ミズクラゲ餌料としての微小動物プランクトン：現場海水中でのポリプ期の増殖と捕食応答

Microzooplankton as a food source for the scyphozoan *Aurelia coerulea*: growth and feeding responses of the polyp stage in field assemblages

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Running head: Growth of *Aurelia* polyps in field seawater

Abstract

To evaluate the growth and feeding responses of the polyp stage of the moon jellyfish *Aurelia coerulea* to natural microzooplankton assemblages, bud production among *A. coerulea* polyps was monitored in field bottle incubation experiments using fractionated field seawater (<200-μm fraction) in summer. During this incubation period, feeding rates were measured twice by examining changes in the abundance of various microzooplankton taxa over two days. The number of buds increased with incubation period, reaching a mean of 5.8–9.3 buds per polyp after 16 days, at which point the carbon content of the new buds and the mother polyp was estimated to be 1.3–2.5 times higher than the carbon content of the initial polyp. Using these carbon content estimates, I calculated specific growth rates of 0.1–0.2 d^{-1} during the first 10 days. The results of the present feeding experiments suggest that polyps utilize diverse groups of microzooplankton and achieve relatively high carbon ingestion rates from ciliates, dinoflagellates, molluscs, and copepod nauplii. Total microzooplankton ingestion rates were estimated to be 4.05 and 3.27 μgC $polyp^{-1}$ d^{-1} in the two experiments, respectively. These findings show that natural microzooplankton assemblages play a role as prey of polyps and can promote asexual reproduction of polyps under natural summer conditions.

Keywords: asexual reproduction; *Aurelia*; feeding selectivity; ingestion; jellyfish

Introduction

The scyphomedusan jellyfish *Aurelia coerulea* von Lendenfeld, 1884 is a common species in temperate waters in the northwestern Pacific, Australia, west coast of the USA, Mediterranean and Atlantic coast of Europe (Lawley et al. 2021). Dense aggregations of this species have hampered commercial fishing by clogging and bursting trawl nets (Yasuda 1988, Uye & Ueta 2004) and have caused problems for coastal power plants by blocking cooling water intake pipes (Rajagopal et al. 1989). In addition, an increase in jellyfish biomass may reduce fish standing stocks and hurt commercial fisheries because most jellyfish compete with planktivorous fish for food resources (e.g., copepods) and are potential predators of fish eggs and larvae (Möller 1980, 1984, Schneider & Behrends 1994, Olesen 1995, Purcell 1997). In view of these negative effects, it is imperative to understand the mechanisms underlying increases in jellyfish biomass and the occurrence of blooms.

To clarify these mechanisms, it is essential to improve our understanding of the polyp and ephyra stages of the jellyfish lifecycle. The polyp stage is the only stage that can generate additional polyps by asexual budding and release numerous planktonic ephyrae through strobilation. So far, several studies of scyphozoan polyps have described potential growth activity during this stage and elucidated the effects of temperature and food availability

52 (Purcell et al. 1999, Ishii & Watanabe 2003, Liu et al. 2009, Han & Uye 2010, Kamiyama
53 2013). However, the growth response of the polyp stage to natural planktonic assemblages
54 remains poorly understood.

55 Microzooplankton are a numerically important component of marine zooplankton
56 communities worldwide (e.g., Pierce & Turner 1992). Although there is some information on
57 the availability of microzooplankton as a food source for scyphozoan jellyfish, quantitative
58 data for the polyp stages are more limited (Kamiyama 2011, 2013). Because polyps are
59 considerably smaller than medusae, it is logical to expect them to feed more efficiently on
60 microzooplankton. Furthermore, because polyps are sessile, they may have fewer
61 opportunities to encounter less abundant prey items. Hence, microzooplankton, which occur
62 at 1–2 orders of magnitude higher abundance than meso- and macrozooplankton (e.g., Uye et
63 al. 1996, Uye & Shimazu 1997), may be an essential prey source during this stage.

