SHORT COMMUNICATION

Bloom or non-bloom in the giant jellyfish *Nemopilema nomurai* (Scyphozoa: Rhizostomeae): roles of dormant podocysts

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We suggest that podocysts are responsible for bloom versus non-bloom years of the giant jellyfish *Nemopilema nomurai*. They were capable of dormancy for at least 6 years. Their excystment was very rare (ca. 1%) in well-aerated seawater at constant temperature (19°C), but increased significantly under exposure to abnormally high temperatures (≥ 27°C), low salinities (8–24), hypoxia (1.0 mg O_2 L^{-1}) and burial in organic-rich mud. Their behavior (e.g. maintaining dormancy or mass excystment) may influence medusa population sizes in any given year.

Massive blooms of the giant jellyfish *Nemopilema nomurai* Kishinouye used to be very rare in the East Asian Marginal Seas (Kishinouye, 1922; Shimomura, 1959; Yasuda, 2004), but have become increasingly frequent recently (Kawahara *et al.*, 2006; Uye, 2008). The recent and recurrent nature of the blooms suggests that

anthropogenic environmental conditions conducive to outbreaks (e.g. over-fishing, global warming, eutrophication and increase of marine construction) have prevailed for some time in Chinese coastal waters, the seeding and nursery grounds of this jellyfish species (Uye, 2008, 2011). Anthropogenic environmental perturbations have been argued to be the major causes for jellyfish blooms elsewhere in the world (Purcell *et al.*, 2007, 2012; Condon *et al.*, 2012).

In the population dynamics of scyphozoan medusae, asexual reproduction by benthic polyps has been considered an important determinant of the medusa population size in the following season (Mills, 2001; Watanabe and Ishii, 2001; Arai, 2009). In *N. nomurai*, as in other rhizostome species, chitin-covered podocysts (average diameter and height: 320 and 128 μm, respectively), which contain stored nutritional reserves and can remain dormant for a prolonged period of time, are the only form of asexual reproduction by the polyps (Arai, 1997; Kawahara *et al.*, 2006; Ikeda *et al.*, 2011). Although the function of podocysts in the formation of medusa populations has been little studied (Blanquet, 1972; Black, 1981; Thein *et al.*, 2012), it is possible that their production and excystment may play important roles in the generation of medusa blooms.

Populations of *N. nomurai* show remarkable year-to-year differences in occurrence and abundance off Japan. Massive blooms have occurred since 2002, but did not occur in 2008, 2010 and 2011, when only countable medusae were observed in Japanese waters. It is possible that conditions off China leading to podocyst excystment and encystment have varied between years (e.g. Tanaka and Suzuki, 2005), and so to further our understanding of the mechanisms behind the large annual variations in the *N. nomurai* medusa population size (i.e. bloom or non-bloom), we have conducted preliminary experiments to examine the production, dormancy and excystment of its podocysts under different environmental conditions.

Polyps of *N. nomurai* were derived from artificially fertilized eggs spawned by three females, respectively, in 2005 and 2006 (see Ohtsu *et al.*, 2007), and these polyps were collectively used in the experiments. Plastic (i.e. polystyrene) Petri dishes diameter: 9 cm used as substrates for polyp attachment were placed upside down, so that polyps were suspended from the bottom of the dish in an aquarium containing ca. 10-L filtered seawater under standard conditions [temperature: 19°C, salinity: 33, dissolved oxygen (DO) concentration: ≥ 5.0 mg O_2 L^{-1}]. Dishes were kept in the dark, except for feeding, water change and observation. At weekly intervals, each dish with polyps was put into a plastic container (200-mL volume), to which 300−500 newly hatched *Artemia* nauplii (Utah, USA) were introduced, and the polyps were allowed to feed for 1 day.

Polyps with 16 tentacles from a month-old stock culture were used in this experiment. Eight dishes, each with 37−155 polyps, were individually placed in a plastic container containing ca. 300-mL filtered seawater (salinity: 33, DO: ≥ 5.0 mg O_2 L^{-1}), and kept at eight different temperatures (i.e. 5, 8, 11, 15 19, 23, 27 and 31°C) in the dark. At 2−3-day intervals, 300−500 *Artemia* nauplii were introduced into each container to feed the polyps for 2 h, and then the seawater was replaced. Prior to the experiment, the location of each polyp on the transparent dish was marked with a pen, and the polyps and podocysts were photographed monthly to count the numbers of podocysts produced. Strobilation was monitored at 1−5-week intervals. The experiment ran for 190 days, a sufficient duration for polyps to exhibit their asexual reproduction modes.

