Contribution to the ICES/PICES Theme Session: 'Interactions of Gelatinous Zooplankton within Marine Food Webs'

SHORT COMMUNICATION

Regulation of *Cyanea capillata* populations by predation on their planulae

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A series of feeding trials was carried out, offering planula larvae of *Cyanea capillata* to three potential predators, *Mnemiopsis leidyi*, *Mytilus edulis* and *Ciona intestinalis*, alone or mixed with algae or copepods. Mussels and ascidians consumed planulae in significant numbers in all prey combination trials, whereas ctenophores did so only when planulae were offered as sole prey. These results suggest that grazing pressure on scyphozoan planulae may play an important role in regulating medusa blooms.

KEYWORDS: jellyfish; grazers; medusa; scyphozoa; bio-regulation; ctenophores; mussels; ascidians

Although it is the largest and most conspicuous life stage, the adult medusa is just one of several stages in the scyphozoan life cycle and control of jellyfish populations may occur at any of them. Many agree that the success of the asexual benthic stage (scyphistoma) and the survival of the released ephyrae are critical in forming jellyfish blooms (Lucas *et al.*, 2012; Fu *et al.*, 2014); however, among the factors that may affect medusa population blooms, predation is the least studied. Nudibranchs graze scyphomedusa polyps, possibly regulating jellyfish populations in some regions (Hernroth and Grondahl, 1985;

Hoover *et al.*, 2012) and other invertebrates, including amphipods, sea spiders and crabs have also been shown to feed on medusa polyps (Oakes and Haven, 1971).

Although essential for ensuring a successful benthic population, very few studies have been conducted on the survival of scyphozoan planula larvae, from release to settlement. Whereas large numbers of planulae are released into the water column and benthos, especially during swarm events, their ecological roles, both as the seed for polyps and as a source of food for various organisms, have not been studied.

Coastal waters in which blooms of scyphomedusae have been observed are often characterized by a rich and diverse macrofaunal community, including suspension feeders such as benthic bivalves and ascidians, or neritic bloom-forming ctenophores. Shiganova (Shiganova, 1998), Purcell *et al.* (Purcell *et al.*, 2001) and others have shown that swarms of ctenophores can efficiently graze planktonic organisms in coastal waters and may have a large impact on the pelagic food web. Likewise, mussels and ascidians, known to feed mostly on phytoplankton, detrital particles and bacteria, have also been found to consume zooplankton passing close to the seabed (Bingham and Walters, 1989; Davenport *et al.*, 2000), but this has never been tested for scyphozoan larvae.

In order to test our hypothesis that suspension feeders can regulate scyphomedusa populations through predation on their planula stage, it is necessary to show that predation on planulae actually exists. In this study we examined, through laboratory feeding experiments, the predation on *Cyanea capillata* planulae by three common representatives of both pelagic and benthic suspension feeders and discuss their potential role as bio-regulators of medusa populations.

This study was performed at the Sven Lovén Centre for Marine Sciences (Kristineberg Station), Sweden. All species chosen for the study; "predators"—the ctenophore *Mnemiopsis leidyi*, the mussel *Mytilus edulis* and the ascidian *Ciona intestinalis* and "prey"—*C. capillata* planulae are native to this site and abundant. Ctenophores ($20-30$ mm aboral−oral length) were obtained from the station's zooplankton laboratory culture, fed on a diet of *Artemia* and copepods. *Mytilus edulis* ($40-60$ mm length) were collected at <1 m depth from the Gullmar Fjord and *C. intestinalis* ($2.5-5$ g wet weight) were collected from the seawater supply of the research facility and both were placed in running natural seawater for several days until the beginning of the experiments. Mussels and tunicates were checked on a daily basis; open siphons indicated healthy individuals.

Cyanea capillata planulae (length ~200 μm) were chosen as the main experimental prey on account of the high local abundance of medusae (>30 individuals m^{-2}) during the time of the study (July−August). The high fecundity of this species made it easy to harvest planulae; suspending a freshly collected sexually mature female in a 10-L bucket for 1 h yielded $>10^5$ planulae. Planulae were concentrated by means of a 105-μm sieve into a 500-mL beaker and their abundance was determined by counting and averaging abundances in 10 aliquots.

To test feeding preferences (i.e. how the presence of an additional food item affects the percentage removal of planulae) as well as to ascertain good physiological condition, all three predators were also offered additional prey (see below) which they are known to consume in the field and in the laboratory (Petersen and Riisgard, 1994; Waggett and Costello, 1999; Riisgard *et al.*, 2003).

The zooplanktivorous *M. leidyi* was offered the calanoid copepod *Acartia tonsa* (length <1000 μm), while mussels and ascidians, known to feed mostly on phytoplankton, were offered *Rhodomonas* sp. microalgae. In preliminary trials, mussels (unlike ascidians) were found to readily consume copepods, whereupon copepods were also used in mussel mixed-feeding trials.

