (GLMM) with the package lme4 (Bates *et al.*, 2011) setting a Poisson error family and a log link. The DTM was treated as a survival analysis because, after a polyp metamorphosed, it was no longer part of the experiment (i.e. the distribution of remaining polyps followed a Kaplan–Meier survivor function) and the transformation to medusa was considered as “death” data. The survival analysis was done using censored data, because all polyps had not metamorphosed into medusae by the end of the experiment. To do this the *Survival* package (Therneau, 2012) fitting a parametric model using the *survreg* (accelerated failure-time models) function was used to estimate the total number of days to become a medusa for the un-metamorphosed polyps using a GLM with an exponential error distribution. Differences in MD were compared using the Pearson’s  $\chi^2$  test and the *P*-values obtained by Monte Carlo simulation (number of simulations = 2000). All analyses and figures were made using the free statistical software R, version 2.15.0. Data are presented as mean  $\pm$  standard error.

Survival of *Carybdea* sp. polyps was 100% at all salinities and the polyps began budding new polyps on the eighth day (Fig. 1A). The BI (buds cubopolyp<sup>-1</sup> day<sup>-1</sup>) showed no differences between tested (32 and 38) and the control (35) salinities (Table I). The AB (buds salinity<sup>-1</sup>) during the experiment did not differ among salinities (Table I), but the interaction between the days of the experiment and salinity was significant at *P* < 0.01. After Day 43, polyps at low salinity (32) had accumulated fewer buds than those at the control salinity (35) (*t* = -4.1, *P* < 0.0001). In contrast, polyps at high salinity (38) after Day 43 had accumulated more buds than the control (*t*-value -6.36, *P*-value < 0.0001) (Fig. 1A).



**Fig. 1.** Accumulated buds (A) and medusae (B) produced by 18 polyps of *Carybdea* sp. during the 74-day experiment at three salinities (low = 32, control = 35, high = 38) at 23°C. The pie charts in (B) represent the proportion of polyps in three metamorphosis states (early, mid and late) at the beginning of the experiment and at 25-day intervals thereafter.

It is important to note that both budding and metamorphosis into medusae occurred simultaneously during each metamorphosis phase (except “a”) at all salinities (Supplementary data, Fig. S1).

Metamorphosis of polyps into medusae was significantly ( $P < 0.05$ ) affected by the salinity treatment. The average number of medusae produced was higher at low salinity ( $0.89 \pm 0.07$  medusae salinity<sup>-1</sup> day<sup>-1</sup>) than at the control and high salinities ( $0.72 \pm 0.1$  and  $0.56 \pm 0.1$  medusae salinity<sup>-1</sup> day<sup>-1</sup>, respectively) (Fig. 1B). The AM (medusae salinity<sup>-1</sup>) also differed among salinities, showing a negative trend with the increase in salinity (Table I). DTM (days) clearly increased with increasing salinity (Fig. 2A). Survival analysis showed that polyps at high salinity took significantly ( $P < 0.01$ ) longer to

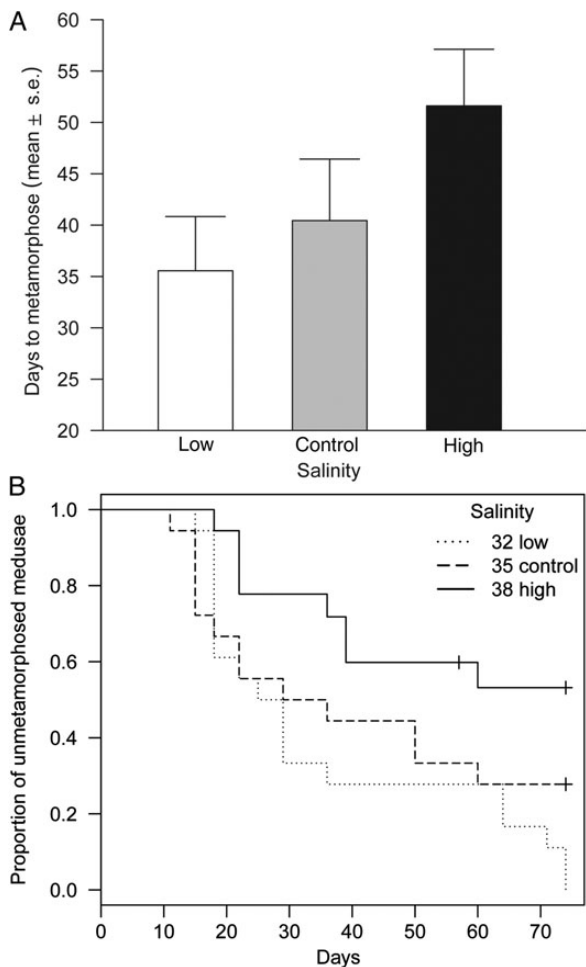
**Table I: Salinity effects on asexual reproduction of *Carybdea* sp.**

