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Planktonic ciliates as food for the scyphozoan *Aurelia aurita* (s.l.): Effects on asexual reproduction of the polyp stage

Takashi Kamiyama *

National Research Institute of Fisheries and Environment of the Inland Sea, Fisheries Research Agency of Japan, 2-17-5 Maruichi, Hatsukaichi, Hiroshima 739-0452, Japan

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ABSTRACT

The value of planktonic ciliates as prey for the asexual reproduction of the polyp stage of the moon jellyfish (*Aurelia aurita*) was investigated by monitoring somatic growth (calyx diameter), bud production and prey consumption of polyps on a diet of the tintinnid ciliate *Favella taraikaensis* labeled with a stable nitrogen isotope (^{15}N). These results were compared with those for polyps on a diet of metazoan larvae (*Artemia* spp.). In addition, nitrogen and carbon specific gross growth efficiencies on the ciliate diet were estimated from increased ^{15}N content of polyps and consumption of ciliate ^{15}N, and from the increase of polyp dry-weight (somatic growth and bud production), a weight:carbon factor and consumption of ciliate carbon, respectively. The calyx diameter of polyps increased with incubation time during the first 7–10 days with ciliate prey and during the first 11–15 days with *Artemia* prey. Bud production started after a lag period of 6–7 days in all prey treatments, and the cumulative bud number increased with incubation time. The mean bud production rates after the initial lag period were higher with larger amounts of prey and estimated at 0.08–0.38 ind polyp^{-1} d^{-1} with ciliate prey and 0.12–0.35 ind. polyp^{-1} d^{-1} with *Artemia* prey. The mean relative change in calyx diameter with *Artemia* prey between day 10 and day 15 was significantly higher than that with ciliates at the same prey carbon supply, but bud production rate on a diet of *Artemia* consumed at 5 μg-C polyp^{-1} d^{-1} was significantly lower than that on a diet of ciliates consumed at the same rate. Promotion of bud production of polyps on a diet of ciliates rather than the somatic growth may be related to fragility characteristic of the ciliate prey, which are easily digested and absorbed. The nitrogen specific gross growth efficiency of *A. aurita* polyps on a diet of ciliates ranged from 59% to 78% (mean 68%). The relatively high values were supported by high carbon specific gross growth efficiencies ranging from 42% to 64% (mean 54%). This characteristic of polyp may be due to the small size and the low metabolic loss of assimilated energy source. These results indicate that planktonic ciliates, which are readily available to polyps in nature, can serve as a sufficient diet for asexual production of *A. aurita* polyps.

1. Introduction

Gelatinous plankton such as the scyphomedusae, ctenophores and cinidarians have increased in abundance in various estuarine and ocean ecosystems worldwide. The mechanisms behind these increases are not well known but climate effects and anthropogenic changes may be important factors (Purcell, 2005; Purcell et al., 2007). For example, jellyfish biomass (primarily medusae of *Chrysaora melanaster*) in the Bering Sea and Gulf of Alaska increased dramatically from 1990 to 1997, possibly in association with a climate shift to increasing solar radiation and sea surface water temperature (Purcell, 2005). In addition, populations of the scyphomedusan jellyfish *Aurelia aurita* (Linnaeus) have apparently increased since the 1980s in the Seto Inland Sea of Japan, partly because of increasing winter minimum sea temperatures, a decline in the planktivorous fish population, evident in the decreased catch, and an increase in polyp-attachment areas resulting from waterfront construction projects (Uye and Ueta, 2004; Uye et al., 2003).

Most jellyfish compete with planktivorous finfish by consuming the same food resources (e.g., copepods) and are potential predators of fish eggs and larvae (Möller, 1980, 1984; Olesen, 1995; Purcell, 1997; Schneider and Behrends, 1994). Hence, an increase in jellyfish biomass may reduce the fish standing stock and commercial harvest. In addition, blooms of large scyphomedusae have hampered commercial fishing activities by clogging and bursting trawl nets (Shimomura, 1959; Uye and Ueta, 2004) and have caused problems to coastal power plants by blocking intakes for cooling water (Rajagopal et al., 1989). Such negative effects of jellyfish blooms make it imperative to understand the mechanisms underlying the increase in their biomass and the occurrence of blooms.