All experiments were conducted in a darkened temperature-controlled room, set to 17.5°C (ambient seawater temperature) using 0.3-μm-filtered seawater ($31-32$ psu) and following variable pre-trial acclimation periods.

The experimental design (Table I) consisted of the introduction (at t_0) to each of the three predators, of a known number of prey items of one or two prey taxa (i.e. "single" or "mixed") for a given duration and then counting the remaining prey at the end of the predation episode (t_1). Controls simulated the experimental design with no predators present in the vessels. Ten feeding trials and five control trials were performed for each predator−prey combination. Due to logistic constraints, the abundances of planulae introduced into the experimental tanks (Nt_0) were not quantified; rather we introduced aliquots from the stock beaker, calculated to contain a predetermined target number of organisms. Planulae at t_1 (Nt_1) were fixed in 2% acid Lugol's solution and counted individually on the day following feeding experiments. Copepods were counted individually and algal abundances were determined by means of an ELZONE 5380 cell counter, at both t_0 and t_1.

Nt_0 of planulae used for the feeding trials as well as feeding durations were determined following a series of preliminary feeding trials with different prey densities and times. The planula abundance chosen was the minimum number of prey which yielded a noticeable "predation signal" (i.e. comparing control versus predators) within the shortest practical experimental duration. Target Nt_0's of copepods and algae used in these trials were based on the results of previous workers (Reeve *et al.*, 1989; Petersen and Riisgard, 1994; Riisgard *et al.*, 2003).

Our experimental design relied on the basic assumption that when predators are absent, Nt_1 (counted) should roughly equal the calculated Nt_0. However, our results occasionally revealed a notable $Nt_1 > Nt_0$ in the five control vessels. A close examination of the planulae in the control vessels at t_1 confirmed their viability, as they exhibited the same swimming pattern and speed as in t_0. Furthermore, the planulae did not metamorphose into scyphistomae in the vessels or in the stock beaker. Thus, we attribute this

Table I: Experimental design: details of the feeding trials that were conducted for the three suspension feeders, M. leidyi, M. edulis and C. intestinalis

| Predator | # of predators per container | Container volume (L) | # of experimental containers (control/ treatment)[a] | Pre-trial starvation duration (h) | Trial duration (h) | Abundance of prey offered per experimental tank | | | Mixed trials | |
						Planulae[b] (ind L^{-1})	Copepods (ind L^{-1})	Algae[b] (cells mL^{-1})	Planulae and copepods	Planulae and algae
Ctenophore	2	10	15 (5/10)	12	6	100	10	–	+	–
Mussel	1	0.5	15 (5/10)	24	0.5	100	100	8000	+	+
Ascidian	1	0.5	15 (5/10)	24	2	200	–	8000	–	+

[a]Control = no predator, treatment = with a predator.
[b]Calculated target number of planulae at t_0.
"+" and "−" signs denote whether a given combination was offered to each predator.

difference to our lack of success in achieving the desired target number of planulae. Therefore, comparisons were made of Nt_1's of treatments against controls. In order to express the degree of predation, we defined, for each trial, an "apparent % prey removal" as:

$$\% \text{ removal} = \frac{\text{mean} Nt_1 \text{ control} - \text{mean} Nt_1 \text{ treatment}}{\text{mean} Nt_1 \text{ control}} \times 100.$$

Predators, the siphons of which were not open at the end of the trial or that were otherwise deemed physiologically compromised, were excluded from the results. The Mann–Whitney U-test and the Kruskal–Wallis test (IBM SPSS statistics 19 software) were used to test the differences between controls and treatments and between Nt_1s of single and mixed diets. Differences were considered significant at $P < 0.05$. Results of all feeding trials are summarized in Table II.

Mnemiopsis leidyi incubated with planulae as sole prey resulted in a significant reduction (17%) in abundances of planulae relative to controls. When offered a mixed diet of planulae and copepods, however, the difference in Nt_1s was not significant. A visual examination of several *M. leidyi* individuals at the end of the mixed-diet experiment revealed the presence of planulae in the ctenophore body cavities (Fig. 1A and B), whereas copepod (natural prey; Burrell and Vanengel, 1976) remains were not visible. An absence of the remains of copepods in the ctenophore gut implies full digestion of this natural/preferred ctenophore prey. In addition, planulae were also observed as ex-corporeal mucus-covered clusters (Fig. 1C and D). When the ctenophores were offered only planulae, no remains were observed, implying that, in the absence of a "preferred food" (e.g. copepods), planulae may be fully digested. In a different study, prey preference was also observed when *M. leidyi* were offered a mix of *Artemia salina* and cod eggs, consuming the brine shrimp efficiently while ejecting most of the ingested eggs (Jaspers *et al.*, 2011).