64 Laboratory culture experiments have shown that *A. coerulea* polyps can feed on ciliates,
65 the main component of microzooplankton, and can assimilate their food energy to efficiently
66 produce buds (Kamiyama 2011, 2013). However, the quantitative effects of microzooplankton
67 on *A. coerulea* polyps under field conditions remain to be clarified.

68 Here, I examined the growth response of polyps of *A. coerulea* in natural
69 microzooplankton assemblages in field experiments. In addition, two field-bottle-experiments

were conducted during the polyp growth experiment to investigate taxon-specific feeding rates in natural seawater. Based on the results of these experiments, the role of microzooplankton on polyp growth under field conditions was evaluated. The present study is the first to examine growth and feeding responses of polyps to a variety of microzooplankton taxa in field seawater.

Materials and Methods

Aurelia coerulea polyps

Three individuals of brooding *A. coerulea* medusae were captured by scooping with a hand net in Hiroshima Bay in summer, 2008 and brought to the laboratory. They were reared in a 100-l *Artemia* hatchery tank at ca. 20 °C by providing *Artemia* nauplii as prey. Then, planulae naturally discharged from the medusae metamorphosed to polyps, and then adhered to the bottom and side of the tank. Some of them were selected to establish a stock culture of *A. coerulea* polyps. The stock polyps were maintained in plastic cups containing filtered seawater at 20–25 °C under irradiances of 30–100 μmol photons m^{-2} s^{-1} with a 12 h:12 h

87 light–dark photocycle and were fed in excess with newly hatched nauplii of *Artemia* sp. two
88 to three times a week.
89
90 **Preparation and experimental design for field growth experiment**
91
92 For the field growth experiment, *A. coerulea* polyps with similar body size were randomly
93 selected from a stock culture, and then inoculated one by one into each well of 4-well plates,
94 which were produced by cutting 24-well multi-well plates (Fig. 1A), and were incubated in
95 filtered seawater without supply of prey at 25 °C in the dark for one week to facilitate
96 settlement of the polyps on the bottom of each well. Then, the end of a nylon line (ca. 10 cm)
97 was connected to the corner of each plate, and a small float was attached to the opposite side
98 of the line to suspend each plate in an incubation bottle (Fig. 1A, B). This system allowed
99 plates to move and helped polyps to encounter prey in the bottles. Seawater for the
100 experiments was collected one day before the experiment with a Van-Dorn water sampler
101 from a depth of 2 m at the study site for the following experiments and was passed through
102 glass microfiber filters (Whatman GF/C, pore size 1.2 μm). The filtrate was used to fill two
103 1,000-mL polycarbonate bottles, and the prepared plates were placed in the bottles. Then, the
104 bottles were suspended at 2-m depth at the study site for one day to acclimate the polyps to

105 natural environmental conditions. Since some polyps did not adhere to the wells on the plates
106 in the settlement process or peeled from the plates and dropped in the bottle in the acclimation
107 process, it was impossible to prepare the plates with 4 polyps attached for all treatments.
108 Hence, plates with 3 polyps attached were also used for the following experiments.
109 The field growth experiment was conducted at the study site (34°16'31"N, 132°16'1"E, the
110 port for research ships in Hatsukaichi field station of the Japan Fisheries Research and
111 Education Agency) located in western Hiroshima Bay, the Seto Inland Sea of Japan. The
112 experiment began on 23 August 2011 and lasted 16 days. Seawater collected with a Van-Dorn
113 water sampler at the site at a depth of 2 m (ca. 12 L) was passed through a 200-µm plankton
114 net (hereafter "the <200-µm fraction"). Temperature and salinity were monitored using a
115 conductivity–temperature–depth (CTD) meter (ASTD687; JEF-Advantec), and the
116 chlorophyll-*a* concentration on a Whatman GF/F filter was measured with a fluorometer
117 (10Au; Turner Designs) using the N,N-dimethylformamide extraction method on 120- or 124-
118 mL seawater samples. Before setting up each growth experiment, a 200-mL seawater sample
119 (the <200-µm fraction) was fixed with Lugol's iodine solution (final concentration: 5%) and
120 stored at ca. 5 °C in the dark to monitor microzooplankton biomass and community
121 composition.