A. aurita is a widespread species in temperate coastal waters around the world, and dense aggregations of this species (jellyfish

Abbreviations: ESD, equivalent spherical diameter; GGE, gross growth efficiency.

* Tohoku National Fisheries Research Institute, Fisheries Research Agency of Japan, 3-27-5 Shinhama, Shiogama, Myagi 985-0001, Japan. Tel.: +81 22 365 9827; fax: +81 22 367 1250.
E-mail address: kamiyama@affrc.go.jp.

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blooms) often occur in spring and summer (Hernroth and Gröndahl, 1985; Ishii et al., 1995; Lucas, 1996; Lucas and Williams, 1994; Möller, 1980; Omori et al., 1995; Schneider, 1989). The ecology of the planktonic (medusa) stage of this species has been studied because dense blooms of this stage are the direct cause of the various problems mentioned above. However, *Aurelia* spp. usually has a benthic (polyp) stage, in which individuals are usually attached to floating material or coastal structures. Polyps can generate additional polyps by asexual budding and release numerous planktonic ephyrae (small medusae) through strobilation. Hence, the size of *A. aurita* populations probably reflects the success of the benthic polyp stage in interspecific competition for space with other sessile organisms and in asexual reproduction (Ishii and Katsukoshi, 2010; Ishii and Watanabe, 2003).

Increases in the number of polyps and the release of ephyrae are strongly influenced by environmental factors such as temperature (Han and Uye, 2010; Liu et al., 2009; Ma and Purcell, 2005; Purcell, 2007; Purcell et al., 1999), light (Liu et al., 2009; Purcell, 2007) and abundance of suitable food (Båmstedt et al., 2001; Ishii and Watanabe, 2003; Purcell et al., 1999) during the polyp stage. Stomach content analysis suggests that the main food of scyphomedusae such as *A. aurita* is mesozooplankton (Arai, 1997). *A. aurita* in particular can feed on food particles over a range of sizes, in contrast to planktivorous fish, which use vision to select large food organisms (Eiane et al., 1999). Therefore, small copepods, such as *Oithona* spp., which often dominate total zooplankton biomass in eutrophic embayments, are considered to be an important food source for *A. aurita* in such habitats (Ishii, 2001).

Microzooplankton, consisting mainly of protozooplankton, is also a numerically important component of marine zooplankton communities worldwide (e.g., Pierce and Turner, 1992). However, there is little information on the availability of microzooplankton as a food source for scyphozoan jellyfish, and the quantitative data are mostly limited to the medusa and ephyra stages (Båmstedt, 1990; Fukuda and Naganuma, 2001; Olesen et al., 1996; Stoecker et al., 1987; Sullivan et al., 1994). Because polyps and ephyrae are considerably smaller than medusae, it is logical to expect that they can more efficiently feed on microzooplankton. Polyps are sessile and ephyrae have limited swimming ability, so a high density of microzooplankton would be needed to increase the chance for these stages to encounter this prey.

In Tokyo Bay, the high abundance of polyps, inferred from the high abundance of medusae, is likely supported by a high density of microzooplankton (Ishii, 2001). By conducting laboratory culture experiments, Kamiyama (2011) confirmed that ciliates, a main component of microzooplankton, are actively utilized as prey by *A. aurita* polyps and assimilated into the polyp body. However, the effects of microzooplankton on the growth of *A. aurita* polyps and the growth efficiency of polyps on a diet of microzooplankton are still unknown.

In the present study, laboratory experiments were conducted to investigate the asexual reproductive ability of *A. aurita* polyps on a diet of planktonic ciliates. The results were compared with those for polyps grown on a diet of nauplii of *Artemia* spp., which is representative of mesozooplankton. Furthermore, the gross growth efficiency (GGE) of polyps on a diet of ciliates was estimated on nitrogen and carbon bases.

2. Materials and methods

2.1. A. aurita polyps and ciliates

Spawning *A. aurita* medusae were captured in Hiroshima Bay and brought to the laboratory, where the released planulae metamorphosed into polyps. The polyps were maintained in plastic cups containing filtered seawater at 18–25 °C under 300 μmol photon m^{-2} s^{-1} with a 12 h:12 h light–dark cycle and provided with newly hatched nauplii of *Artemia* spp. (hereafter, '*Artemia*') as a food source.

