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Evolutionary Meaning of Non-synchronous Medusa Release and Spawning in the Most Advanced Bivalve-Inhabiting Hydrozoan, *Eugymnanthea japonica*

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Circadian spawning times of medusa of the bivalve-inhabiting hydrozoans *Eugymnanthea japonica* Kubota and *Eutima japonica* Uchida are confirmed to be morning (possibly sunrise) for the former species and night for the latter. *Eugymnanthea*, with small, short-lived, univoltine medusae, seems to have evolved from a form similar to *Eutima japonica*, with larger, longer-lived, multivoltine medusae; the morning spawning of medusae in *Eugymnanthea* may therefore be a newly evolved trait. Medusa release from polyps and spawning of medusae are not synchronous in *Eugymnanthea japonica*. This non-synchrony may represent an evolutionarily transitional state leading to the most advanced state, synchrony of these two reproductive events, as in certain other ephemeral hydrozoan medusae.

Key words: bivalve-inhabiting hydrozoan, spawning time, ephemeral medusa, medusa release, transitional evolutionary state

INTRODUCTION

In the Hydrozoa, the release of a mature medusa from a polyp and the spawning of this medusa at specific times of day are ecologically meaningful events for hydrozoans with degenerative, ephemeral mature medusae (medusoids, eumedusoids, swimming gonophores, etc.) (Genzano and Kubota, 2003). The life spans of such medusae are very short and sexual reproduction is carried out once in their life (Yoshida, 1954; Kubota, 1991, 1996; Genzano and Kubota, 2003). Spawning at the time of medusa release is an essential behavior for such medusae if an adequate number of surviving offspring is to be assured. One might expect the most advanced bivalve-inhabiting hydrozoans of the genus *Eugymnanthea*, which have such medusae, to behave in this fashion, but this proves not to be the case (cf. Kubota, 1991). In the present study, the circadian time of spawning of *Eugymnanthea japonica* Kubota, and also that of its putative ancestral form of *Eutima japonica* Uchida, are elucidated for the first time. The differences between species in the timing of these two reproductive events are discussed in an evolutionary context.

MATERIALS AND METHODS

1. *Eugymnanthea japonica* Kubota

Observations on spawning of *Eugymnanthea* medusae are based on specimens obtained in the 1990s at Shirahama, Wakayama Prefecture, Japan, where the hydroids were associated with *Mytilus galloprovincialis* attached to the buoys of a raft (cf. Kubota, 1993b, 1994, 1996). Spawning time is unclear, as the for-

mer supposition (night spawning) is incorrect (Kubota 1991, 2004); the three observations were therefore conducted in the following conditions, using natural seawater (c 32 psu) at Shirahama, Wakayama Prefecture, Japan.

Observation 1

Two individuals of the host bivalve were collected and hydroids with well-developed medusa buds were picked out from the hosts in the daytime. These hydroids were kept in a laboratory aquarium for 12 hrs (at night) under artificial light, and then transferred to a dark room (temperature maintained at 21°C), where they released medusae. Spawning was observed in a total of 64 female medusae (8–11 individuals per trial) during daytime after various time durations in the dark (seven different durations ranging 1 hr to 24 hr). Observations, made at room temperature (ca 26°C) using a stereoscopic microscope, lasted at least 30 min after returning medusae to the light. The temperature of the medium maintained ca 21°C during observations.

Observation 2

One individual of host bivalve attached to a raft was collected in the daytime and kept it in a laboratory aquarium. In the next morning, hydroids with well-developed medusa buds were picked out from the host and transferred to the dark conditions. Medusae obtained were kept for 22 hours under the artificial light. Then most were transferred to a dark room (21°C) for various durations (0.25–3.0 hr) of dark conditions, but some were exposed to light continuously (maximally 31.5 hr in total) as a control. Spawning of all medusae was observed in the daytime following these treatments.