A portion of the seawater (ca. 4 L) was filtered through Whatman GF/C filters (hereafter "the GF/C filtrate fraction"). The <200-μm fraction was used to fill four 1,000-mL polycarbonate bottles for the experimental treatment, and the GF/C filtrate fraction was used to fill three bottles for the no-prey control treatment. In both cases, each bottle was filled to the brim (seawater volume: 1,090 mL). Then, a plate with 4 polyps was set in each experimental bottle, and with 3 polyps was put in the control bottle. All the bottles were then suspended at a depth of 2 m from a float on the sea surface, being tied to a stainless-steel ring with a rope, which was intended to facilitate the movement of seawater inside the bottles (Fig. 1C). Two days later, all bottles were retrieved, and the plates were taken out. Filtered seawater was then poured into all wells of a given plate, and all polyps were photographed using a stereomicroscope (SZX9, Olympus) and a digital camera (Camedia SP 350, Olympus), as described below. Subsequently, the experimental and control bottles were filled again with freshly collected seawater (< 200 μm fraction and GF/C filtrate fraction, respectively) and the polyp plates were re-deployed into them. This procedure was repeated every other day for 16 days. Unfortunately, 2 out of 3 control treatment bottles were lost to waves during the field incubation period (day 4 and 10). Hence, one remaining bottle containing 3 polyps was used to evaluate the no-prey control treatment.

Polyp growth analysis

Before plated polyps were placed in each bottle, I took photographs of the original polyps and of any new child polyps (buds) produced from the stalk or pedal stolon in filtered seawater, and then the number of buds produced by the original polyps and their calyx diameters were examined on the digital images using Scion image software (Scion Corp.) and Image J software (NIH, USA; http://rsbweb.nih.gov/ij/). Carbon contents of polyps and buds (PC; μgC ind^{-1}) were estimated using the following calyx diameter–dry weight equation for *A. coerulea* polyps (Kamiyama 2013) and a carbon–dry weight conversion factor of 0.233 μgC μg^{-1} (Kamiyama 2011):

$$PC = 0.233 \times e^{(1.81 \times \ln(CD) - 8.06)} \quad (1)$$

where CD (μm) is calyx diameter. Here, it was assumed that the equation and conversion factor were also applicable to buds produced from a polyp.

To evaluate the growth of polyps, bud production rates and carbon growth rates were calculated from the slopes of linear regressions of cumulative bud number per polyp versus incubation period and of summed carbon contents of polyp and buds versus incubation time during the first certain period, respectively.

158 **Experimental design for field feeding experiments**

159

160 To elucidate feeding responses of the polyps to microzooplankton assemblages, the
161 following experiment was performed twice (27 August and 6 September 2011) during the
162 field growth experiment. Three additional bottles without polyps were prepared using the
163 <200-μm seawater fraction as a no-polyp control treatment. These were suspended at a depth
164 of 2 m at the study site and incubated for 2 days in accordance with the procedures used in the
165 field growth experiment. At the start of the experiments, three 200-mL seawater samples (the
166 <200-μm fraction) were fixed with Lugol's iodine solution (final concentration: 5%) to
167 determine the initial microzooplankton density and biomass. Following a 2-day incubation,
168 the same volume of seawater was collected from each bottle in the no-polyp control and
169 experimental treatments to determine the final microzooplankton abundance and biomass.
170 Changes in the abundance and biomass of each microzooplankton taxon over the 2-day
171 incubation were examined to estimate feeding parameters.