Podocysts in two age groups, $\leq$4-month-old and 12−20-month-old, were prepared for this experiment. The older group was produced by the stock culture of polyps during 12 months, when all polyps were removed with a thin metal blade, and the podocysts then kept for 8 more months at 19°C. To identify the environmental factors inducing excystment of these podocysts, 27 dishes, each with 24−204 podocysts, were individually placed in various environmental conditions for various durations as shown in Table I. We assumed the environmental parameters during the stock culture (19°C, 33-salinity, ≥ 5.0 mg O_2 L^{-1}-DO) to be standard conditions suitable for polyp survival. When one parameter was varied, the other parameters remained the same. Hypoxic conditions were created by bubbling nitrogen gas in filtered seawater in glass bottles with a stopper, into which the podocysts were placed for 12 days until return to the standard conditions. They were also buried under a 2-cm thick layer of organic-rich mud, which had been taken from the tidal flat of the Inland Sea of Japan, assuming that burial in mud might create the same condition of hypoxia naturally experienced in the field. They were buried for periods of 12 days and 3 years (only for 12−20-month-old podocysts), before the silt was removed by gentle washing and they returned to the standard conditions. The monitoring of podocyst excystment under the standard conditions ran for 80 days.

Following the excystment experiments, the cuticle covering unexcysted podocysts was artificially removed with a dissecting needle, in order to examine whether the naked cell mass would transform into a polyp or not (Black *et al.*, 1976; Ikeda *et al.*, 2011). In addition, a similar cuticle removal was applied to 10 podocysts at

Table I: Effects of various environmental factors that induce excystment of N. nomurai podocysts

Environmental factors examined	Experimental condition				% of excystment (podocyst numbers)	
	Temperature (°C)	Salinity	DO (mg $O_2\ L^{-1}$)	Duration	1–4-month-old	12–20-month-old
Temperature	5	33	>5.0	80 days	0 (50)	0 (47)
	7				0 (190)	0 (61)
	11				0 (170)	0 (111)
	15				0 (109)	0 (69)
	19 (control)				1 (204)	1 (84)
	23				4 (200)	4 (77)
	27				55 (49)	0 (50)
	31				39 (46)	4 (57)
Salinity	19	8	>5.0	80 days	19 (54)	3 (59)
		16			20 (59)	0 (51)
		24			7 (27)	4 (46)
DO	19	33	1.0	12 days (80 days observation)	13 (24)	8 (80)
Sediment	19	33	(No data)	12 days	14 (45)	25 (60)
				3 years (80 days observation) (80 days observation)	(No data)	9 (43)

See text for detailed experimental methods.

each of various ages (1, 2, 4 and 6 years old) that had remained dormant in the stock culture.

Temperature was a primary determinant of the mode of asexual reproduction in *N. nomurai*. Strobilation was confined to temperatures ≤15°C, while podocyst production occurred mainly at temperatures ≥19°C. As shown in Fig. 1, at both 11 and 15°C, strobilation started a month after cooling from 19°C, followed by a rapid and then gradual increase of strobilation, so that all the polyps strobilated within 4 months. At 8°C, strobilation started 3–4 months later and was complete by the end of the experiment (190 days). At 5°C, strobilation occurred 5–6 months later. In our previous paper (Kawahara *et al.*, 2006), we reported that a sudden temperature increase from 13 to 23°C was a trigger for strobilation of *N. nomurai* polyps, since strobilation took place when polyps were transferred to 23°C after they had been kept at 13°C for 2 months. However, as demonstrated in this study, the physiological cue for

Fig. 1. Temporal changes in frequency of strobilation of *N. nomurai* polyps at various temperatures.

strobilation builds up in the polyps while they are kept at lower temperatures (e.g. ≤15°C) for at least a month.

Polyps of *N. nomurai* only produced podocysts as a means of asexual reproduction by protruding a stolon that attached to a new site 10–300 μm away from the base and the body mass gradually moved to the new position, leaving a podocyst at the former position. Podocyst production was performed by polyps before strobilation, and was confined, in general, to the first 3 months after larval settlement even at 19 and 23°C. Approximately 20% of polyps produced podocysts at 5°C, the proportion producing them increasing at higher temperatures: 77% at 19°C and 74% at 23°C. Polyps placed at 27 and 31°C gradually deformed and detached from the plates within a week without producing any podocysts. The average podocyst production was very low (range: 0.1–0.7 podocysts $polyp^{-1}$) at temperatures ≤15°C, and increased to 1.7 and 3.2 podocysts $polyp^{-1}$ at 19 and 23°C, respectively. The maximum production (16 podocysts) was attained by a polyp kept at 23°C.