Salinity	Polyp production			Medusa production						
	BI (buds cubopolyp <sup>-1</sup> day <sup>-1</sup> )	AB (buds salinity <sup>-1</sup> )	AM (medusae salinity <sup>-1</sup> )	DTM (days)	MD (Early/mid/late)	Day 1 E/M/L	Day 25 E/M/L	Day 50 E/M/L	Day 74 E/M/L	
Low (32)	0.12 ± 0.014	18	35.6 ± 5.3	4/3/11	3/2/13	0/0/18				
Control (35)	0.15 ± 0.016	13	40.4 ± 5.9	7/1/10	4/2/12	4/1/13				
High (38)	0.16 ± 0.017	10	51.6 ± 5.5	10/3/5	5/5/8	8/0/10				
Comparisons	35 vs. 32	35 vs. 38	35 vs. 38	35 vs. 32	35 vs. 32	35 vs. 38	All	All	All	
Statistical test (df)	$F = -1.23$	$F = 0.41$ (2)	$t = -1.26$ (51)	$t = -5.87$ (42)	$z = 1.25$ (2)	$z = 2.79$ (2)	$\chi^2 = 1$ (4)	$\chi^2 = 6.1$ (4)	$\chi^2 = 3.8$ (4)	$\chi^2 = 12.4$ (4)
Significance (P)	0.23 ns	0.82 ns	0.21 ns	<0.001 <sup>c</sup>	0.21 ns	0.005 <sup>b</sup>	1 ns	0.22 ns	0.44 ns	0.004 <sup>c</sup>
Code	32 = 35 = 38	32 = 35 = 38	32 = 35 < 38	32 = 35 = 38	32 = 35 = 38	32 ≠ 35 ≠ 38				

ns, non-significant; BI, budding index (buds cubopolyp<sup>-1</sup> d<sup>-1</sup>); AB, accumulated buds produced (buds salinity<sup>-1</sup>); AM, accumulated medusae produced (medusae salinity level<sup>-1</sup>); DTM, days to metamorphosis (days); MD, metamorphosis development (early/mid/late). Means ± standard error.



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**Fig. 2.** Effects of salinity on the days to metamorphosis for *Carybdea* sp. (A) Number of days until metamorphosis in each salinity and (B) the survival model in which the decay rates of the curves are proportional to the numbers of polyps that transformed into a medusa.

metamorphose than at the low and control salinities (Fig. 2B). The survival model with censored data estimated times for all polyps to metamorphose into medusae to be 36, 56 and 116 days for the low, control and high salinities, respectively (Table I). Differences in the medusa development stage (MD) among salinities were obvious and significant ( $P < 0.01$ ) at the end of the experiment (Fig. 1B, Table I).

Our results show that salinity significantly affects the asexual reproduction of the cubozoan *Carybdea* sp. Salinity variability along the coast of La Parguera (SW Puerto Rico), where the polyps live (Studebaker, 1972), is governed mostly by weather conditions; thus clear weather conditions can increase salinity to 37.7, comparable to our high salinity of 38, and rain storms can reduce salinity  $< 31$ , even in bottom waters (Otero, 2009). These and the annual average coastal salinity (35)

as the control (Hertler, 2002; Otero, 2009) provided realistic levels for our experiment.

*Carybdea* sp. polyps produced medusae more rapidly at low salinity than at control and high salinities. Significant effects of salinity on medusa production have also been shown in hydrozoans (Ma and Purcell, 2005) and scyphozoans (Rippingale and Kelly, 1995; Purcell, 2007; Holst and Jarms, 2010).