172

173 **Microzooplankton analysis**

174

175 Preserved microzooplankton samples in the 200-mL field seawater (which was collected
176 every other day and at the start and end of the feeding experiments) were concentrated into a
177 1-mL volume using the settling method, and then were observed under a phase-contrast
178 microscope at 150 × magnification using a Sedgewick Rafter counting chamber. The
179 coefficient of variation (the ratio of the standard deviation to the mean) of total
180 microzooplankton abundances in five subsamples of seawater collected in the field growth
181 experiment was 10% at 4.76 x 10^3 inds L^{-1} of the mean abundance. In this study, zooplankton
182 in the <200-µm seawater fraction, including plastidic and aplastidic dinoflagellates, were
183 defined as microzooplankton. These organisms were identified to the species, genus, or other
184 taxonomic level, and these groupings were used for the taxon counts. Aloricate ciliates and
185 dinoflagellates (including thecate and athecate) were categorized into three size groups based
186 on cell length viz. 15-30 µm (<30 µm), 30-50 µm and >50 µm for the former and 20-50 µm
187 (<50 µm) and ≥50 µm for the latter. For each group, the cell/body dimensions of up to 10
188 individuals were measured using a calibrated ocular micrometer in all timeseries samples in
189 the field growth experiment and all samples in each field feeding experiment. Mean cell
190 volume was calculated from cell length and width assuming appropriate geometric shapes for
191 ciliates, dinoflagellates, and other protists. Carbon contents were calculated using volume-to-
192 carbon conversion factors (Putt & Stoecker 1989, Menden-Deuer & Lessard 2000). Metazoan

193 body weights were estimated from overall body length or the length of specific body parts
194 using length-to-dry weight conversion factors for each taxon (Hirota 1986) and converted into
195 carbon content using suitable weight-to-carbon conversion factors/equations for each group
196 (Hirota 1986, Fisheries Agency 1989). For copepod nauplii and *Oikopleura* spp., carbon
197 content was directly estimated from the length of specific body parts using the appropriate
198 length-to-carbon conversion equations for each taxonomic group (Uye et al. 1996, Sato et al.
199 2001). Abundance and biomass were estimated for eight microzooplankton groups: ciliates,
200 dinoflagellates, molluscs, rotifers, copepods, copepod nauplii, *Oikopleura* spp., and others.
201
202 **Feeding rate calculation**
203
204 Feeding rates of *A. coerulea* polyps were estimated from the change in abundance of each
205 taxon during the incubation period in the experimental treatment, and values were corrected
206 based on abundance changes observed in the control treatment. Assuming an exponential
207 decline in taxon abundance, the clearance rate (CR_t, L bottle^{-1} d^{-1}) was calculated according
208 to Frost (1972):
209 $$CR_t = \left(\frac{\ln Z_0 - \ln Z_t}{t} + k_t\right) \times V \quad (2)$$

where t(d) is the sampling duration; Z_0 and Z_t (individuals L^{-1}) are the mean abundance of each taxon in the experimental treatment at time 0 and time t, respectively; and V (L) is the volume of seawater in the bottle. Finally, k_t (d^{-1}) is the growth or mortality rate in the control treatment during time t, calculated from the following equation:

$$k_t = \frac{lnCZ_t - lnCZ_0}{t} \quad (3)$$

where CZ_0 and CZ_t (individuals L^{-1}) are the mean abundance at time 0 and time t, respectively, of each taxon in the control treatment. The sampling period (2 days) was less than the generation time of all metazoans except rotifers. Hence, if k_t was non-negative, k_t was assumed to be 0.

The CR_t was contributed by initial mother polyps (4 polyps) and buds produced by them. To estimate clearance rate per individual polyp (standardized clearance rate), the number of polyps in the bottle (N_{std}; polyps $bottle^{-1}$) was standardized with the following equations based on the ratio of carbon contents of them to the initial polyp carbon:

$$N_{std} = \frac{PC_d}{PC_0} \quad (4)$$

where PC_d (µgC $bottle^{-1}$) is the total carbon content of polyps and buds in the bottle at the start of each feeding experiment (day 4 and day 14), and PC_0 (µgC ind^{-1}) is the mean carbon content of a polyp at the start of the filed growth experiment (day 0), which were calculated from calyx diameter and the Eq (1).