When podocysts were kept under the standard conditions throughout, they excysted very rarely (1%, Table I). No podocysts excysted at temperatures ≤15°C. The podocysts kept at temperatures ≥23°C excysted substantially; especially those ≤4 months of age kept at 27 and 31°C (55 and 39% of excystment, respectively; Table I), although these temperatures are sublethal to polyps as shown above. Exposure to lower salinities (8–24) also induced excystment, particularly for the ≤4-month-old podocysts (ca. 20%, Table I), and these salinities are suitable for polyps' survival and growth (unpublished data). The podocysts that had
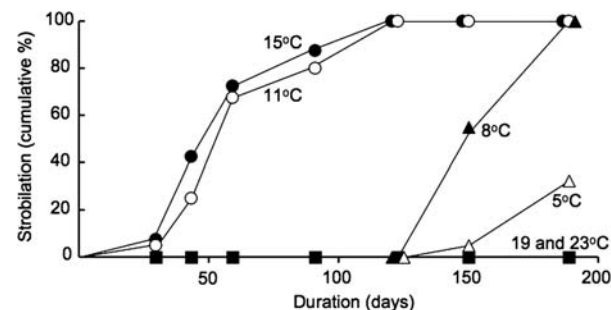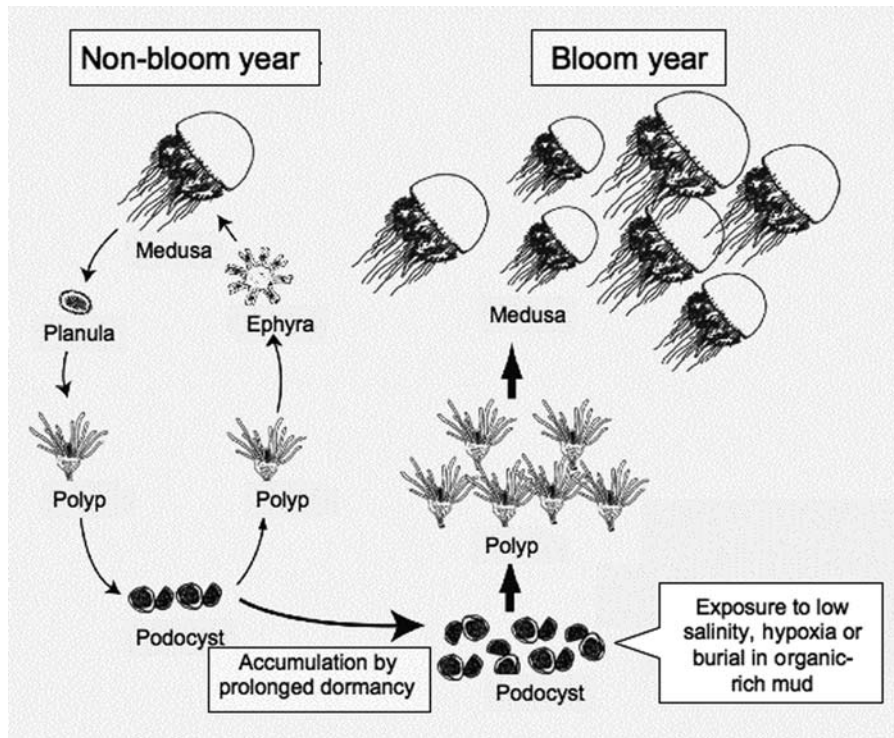
Fig. 2. Schematic representation of the possible distinction in excystment of podocysts into polyps between bloom or non-bloom years for *N. nomurai* medusae in the East Asian Marginal Seas. In non-bloom years, a few podocysts may excyst, whereas in bloom years, a large number of podocysts may excyst.

been exposed to either hypoxia or burial in mud for 12 days were induced to excyst upon return to the standard conditions (Table I). The 12−20-month-old podocysts excysted upon return to the standard conditions, even after 3 years of burial in silty mud (Table I). We confirmed in preliminary experiments that viable podocysts will successfully transform into polyps within 2−7 days after artificial removal of their chitin covering (Ikeda *et al.*, 2011). More than 80% of the podocysts that had remained unexcysted during experiments with changed culture conditions, transformed to polyps on removal of their covering, indicating that most of them were viable.

All podocysts that had been dormant for 1, 2 and 4 years and 80% of the oldest (6-year-old) were successfully transformed to polyps by artificial removal of their covering, indicating that they are capable of dormancy for at least 6 years. This great longevity is due to extremely low metabolism of the dormant cells (Ikeda *et al.*, 2011), in addition to their physical robustness against bacterial attack even in organic-rich silt as well as polyp predators such as the sea snail *Calliostoma unicum* and the majid crab *Hyastenus diacanthus* (unpublished observations). After some, as yet indefinite, dormant period, the podocysts are capable of excysting into newly active polyps, which grow larger and transform into strobilae-producing ephyrae.

Recurrent blooms of *N. nomurai* may have certainly increased its polyp population size in the Bohai Sea, Yellow Sea and East China Sea, although the locations of polyps have not yet been established. Given the characteristics of podocyts of this species, large numbers of them may exist in the benthos of these shallow, murky and productive systems. Thus, their behavior (i.e. encystment and excystment) may very likely play a significant role in influencing medusa population sizes in the following season. As schematically represented in Fig. 2, if only a few podocysts excyst (or the majority of them remain dormant), the total polyp population stays at a low level, and hence a non-bloom year would be expected. On the other hand, if massively accumulated podocysts excyst extensively to build up a large polyp population size, a bloom year would be forecast. *In situ* factors inducing dormant podocysts to excyst are still unclear, but exposure to lowering salinity (Jijun *et al.*, 2008), recovery from hypoxia (Chen *et al.*, 2007; Ning *et al.*, 2011) as well as burial in the mud may be important. Therefore, tiny podocysts may play important roles not only to maintain the population for prolonged periods (at least for 6 years) but also determine the polyp population size, which would lead to problematic blooms of giant medusae.

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