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Ecophysiological characteristics of podocysts in *Chrysaora pacifica* (Goette) and *Cyanea nozakii* Kishinouye (Cnidaria: Scyphozoa: Semaeostomeae): Effects of environmental factors on their production, dormancy and excystment

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ABSTRACT

Apart from strobilation, podocysts are the only form of asexual reproduction in the semaeostome jellyfishes *Chrysaora pacifica* and *Cyanea nozakii*; they function as means of both the propagation and persistence of their polyp populations. We investigated the effects of temperature, food supply, salinity and dissolved oxygen concentration on the production, dormancy and excystment of podocysts in those two species. In both, podocyst formation by polyps accelerated with increasing temperature and greater food supply, suggesting that summer is the main encystment season. Excystment from podocysts and development of active polyps were induced by lowering temperature or exposure to hypoxia followed by return to aerobic conditions, suggesting that autumn is the main excystment season. Podocysts were capable of dormancy for at least a year, encapsulated in a chitinous capsule and using nutrients stored in their cell mass; thus protecting them from adverse environmental conditions. Global warming and coastal eutrophication (i.e. increase in food supply and frequent hypoxia) may benefit the production and later excystment of podocysts, enabling the medusa populations to bloom more often and extensively.

1. Introduction

Massive blooms of scyphozoan jellyfishes have often been reported as degrading marine ecological services in various areas of the world oceans (Arai, 2001; Brotz et al., 2012; Mills, 2001; Purcell, 2012). Among those, the East Asian seas are one of the hot spots of jellyfish outbreaks, since the semaeostome jellyfish *Aurelia aurita* s.l. and the rhizostome jellyfish *Nemopilema nomurai* have repeatedly caused nuisance blooms that have damaged fisheries and coastal power plant operations (Kawahara et al., 2006; Nomura and Ishimaru, 1998; Uye, 2008). In addition to those species, *Chrysaora pacifica* and *Cyanea nozakii*, both semaeostomes, have often caused problematic blooms that hampered local fisheries (Dong et al., 2008; Dong et al., 2010; Morandini and Marques, 2010; Uye and Ueta, 2004; Xian et al., 2005). Although climatic regime shifts are thought to be responsible for the fluctuations of jellyfish populations (Brodeur et al., 2008; Condon et al., 2012; Lynam et al., 2010), various anthropogenic impacts to the marine environment, such as global warming, eutrophication, overfishing and marine constructions, are also argued to be drivers of the recent increase and expansion of jellyfish blooms (Duarte et al., 2013; Purcell et al., 2007; Richardson et al., 2009; Uye, 2011).

The typical life cycle of scyphozoan jellyfishes alternates between a sexual medusa phase and an asexual polyp phase (Arai, 1997). The polyp phase has recently been shown to affect the population dynamics of the medusa phase, because polyps have high reproductive potential and survivability (Lo et al., 2008; Lucas et al., 2012; Watanabe and Ishii, 2001). Polyps reproduce asexually by direct budding, fission, cyst formation and finally by strobilation to release ephyrae, the earliest planktonic stage (Arai, 1997). Although medusae of *C. pacifica* and *C. nozakii* are largely sympatric with *A. aurita* (Kinoshita et al., 2006; Zhang et al., 2012), one of the most studied semaeostome species, knowledge of their polyp ecology is extremely limited.

A common mode of asexual reproduction by semaeostome polyps is podocyst formation (Dong et al., 2008; Kakinuma, 1967; Thein et al., 2012). Podocysts consist of a mass of cells having rich nutrient reserves and few organelles, and enclosed by a robust chitinous capsule to avoid predation by the natural enemies of polyps, such as nudibranchs, and to prevent infestation by bacteria and fungi (Black, 1981; Blanquet, 1972; Chapman, 1968; Ikeda et al., 2011). Furthermore, podocysts can survive severe hypoxia (<0.5 mg O_2 l^{-1}) in which polyps die (Ishii et al., 2008; Levin et al., 2009; Thein et al., 2012). These physiological characteristics enable podocysts to remain

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dormant for long periods, for example, 3.2 years in *A. aurita* (Thein et al., 2012) and >2 years in *Chrysaora quinquecirrha* (Black et al., 1976). Production and excystment of podocysts are affected by environmental factors such as temperature, food supply, salinity and dissolved oxygen concentration (Guo, 1990; Kawahara et al., 2013; Lu et al., 1997; Thein et al., 2012). Consequently, podocysts compose a regular seasonal stage in semaeostome life cycles; they are produced in summer and excyst to polyps in winter in *Cyanea* sp. in Niantic River estuary, Connecticut, USA (Brewer and Feingold, 1991), and their encystment and excystment occur in late summer and early summer, respectively, in *C. quinquecirrha* in Chesapeake Bay, USA (Cargo and Rabenold, 1980; Cargo and Schultz, 1966, 1967).

Podocyst formation is the exclusive method of asexual reproduction in both *C. pacifica* and *C. nozakii* (Dong et al., 2008; Kakinuma, 1967), as is the case in the rhizostome *N. nomurai* (Kawahara et al., 2013). In *A. aurita* polyps, however, direct budding is the primary reproductive means, and podocysts are only formed by its polyps when they are exposed to scarce food (Han and Uye, 2010; Thein et al., 2012). We hypothesize that the environmental factors influencing podocyst production and excystment differ among the three semaeostome species. Therefore, we have investigated the ecophysiological characteristics of *C. pacifica and C. nozakii* podocysts, their production, dormancy and excystment. In our report here we first survey the morphology of their podocysts. Second, we report our tests of temperature, food supply and salinity effects on podocyst production. Third, we consider environmental cues that could induce podocyst excystment and polyp development. Finally, we discuss the roles of podocysts in the seasonal population dynamics and persistence of *C. pacifica and C. nozakii*, including their medusa population outbreaks.