228 Hence, standardized clearance rate per polyp ($CR_{t\text{-}std}$, L polyp^{-1} d^{-1}) was estimated from

229 Eq (2) and Eq (4):

230 $$\mathrm{CR}_{t-std} = \frac{CR_t}{N_{std}} \quad (5)$$

231 Carbon-based ingestion rates (I_c, μg C polyp^{-1} d^{-1}) for each taxon were calculated based

232 on the standardized clearance rate ($CR_{t\text{-}std}$) and geometric mean of carbon biomass (C_{avg}, μg C

233 L^{-1}) of each taxon during the incubation period (Heinbokel 1978):

234 $$I_c = CR_{t-std} \times C_{avg} \quad (6)$$

235 where $C_{avg} = \frac{C_t - C_0}{ln\, C_t - ln\, C_0}$, and C_0 (μg C L^{-1}) and C_t (μg C L^{-1}) are the biomass of a given

236 taxon at time 0 and time t (= 2 days), respectively.

237 The student t-test ($p < 0.05$) was used to evaluate whether the means of the abundance of

238 each taxon in each experimental treatment was significantly different from the value in the

239 control treatment at the end of each feeding experiment. One sample t-tests ($p < 0.05$) were

240 used to evaluate whether the means of $CR_{t\text{-}std}$ and I_c were significantly different from zero.

241

242 **Prey selectivity**

243

Ivlev's electivity index (Ivlev 1961) was used to evaluate feeding selectivity for each microzooplankton taxon and each category of protists (ciliates and dinoflagellates) in the two feeding experiments. Ivlev's index (E) is defined as

$$E_i = \frac{r_i - p_i}{r_i + p_i} \quad (7)$$

where r_i is the individual-based proportion of prey type i ingested by polyps, and p_i is the individual-based proportion of prey type i in the environment. E can range from −1.0 to +1.0, with positive values indicating a preference for a prey item and negative values indicating avoidance. An E of 0 indicates that the ingestion rate is in proportion to availability (i.e., no selection). One sample t-tests ($p < 0.05$) were used to evaluate whether per-bottle means were significantly different from zero.

Results

Environmental conditions, microzooplankton abundance, and biomass in the field growth experiment

The temperature of seawater samples collected during the field growth experiment ranged from 25.3 to 27.0 °C, and salinities ranged from 28.2 to 30.9 (no data were collected on 23

August and 25 August) (Fig. 2A). Chlorophyll-*a* concentrations fell within the range of 3.5–27.9 μg L^{-1} (no data were collected on 23 August). Relatively low chlorophyll-*a* concentrations (<5.3 μg L^{-1}) were observed from 2 September to 4 September (days 10–14).

Microzooplankton abundance was extremely high (346.2 × 10^3 inds L^{-1}) on 23 August (day 0), and then ranged from 3.39 × 10^3 to 17.2 × 10^3 inds L^{-1} during the field growth experiments (Fig. 2B). Dinoflagellates were the most abundant taxa throughout the experimental period, except on 6 September when the abundance of ciliates was comparable to that of dinoflagellates. Microzooplankton biomass varied greatly during the experiments and ranged from 12.8 to 553.2 μgC L^{-1} (Fig.2C). The biomass of dinoflagellates was also large until 27 August, and that of ciliates, molluscs, and/or copepod nauplii was comparable to dinoflagellates.

Abundance of ciliates ranged from 0.64 × 10^3 to 2.39 × 10^3 inds L^{-1}, dominated by the smallest size group of aloricate ciliates and tintinnid ciliates (Fig. 3A, B). Biomass of ciliates ranged from 2.3 to 17.6 μgC L^{-1}, more than 50% of which was constituted by the largest size group of aloricate ciliates and tintinnid ciliates (Fig, 3C, D). Abundance and biomass of dinoflagellates exhibited extremely high values on the first day of the growth experiment (344.66 × 10^3 inds L^{-1} and 510 μgC L^{-1}, respectively), 95 % and 89% of which were constituted by the small thecate group, respectively (Fig. 4). Those values were maintained at

280 more than 13.00 × 10^3 inds L^{-1} and 36 µgC L^{-1} until August 27, and then ranged from 2.10 ×

281 10^3 to 3.09 × 10^3 inds $L^{-1}$and from 3.3 to 10.9 µgC L^{-1}, respectively. After the first day of

282 incubation, dinoflagellates were numerically dominated by small thecate and athecate types,

283 while interms of biomass they were dominated by small thecate and large athecate types

284 throughout the whole incubation period.