The budding index (BI) of *Carybdea* sp. showed no significant differences among salinities (Table I). This BI reflects the numbers of buds produced per polyp before metamorphosis, comparable to the budding rate commonly measured for scyphozoan polyps (Purcell, 2007). The lack of differences among salinities for BI suggests that salinity does not change the budding process; however, salinity affected the time span in which a bud was produced, as reflected by the significant interaction between days and treatment levels in AB, which resulted in a lower proportion of accumulated buds after Day 43 at low (32) salinity.

Salinity affected the time required by *Carybdea* sp. to complete metamorphosis. High salinity extended the days to metamorphosis (DTM). In previous studies, metamorphosis of *Carybdea* sp. polyps was induced by increasing the temperature above a critical threshold ( $24^{\circ}\text{C}$ ) and by suspension of feeding (Stangl *et al.*, 2002). Gordan *et al.* (Gordon *et al.*, 2004) found young medusae of *Chiropsalmus* sp. (= *Chiropsella bronzie*; Gershwin, 2006) after rain events, suggesting enhanced metamorphosis at low salinity conditions. The opposite may be true for the sea wasp (*Chironex fleckeri*; Southcott, 1956), for which small ( $< 2$  mm) medusae appeared when river waters were  $> 25^{\circ}\text{C}$  and at the annual salinity peak ( $> 40$ ) (Hartwick, 1991). Kingsford *et al.* (Kingsford *et al.*, 2012) similarly suggested that high rainfall may negatively affect this species. In contrast, polyps of *Alatina nr mordens* budded at all combinations of temperature ( $18\text{--}31^{\circ}\text{C}$ ) and salinity (22–40); however, metamorphosis was not induced in any of the temperature–salinity combinations over 6 weeks, even when polyps were returned to pre-experimental conditions ( $25^{\circ}\text{C}$  and 32.6) (Courtney and Seymour, 2013). Thus, the importance of salinity changes may be related to the habitat of some species living where salinity changes occur seasonally (i.e. mangroves, estuaries) (Studebaker, 1972; Kingsford *et al.*, 2012).

The effect of salinity seemed to be most important for the rate of metamorphosis of *Carybdea* sp. This idea is supported by both (i) the results of the survival model and (ii) the results from MD. The survival model showed that the DTM at high salinity (38) was more than three times longer than at low (32) salinity (116 vs. 36 d). Although all polyps followed the stages of metamorphosis described for *Carybdea* sp. (Straehler-Pohl and Jarms, 2005), those at

the low salinity completed metamorphosis in significantly fewer days and, consequently, were in a significantly different stage at the end of the experiment (Fig. 1B, Table I). Furthermore, metamorphosis reversed in high salinity (38) during the last week of the experiment; between Days 57 and 67, three of five remaining polyps reverted from mid- to early metamorphosis stage. The other two polyps continued metamorphosis to late stage.

Our results clearly show that salinity affected the asexual reproduction of *Carybdea* sp., mainly modifying the rate of metamorphosis, but also the total numbers of buds and medusae accumulated. Moreover, if we use the total days predicted by the survival model, the numbers of buds generated at the three salinities would be 78, 151 and 334 for the low, control and high salinities, respectively. Thus, the population of *Carybdea* sp. responds to changes in salinity by transforming into medusae at low salinity regimes and by increasing polyp population size with asexual budding at high salinity conditions.

For some cubozoans, salinity changes may be an important signal for metamorphosis. Kingsford *et al.* (Kingsford *et al.*, 2012) noted that planktonic food abundance as well as polyp substrates and retention areas for medusae ultimately may be related to cubozoan abundance. In tropical waters, salinity changes in coastal areas are correlated with rains and river run-off and, consequently, increased coastal productivity (Rippingale and Kelly, 1995; Hertler, 2002; Otero, 2009). As part of the natural seasonal cycle in Puerto Rican coastal surface waters, zooplankton abundance increases in July, following the peak of Chl *a* and the decline in salinity (Yoshioka *et al.*, 1985). In a similar context, Bordehore *et al.* (Bordehore *et al.*, 2011) described an unusually high abundance of *C. marsupialis* associated with low salinity of natural freshwater discharges with increased Chl *a* due to the enhancement of nutrients from anthropogenic (agricultural, sewage discharges) activities along the coast of Denia (Alicante, Spain). Thus, *Carybdea* sp. polyps metamorphose in the wet season and release medusae that benefit from high levels of production in the coastal water column.

## SUPPLEMENTARY DATA

Supplementary data can be found online at <http://plankt.oxfordjournals.org>.

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