2. Material and methods

2.1. Effects of environmental factors on podocyst production

Fertilized eggs and planulae were obtained by means of artificial fertilization using gametes from mature medusae of *C. pacifica* and *C. nozakii* collected in the Inland Sea of Japan (Ohtsu et al., 2007). Swimming planulae were transferred to plastic vessels (diameter: 130 mm, depth: 55 mm) containing 500 ml of filtered (0.2 μm) seawater (salinity: 32). They were held in darkness at 22 °C to induce settlement on the bottom. The settled polyps were fed newly hatched *Artemia* nauplii (Utah, USA) at intervals of 2–3 days to establish stock cultures. Polyps 2–3 months old with calyx diameters of approximately 300 μm and 150 μm in *C. pacifica* and *C. nozakii*, respectively, and before podocyst production started, were detached from the bottoms of vessels using a thin metal blade and subjected to the experiments described below.

Effects of temperature and food supply on the asexual reproduction of 5 polyps were examined in each of 30 multifactorial experiments, consisting of 6 different temperatures (established by EYELA MTI-202 incubator, Table 1) and 5 different food regimes (series of carbon supplies correspond to 0, 1, 2, 5, and 7 *Artemia* nauplii polyp^{-1} d^{-1}, respectively, Table 1). Detached polyps were individually placed in the wells of 6-well polystyrene culture plates, each containing 10 ml of filtered, oxygen-sufficient (≥5.0 mg O_2 l^{-1}), seawater of salinity 32 and held in darkness, except during feeding, water change, and observation. The specified numbers of *Artemia* nauplii were provided near the polyp tentacles using a pipette. Numbers of podocysts produced and of strobilating polyps were counted daily for 8 weeks. Combined effects of temperature and food supply on the podocyst production were examined with two-way analysis of variance (ANOVA) using SPSS 10.0 software. When the overall ANOVA results were significant, Tukey's pair-wise comparisons were performed to test among experimental combinations.

Under constant temperature (22 °C) and a low-food regime (2.4 μg C polyp^{-1} d^{-1}), the effect of salinity was examined by placing polyps individually in 6-well plates containing seawater of 7 different salinities (5 polyps at each level, Table 1) for 8 weeks. The cumulative numbers of podocysts produced in different salinities were analyzed with one-way ANOVA. When the overall ANOVA results were significant, the means were compared using Tukey's pair-wise comparisons.

2.2. Effects of environmental factors on podocyst excystment

Prior to the experiment, polyps of both species were cultured in polystyrene dishes (92 × 92 × 18 mm) at both 18 and 28 °C with excess *Artemia* nauplii (>16.5 μg C polyp^{-1} d^{-1}) for a month to obtain enough podocysts for the experiment. Thereafter, all the polyps were removed with a blade and only podocysts were left on the dishes until subjected to the experimental conditions.

Batches consisting of 20–30 *C. pacifica* podocysts and 10–14 *C. nozakii* podocysts, all ≤1 month old, were prepared in triplicate for experiments to examine the effect of temperature on their excystment. The podocysts produced at 28 °C were transferred directly to 19 and 11 °C, as well as in stepwise fashion to 25, 22, 19, and 13 °C for 3 weeks each, mimicking the seasonal temperature decline (Table 1). The podocysts produced at 18 °C were directly transferred to 22 and 28 °C (Table 1). Excystments were monitored for 12 weeks. Additional podocysts were maintained and monitored as controls at 28 and 18 °C throughout the experiments.

To examine the effect of dissolved oxygen concentration (DO) on excystment, 30 podocysts (≤1 month old) of each species were detached from the stock culture dishes. They were transferred to BOD bottles (ca. 100 ml volume) containing seawater of low dissolved oxygen concentration (target DO: 0.5 mg O_2 l^{-1}) created by bubbling nitrogen gas at 22 °C (Table 1). After 14 days of incubation in hypoxia, they were returned to aerated (≥5.0 mg O_2 l^{-1}) seawater to monitor excystment for 14 days. Additional podocysts were kept in aerobic

Table 1
Experimental conditions to examine the effects of temperature and food supply, salinity, and dissolved oxygen concentration (DO) on the podocyst production and excystment of *Chrysaora pacifica* and *Cyanea nozakii*.

Environmental factor tested	Experimental conditions				
	Temperature (°C)	Food supply (μg C polyp^{-1} day^{-1})	Salinity (mg O_2 l^{-1})	DO (weeks)	Duration
1. Podocyst production					
Temperature and food supply	5, 11, 18, 22, 26, 28 ± 0.3	0, 2.4, 4.8, 12.1, 16.9	32	>5.0	8
Salinity	22	2.4	5, 10, 15, 20, 25, 30, 32	>5.0	8
2. Podocyst excystment					
Sudden temperature increase	28 to 19 or 11	0	32	>5.0	12
Sudden temperature decrease	18 to 22 or 28	0	32	>5.0	12
Stepwise temperature decrease	28-25-22-19-13	0	32	>5.0	3 per step
DO	22	0	32	0.5	2 (followed by 2 weeks in aerated seawater)