285

286 **Growth responses of polyps in field seawater**

287

288 The number of buds produced per polyp in the experimental treatment significantly

289 increased with incubation period until days 12–14 ($p < 0.05$, *t*-test for the slope) and then

290 reached a plateau at 5.5–9.3 buds $polyp^{-1}$(Fig. 5A). Podocysts were not observed during the

291 field incubation period. Bud production rates calculated from data during the first 12 days by

292 fitting to the linear regression model ranged from 0.48 to 0.83 buds $polyp^{-1}$ d^{-1} (mean ± SE:

293 0.65 ± 0.09 buds $polyp^{-1}$ d^{-1}). Cumulative buds per polyp in the no-prey control treatment

294 also increased until 29 August (day 6) before leveling off at 3.3 buds $polyp^{-1}$.

295 At the start of incubation, carbon contents for a single polyp in the experimental treatment

296 ranged from 35.4 to 40.9µgC $polyp^{-1}$ (mean±SE: 37.0 ± 2.5 µgC $polyp^{-1}$, n=16), which was

297 almost the same as the value in the control treatment (38.3±9.1 µgC $polyp^{-1}$, n=3). Then, the

298 carbon biomass of the original polyps and their buds significantly increased with incubation
299 period until days 8–12 ($p < 0.05$, *t*-test for the slope) (Fig. 5B), and the carbon growth rate
300 during the first 10 days by fitting to the linear regression model ranged from 3.5 to 6.7 μgC
301 d^{-1} (mean ± SE: 4.92 ± 0.82 μgC d^{-1}). This translates to a specific growth rate for the polyps
302 of 0.1–0.2 d^{-1} (mean ± SE: 0.14 ± 0.02 d^{-1}). Subsequently, the carbon biomass reached a
303 plateau at a value 1.7–3.0 times higher than the initial carbon content, and the carbon growth
304 rate after day 10 was not significant *(p* >0.05, *t*-test for the slope). Total carbon content in the
305 control treatment did not increase significantly during the incubation period ($p > 0.05$, *t*-test
306 for the slope).
307
308 **Calyx diameter of polyps and buds**
309
310 The initial mean calyx diameter of polyps in each bottle ranged from 1,370 μm to 1,525
311 μm, and then was stable until day 6 in the field growth experiments (Fig. 6A). After that day,
312 the mean calyx diameter slightly decreased and ranged from 1,110 μm to 1,199 μm at the end
313 of experiments. The values of buds ranged from 328 μm to 744 μm and did not show any
314 clear trends in variation throughout the whole period of the experiment (Fig. 6B). The mean ±
315 SE of calyx diameters of polyps and buds in the experimental treatment were 1,477 ± 43 μm

316 and 622 ± 55 µm in the first feeding experiment, respectively, and 1,146 ± 47 µm and 522 ±
317 22 µm in the second feeding experiment, respectively.
318
319 **Microzooplankton abundance and biomass and polyp feeding responses in field feeding**
320 **experiments**
321
322 Abundances of microzooplankton on day 4 (August 27) and day 14 (6 September) were
323 mostly dominated by ciliates and dinoflagellates, which accounted for over 90% of total
324 microzooplankton abundance during both periods. The biomass of the microzooplankton
325 assemblages consisted mainly of ciliates, dinoflagellates, molluscs (mainly bivalve larvae),
326 copepods (consisting mostly of *Oithona* spp.), and copepod nauplii (Table 1). Molluscs
327 accounted for the largest or second-largest portion of the total biomass. There was no
328 difference between the abundances of ciliates in the two periods, although the biomass on day
329 14 was higher than that on day 4. Abundances and biomasses of dinoflagellates on day 4 were
330 6.6 and 7.7 times higher than those on day 14, respectively.
331 Among the microzooplankton taxa, ciliates and dinoflagellates were both numerically
332 dominated by small types of aloricate ciliates (<30 µm) and dinoflagellates (<50 µm) on day
333 4 (Table 2). On day 14, tintinnid ciliates and small thecate dinoflagellates (<50 µm)

334 constituted the greatest portion of the total abundance of protists. Biomass of all protists was
335 mostly attributable to large athecate dinoflagellates (≥50 μm) on day 4 and to large aloricate
336 ciliates (>50 μm) on day 14.