The non-motile planulae embedded in mucus were most likely the result of ingestion and regurgitation rather than digestion, and ultimately, these planulae were considered inactive and incapable of settling and developing into polyps. Similarly, Davenport *et al.* (Davenport *et al.*, 2000) found that brine shrimp nauplii ejected as pseudofaeces by mussels could not break free from the sticky mucus in these aggregates. Although our findings suggest that *C. capillata* planulae are not favoured by *M. leidyi*, Javidpour *et al.* (Javidpour *et al.*, 2009) proposed that planulae of *Aurelia aurita* were a major food source for *M. leidyi* in the Kiel Fjord in October, when planulae were most abundant. Inconsistencies in these observations may be the result of different environmental conditions and/or the studied species, but regardless, both studies suggest that ctenophores eliminate planulae from the water column and thus reduce the number of successful scyphistomae.

Unlike the ctenophores, which are tactile predators, both filter feeders, *M. edulis* and *C. intestinalis*, consumed significant numbers of planulae in the various diet combinations offered (i.e. planulae alone, with algae, or with copepods). An increased number of planulae was removed by the mussels in the presence of algae (46% as sole prey versus 64 and 71%, when mixed with algae); however, this difference was not significant ($P = 0.101$, Kruskal–Wallis test). This trend may be explained by findings of Riisgard *et al.* (Riisgard *et al.*, 2003), showing that starved bivalves offered microalgae exhibit an increase in filtration activity, which may have led to greater planulae loss. In contrast, the number of planulae consumed by the ascidian *C. intestinalis* was not affected by the presence of algae, but this may be related to different feeding behaviours and stimuli among different taxa. Planulae remains were not found in either of the filter feeders' experimental containers, suggesting that the ingested planulae were fully digested in both cases. It is noteworthy that both sessile filter feeders were in good physiological condition, based on similar removal proportions of *Rhodomonas* sp. when algae were offered as sole prey.

Table II: Feeding trial results and statistical analyses: Top panel compares the abundances of remaining planula and/or alternative prey numbers (Nt_1) at t_1 between control (=no predator) and treatment (=with a predator) trials

| Predator | Prey (single or in a mixed diet) counted "added for clarity" | Nt_1 control | | | | Nt_1 treatment | | | | | P-value (Mann–Whitney U-test): control versus treatment |
		Mean	Median	±SE	n	% of prey removed	Mean	Median	±SE	n	
Ctenophore	Planulae	738	752	14	5	17	612	630	15	9	0.030
	Planulae (with copepods)	721	727	23	5	8	665	687	26	10	0.327
	Copepods (with planulae)	102	101	2		43	58	60	3		0.020
Mussel	Planulae	41	40	3	5	46	22	22	4	9	0.009
	Algae	7983	7995	68	5	96	288	278	31	10	0.002
	Planulae (with algae)[a]	42	43	4	5	64	15	13	4	10	0.007
	Planulae (with algae)[b]	41	41	2	5	71	12	10	3	9	0.007
	*Algae (with planulae)[b]	7346	7360	93		97	221	224	36		0.003
	Planulae (with copepods)	41	40	2	5	52	20	18	5	10	0.014
	Copepods (with planulae)	53	54	3		54	25	21	6		0.017
Ascidian	Planulae	86	84	3	5	54	39	38	5	10	0.002
	Algae	7982	8002	22	5	65	2780	1464	783	10	0.002
	Planulae (with algae)[a]	101	96	5	5	50	51	51	4	10	0.002
	Planulae (with algae)[b]	89	89	4	5	51	43	47	6	9	0.003
	Algae (with planulae)[b]	9803	9930	116		69	3030	3555	445		0.003

Predator	Trials compared	n*	P-value
Ctenophore	Planulae only versus planulae in the presence of copepods	9/10	0.142 (Mann–Whitney U-test)
Mussel	Planulae only versus planulae in the presence of algae or copepods	9/19/10	0.338 (Kruskal–Wallis test)
Ascidian	Planulae only versus planulae in the presence of algae	10/19	0.168 (Mann–Whitney U-test)

The remaining prey type counted, in each row, were those specified outside the parentheses.
The bottom panel compares planulae predation in single- versus mixed-diet trials. See the text for definition of "% prey removed."
*n, number of replicates of planulae only trials/replicates of mixed-diet trials.
[a]Only planulae were counted at t_1.
[b]Planulae and algae; both counted at t_1.

Our knowledge concerning the regulation of jellyfish populations through predation on their different life stages is still limited and as far as we are aware this is the first study to examine the potential significance of planula predation on the development of jellyfish populations. Our findings may be summarized as follows:

(i) The potential for regulating medusa populations through actual predation on their planulae or through any other manner of inactivation preventing successful settlement exists, as demonstrated by three common representatives of pelagic and benthic suspension feeders.