Observation 3

Many mature *Eugymnanthea* medusae of both sexes were collected in the sea at sunset at Shirahama by towing a plankton net in the vicinity of host mussels (Kubota, 1996 etc.). They were transferred into a 60- or 80-cc polystyrene rearing container wrapped with aluminum foil to exclude light. Whereas male and female medusae were kept separate in Observations 1 and 2, medusae of

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the two sexes were placed together in the same container in Observation 3. The container was opened in the next morning after sunrise. Medusae were kept under the natural light conditions and the following day presence or absence of planulae larvae (proof of spawning and fertilization) was checked after confirming heart-shaped cleavage (up to 16 cells) under a stereoscopic microscope. In other trials, the container was kept shut for several days, after which the presence or absence of planulae was checked. Of course the spawning of medusae of both sexes is confirmed in natural light conditions using a stereoscopic microscope.

2. *Eutima japonica* Uchida

The spawning time of female medusae of *Eutima* medusae (intermedia form + northern form: Kubota, 1992a, 1993a, 1997a) was investigated utilizing hydroids from three individuals of *Mytilus galloprovincialis*, one from Atami, Shizuoka Prefecture, Japan, and two from Zagashima island, Mie Prefecture, Japan, collected in the 1990s. The medusae examined included four specimens of the northern form originating from the Atami mussel and six specimens of the intermedia form originating from the Zagashima mussels. These medusae were reared in the laboratory in artificial seawater (Jamarine U) at 21–22°C and under a 12L:12D light-dark cycle (9:00–21:00 L : 21:00–9:00 D), being fed with *Artemia* nauplii every day. They were cultured individually in an 80-cc polystyrene rearing container, and Jamarine U was changed every day. Spawning of eggs was observed over various durations (within 12 hr) of darkness, in medusae of several different ages.

RESULTS

In laboratory, female medusae of *Eugymnanthea japonica* (< 24 hours old) did not spawn eggs under dark conditions for less than 11 hours, although they spawned eggs under the artificially extended darkness (Table 1). When exposed to light after a period of darkness of at least 1 hour, they spawned their eggs (130–160 eggs when all eggs spawned from a medusa) immediately. This represents the sole spawning event in its short life. Spawning began 21–27 minutes ($n = 42$) after exposure to light and finished rapidly, lasting only 1–3 minutes ($n = 10$) at 21°C.

In Observation 2, spawning of all (sometimes in part) eggs in old medusa (> 22 hours old) can also be induced by dark treatment for at least 15 minutes, after an initial light exposure at ca 21°C during observations (Table 2). These old medusae spawned under the artificially elongated lighting (> 31.5 hr) (Table 3).

Table 1. Spawning of medusae of *Eugymnanthea japonica* (Observation 1).

Duration of dark treatments (hr)	No. of medusae examined	No. of spawned medusae*
1	10	9S + 1(S)
2	8	8S
3	11	10S + 1(S)
11	9	5S + 1(S) + 1(S)(A) + 1N + 1N(A)
12	8	4S + 1N + 2N(A) + 1A
18	8	2S + 1N(A) + 5A
24	10	10A

*S: All eggs spawned from all four gonads.

(S): Well-spawned, but not all eggs spawned from some gonads.

N: No spawning from any gonads.

A: All gonads already spawned under dark conditions.

(A): Some gonads already completely spawned under dark conditions.

Table 2. Spawning of medusae of *Eugymnanthea japonica* after dark treatment (Observation 2).

Duration of dark treatments (hr)	No. of medusae examined	No. of spawned medusae*
0.25	7	2S + 1(S) + 4N
0.50	7	3S + 4N
0.75	7	4S + 3N
1	7	5S + 1(S) + 1N
2	7	6S + 1(S)
3	7	6S + 1N

*S: All eggs spawned from all four gonads.

(S): Well-spawned, but not all eggs spawned from some gonads.

N: No spawning from any gonads.

Table 3. Spawning of medusae of *Eugymnanthea japonica* under constant light (Observation 2).

Duration of lighting treatments (hr)	No. of medusae examined	No. of spawned medusae
22	3	3N
23	6	6N
24	3	3N
31.5	3	3A

N: No spawning from all gonads.