337 At the end of the feeding incubations, the abundances of molluscs in the first experiment
338 and of copepods, copepod nauplii, and *Oikopleura* in the second experiment did not decrease
339 significantly in the experimental treatment compared to in the control treatment. However, in
340 both experiments, the clearance rates of ciliates, dinoflagellates, and molluscs were
341 significantly higher than zero ($p < 0.05$, one sample *t*-test), and the highest significant
342 clearance rate was recorded with respect to rotifers (mean ± SE: 0.28 ± 0.03 L polyp^{-1} d^{-1}) in
343 the first experiment (Table 1). High ingestion rates (> 1 μgC polyp^{-1} d^{-1}) were found for
344 dinoflagellates in the first experiment and for ciliates and molluscs in the second experiment,
345 and significant carbon ingestion rates were recognized for ciliates, dinoflagellates, molluscs,
346 and copepod nauplii in both experiments (Table 1). Carbon ingestion rates for total
347 microzooplankton in the first and second experiments were 4.05 and 3.27 μgC polyp^{-1} d^{-1},
348 respectively.

349 Significantly positive or negative electivity ($p < 0.05$, one sample *t*-test) on
350 microzooplankton groups was observed for rotifers and copepods in the first experiment and

for molluscs and copepods in the second experiment (Table 1). Copepods were the only taxon for which significantly negative electivity was recognized in both experiments.

As for the feeding activities of polyps on ciliates and dinoflagellates, significant clearance rates and ingestion rates ($p < 0.05$, one sample *t*-test) were found towards all groups, except for feeding on small athecate dinoflagellates in the first experiment and large athecate dinoflagellates in the second experiment (Table 2). Ingestion rates on large aloricate ciliates were relatively high in both experiments (Fig. 7). Relatively high ingestion rates on small thecate dinoflagellates and large athecate dinoflagellates were recorded in the first experiment, whereas such high rates with respect to this taxonomic group were not found in the second experiment (Fig. 7).

Among all size groups of ciliates and dinoflagellates in the first experiment, significantly positive electivity was observed towards large sized (> 50 μm) and medium sized (30-50 μm) groups of aloricate ciliates, and on both size groups of thecate dinoflagellates, whereas significant negative electivity was recognized towards both size groups of athecate dinoflagellates. In the second experiment, the only electivity was on small athecate dinoflagellates indicating a significant positive value ($p < 0.05$, one sample *t*-test) (Table 2).

Discussion

369

370 **Growth responses of polyps in microzooplankton assemblages**

371

372 Clear increases of bud production observed during the first 12–14 days of incubation in the
373 <200-μm seawater fraction suggested that polyps performed active asexual reproduction when
374 reared in field microzooplankton assemblages. *Aurelia coerulea* polyps fed ciliates (3.8–5.8
375 μgC ind^{-1}) in a laboratory experiment, by providing cultured ciliates, were reported to have
376 carbon specific growth rates of 0.11–0.24 d^{-1} (Fig. 7 in Kamiyama 2017), which is consistent
377 with the growth rates (0.1–0.2 d^{-1}) observed in the present study.

378 The bud production slowed down or stopped after 10–14 days of incubation. It may be
379 possible to speculate two explanations for this result: limitations on the energy uptake
380 necessary to support production, and the carrying capacity of the space within each well for
381 produced buds. Microzooplankton biomass declined on days 10–14, possibly limiting energy
382 uptake and making it impossible to continue bud production at the rate observed in the first 10
383 days. The limitation of total energy uptake may involve a decrease in the feeding frequency of
384 polyps, which would reduce bud production (Keen & Gong 1989). In addition, the large
385 number of buds produced may have limited the space available for new buds (Willcox et al.
386 2007).