The planktonic tintinnid ciliate *Favella taraikaensis* was isolated from natural coastal seawater from Sendai Bay, northern Japan, in June 2009, and a clone strain of this species was established. Stock cultures of ciliates were maintained at 20 °C in 250-ml polycarbonate flasks containing 150 ml of autoclaved filtered seawater enriched with ethylenediaminetetraacetic acid (EDTA) and a trace-metal solution (ciliate culture medium; Stoecker et al., 1988). The ciliates were fed with a mixture of the dinoflagellates *Heterocapsa triquetra* and *Gyrodinium* sp., which had been maintained in F/4 medium (Guillard and Ryther, 1962) at 20 °C, at a final density on the order of 10^3 and 10^4 cells ml^{-1}, respectively.

For the growth experiments described below, the stock culture of *F. taraikaensis* was labeled with the stable nitrogen isotope ^{15}N via prey consumption. First, to label the ciliate diet, 0.5 ml stock culture of *H. triquetra* or *Gyrodinium* sp. was poured into 60 ml of F/4 medium containing ^{15}N–$NaNO_3$ (^{15}N: 98 atom%, Isotec Inc.) as a nitrogen source, and these cultures were incubated at 18 °C for 2–3 weeks under an irradiance of about 100 μmol photons m^{-2} s^{-1} on a 12 h:12 h light–dark cycle. The ^{15}N-labeled *H. triquetra* (^{15}N-*Ht*) and ^{15}N-labeled *Gyrodinium* sp. (^{15}N-*Gy*) were maintained by transferring some of the culture every 14–21 days into fresh F/4 medium containing ^{15}N–$NaNO_3$.

A portion of the stock culture of *F. taraikaensis* (1–2 ml) was added to a 250-ml polycarbonate flask containing 150–170 ml of fresh ciliate culture medium and ^{15}N-*Ht* and ^{15}N-*Gy* on the order of 10^3 and 10^4 cells ml^{-1}, respectively. When the ^{15}N-labeled *F. taraikaensis* (^{15}N-*Ft*) grew to the density of more than 10 cells ml^{-1}, part of the culture was transferred into a new flask prepared as described above. This procedure was repeated to maintain the ^{15}N-*Ft* stock culture. During the growth experiments described below, 10 ml ^{15}N-*Ft* stock culture was transferred into two or three new culture flasks every 2 days, and at least one flask containing a high-density ^{15}N-*Ft* suspension (7–70 cells ml^{-1}, $n = 34$) was prepared every day until 2 days before the end of incubation (13 days).

2.2. Experimental design

For the following experiments, individual polyps in the stock culture were moved one at a time into the wells of three 12-well multi-plates, each containing about 3 ml of filtered seawater. All three plates were then stored at 20 °C for 10 days to allow the polyps to attach to the bottom of each well. Polyps with similar body size (mean ± standard deviation of calyx diameter, 843 ± 134 μm, $n = 30$) were selected randomly for the following experiments but their ages in day after asexual reproduction were not able to be determined.

Somatic growth and asexual reproduction were monitored for polyps under two types of prey treatments (^{15}N-*Ft* and *Artemia*) with different prey densities. For the ciliate prey treatment, ^{15}N-*Ft* was diluted with filtered seawater to four density levels of 33%, 67%, 83% and 100% of the stock culture density each day, equating to mean densities over the entire incubation period of 13, 25, 32 and 38 cells ml^{-1}, respectively. Three milliliters of each ciliate dilution was then added to 4 wells. For the *Artemia* prey treatment, 12 wells containing 3 ml of filtered seawater were prepared. Then 2, 5 or 10 individual *Artemia* were inoculated into each of four wells to final densities of 0.7, 1.7 or 3.3 ind ml^{-1}, respectively. Another 5 wells containing 3 ml of filtered seawater only were prepared as a treatment with no prey (i.e., control).

All treatments were incubated with gentle agitation using a rocking mixer at 20 °C under an irradiance of around 60 μmol photons m^{-2} s^{-1}. For the ciliate prey treatment, the prey remaining after 2 to 4 h of incubation was counted as described below. Then, each ciliate treatment was replaced with a new ciliate suspension at the same dilution level of the stock culture. This procedure was repeated one to three times per day. The daily carbon supply at the four ciliate density levels corresponded to 2.3 to 6.8 μg-C $polyp^{-1}$ d^{-1} (Table 1).