A: All gonads already spawned.

Table 4. Number of eggs spawned from individual medusae of northern form of *Eutima japonica* of different ages.

Specimen no.	Age of medusa (in days)	Time (hr) after dark			
		0–2.5	2.5–3.5	3.5–4.5	4.5–12
1	37	26	274	15	1
	41	1	327	23	6
	47	1	952	9	1
2	37	0	494	7	30
	41	0	323	7	1
	47	0	564	2	0
3	37	179	1446	2	0
	47	0	789	2	0
4	37	242	753	1	3
	41	11	540	1	0

Table 5. Number of eggs spawned from individual medusae of intermedia form of *Eutima japonica* of different ages.

Specimen no.	Age of medusa (in days)	Time (hr) after dark			
		0–2.5	2.5–3.5	3.5–4.5	4.5–12
1	15	991	0	0	0
2	15	364	0	0	0
	16	671	0	0	0
3	15	231	0	0	0
	16	682	1	0	0
4	15	86	0	0	0
	16	140	0	0	0
5	15	204	1	0	1
	16	127	3	0	0
6	9	0	196	0	0
	12	528	1	0	0

In the breeding trials (Observation 3), many planulae were produced by the next day after only overnight darkness and subsequent light exposure, but none was ever produced

by medusae kept for several days in the dark. Spawning time of both sexes was the same as the above, i.e. about 30 minutes after light illumination at ca 21°C. Presence of the other sex does not trigger any differences in spawning pattern.

In contrast, *Eutima japonica* consistently spawned eggs in the dark (early night). The northern form of 37–47 day-old medusae spawned 300–1625 eggs in 0–3.5 hours (mainly 2.5–3.5 hours) after dark (Table 4), while the intermedia form of 12–16 day-old medusae (smaller than the former form) spawned 86–991 eggs in 0–2.5 hours after dark (Table 5).

DISCUSSION

Medusae of *Eugymnanthea japonica* spawn within 30 minutes after exposure to light, although these medusae mistake their spawning time under artificially elongated darkness or lighting. This indicates that medusae of this species spawn soon after sunrise in the natural conditions, and that the spawning time is different from the medusa release time (early night: Kubota, 1996, 2008). This time lag between medusa release and spawning has not been shown in other short-lived medusae (Ballard, 1942; Yoshida, 1954; Yoshida et al., 1980; Honegger et al., 1980).

Of the two related species *Eugymnanthea japonica* and *Eutima japonica*, the latter, with a larger, longer-lived, free-swimming medusa, represents the ancestral form of the former, with a small, ephemeral medusa (Kubota, 2000), and it spawned its eggs in the dark (early night), in contrast to *Eugymnanthea*, suggesting that the former's morning spawning (possibly sunrise spawning) may be a newly evolved trait.

However, this trait will likely not contribute to the production of more offspring due to the delay between the release of medusae and spawning, the resulting dispersal of the medusae, and the medusa's short lifespan. Distribution patterns of female and male of *Eugymnanthea* are the same. Both are released as mature medusae very near the mussel bed, ensuring encounters between the sexes. Dispersal of medusae at night, at least in places where water flow is strong, is disadvantageous. Indeed, it is difficult to collect medusae of *Eugymnanthea japonica* at most times of day including night, morning, and daytime, by plankton sampling in Tanabe Bay, Wakayama Prefecture, Japan, excluding sunset, as mentioned above (Kubota, unpublished data).

Synchronous timing of both the release of mature medusae and spawning, to assure fertilization of the eggs before the medusae can be dispersed, would seem to be the most adaptive sexual reproductive system in species with small, ephemeral, univoltine medusae such as *Pennaria disticha* (Genzano and Kubota, 2003). Therefore, *Eugymnanthea japonica* may be considered to represent a transitional evolutionary state prior to the most advanced state, which is synchrony of the two events, as exhibited by such species as *Pennaria disticha*.

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