(ii) Under laboratory conditions, presenting the planulae to the predators mixed with other prey known to be readily consumed by them does not significantly alter the proportions of planulae removed when offered as sole prey.

For decades, coastal regions and near-shore marine ecosystems throughout the world have been subjected to heavy development pressures, and the impact of the anthropogenic activity on key biota in these areas is likely underestimated. Since most researchers attribute the apparent increase in jellyfish populations worldwide to the deterioration of marine ecosystems, depletion of populations of near-shore epibenthos, such as mussels and ascidians, may enhance the proliferation of medusae by reducing grazing pressure on their planulae. The increase in the abundance of pelagic ctenophores in many marine systems (Link and Ford, 2006; Brotz *et al.*, 2012) may also play a role in regulating medusae; even if not their preferable prey, massive consumption of the planula larvae by a large number of ctenophores may diminish their numbers, leading to a reduction in adult medusa populations. Observations from recent years in the eastern Mediterranean Sea (D. Angel and D. Edelist, unpublished data) suggest that alterations between the two taxa may exist where high abundances of ctenophores were usually followed by low numbers of scyphomedusae and vice versa.
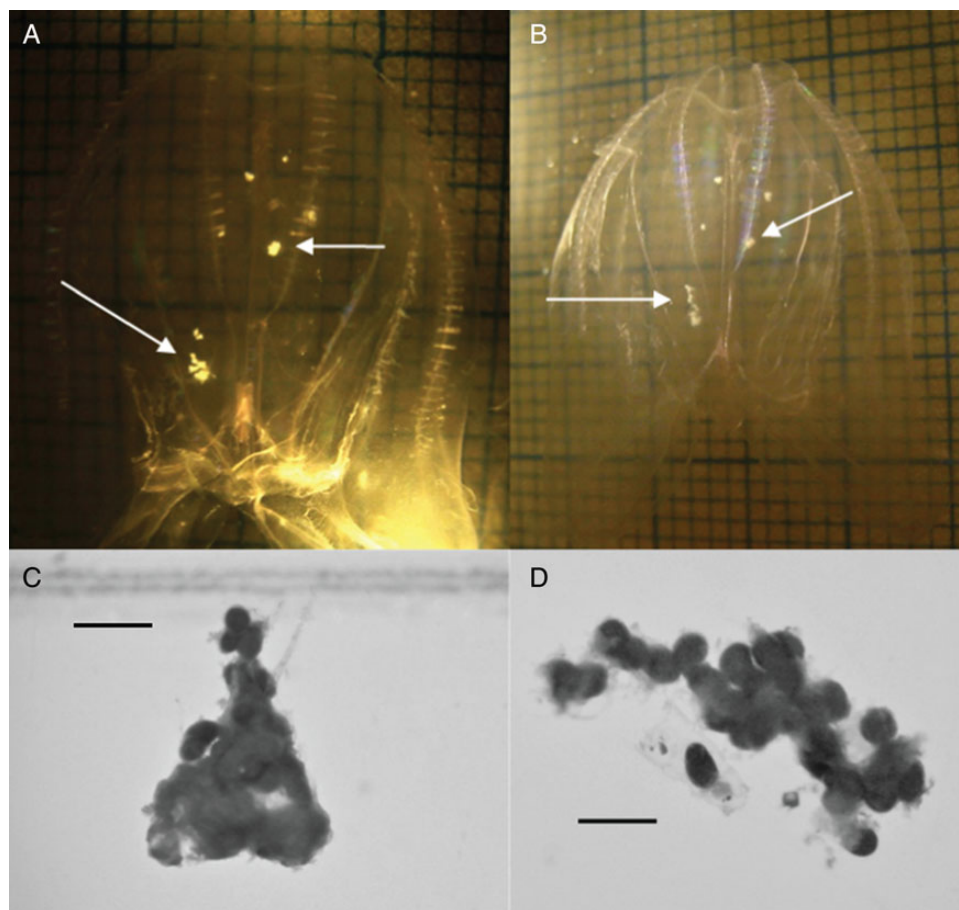
Fig. 1. Planulae observed within *M. leidyi* (**A** and **B**; photographs taken by Nikon Coolpix AW100; squares in the background are 1 mm × 1 mm) at the end of the mixed-diet (planulae and copepods) trials. Arrows indicate the location of the planula clusters. Copepod remains were not observed. Several mucus-covered clusters of planulae were recovered from the bottom of the experimental containers (**C** and **D**; micrographs taken by means of dissecting microscope; scale bars are 200 μm).

The results of this study suggest that predator–prey interactions of some of the key species with scyphozoan planulae in these changing environments may partake in regulating medusae populations and that upsetting of the natural balance of such interactions may be an additional factor promoting their propagation. Assessing the relative importance of the latter among other contributing factors will require further study.

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