387 Contrary to expectations, a small number of buds were produced in the control treatment
388 (GF/C filtration fraction) during the first 4 days. The reason for this is difficult to explain.
389 Since polyps used in this growth experiment were reared without prey in filtered seawater
390 during one week to get them to adhere to the plates, it is difficult to consider that carry-over
391 from prey energy obtained in the stock culture caused such bud production in the control
392 treatment. Energy contributions from dissolved organic matter (Schick 1973, 1975) cannot be
393 ruled out. However, because polyp carbon biomass did not increase in the control treatment
394 during the incubation period, it is reasonable to speculate that physiological changes in the
395 polyps induced by a certain factor in natural seawater temporarily stimulated polyp
396 reproduction at the expense of reducing body energy stores.
397 The calyx diameter of parent polyps in the experimental treatment decreased after day 10
398 in the field growth experiment, when the increase of total carbon contents of parent polyps
399 and their buds was stagnant. However, new bud production partly continued in this period.
400 Since parent polyps dispensed prey energy into bud production, they might decrease in size to
401 save metabolic energy. The calyx diameter of *Aurelia* polyps is also influenced by differences
402 in prey organisms (Kamiyama 2013), and by the frequency of prey consumption, genetic
403 characteristics and interactions of these factors (Keen & Gong 1989).
404

405 **Feeding responses**

406

407 Because polyps are generally considered to be carnivorous (e.g., Arai 1997), *Artemia*
408 nauplii have been used as a representative zooplanktonic prey item in eco-physiological
409 studies (Lucas et al. 2012). However, studies on the actual natural prey items and feeding
410 habits of *A. coerulea* polyps are severely lacking. Recent studies have reported that polyps
411 can feed on microzooplankton such as ciliates and dinoflagellates in the laboratory, but they
412 cannot do so with nanoplankton (Kamiyama 2011, Huang et al. 2015).

413 In the present study, the total microzooplankton ingestion rates estimated from the two
414 experiments were estimated as 4.1 and 3.3 μgC polyp^{-1} d^{-1}, respectively. These rates account
415 for 9–11% of the polyp's body carbon (mean 37.0 μgC ind^{-1}) estimated from their initial
416 calyx diameter. Previous quantitative estimates of polyp feeding activity on microzooplankton
417 have been limited to ciliates (Kamiyama 2011). Kamiyama (2011) reported a maximum
418 feeding rate of *A. coerulea* polyps on a large ciliate species (*Favella taraikaensis* Hada, 1932)
419 in laboratory experiments of 0.33 μgC polyp^{-1} h^{-1}, or 7.92 μgC polyp^{-1} d^{-1}. There data are ca.
420 two times higher than the rates observed in the present study. For this comparison, it is
421 necessary to pay attention to differences in prey source and prey biomass. If the carbon
422 biomasses of microzooplankton in the present feeding experiments (106.8 and 86.3 μgC L^{-1})

423 are fitted to the feeding response equations of *A. coerulea* polyps in the function of ciliate
424 carbon biomass in laboratory experiments (Kamiyama 2011, Table 3), the feeding rates are
425 estimated to be 2.5 and 2.2 μgC $polyp^{-1}$ d^{-1}. The ingestion rate estimated in the present study
426 accounted for ca. 1.5 times higher than the values estimated in the same carbon biomass of
427 ciliate prey. This implies that organisms other than ciliates were able to contribute to the prey
428 energy source for *A. coerulea* polyps as well.
429
430 **Potential errors in estimating the feeding rates of polyps**
431
432 The bottle incubation experiment applied in the present study is advantageous for
433 examining feeding rates on some prey items simultaneously, and allows us to examine a
434 predator's selectivity for prey items. However, this method potentially has the possibility of
435 causing several artificial errors in estimating feeding rates. In particular, the following errors
436 should be considered for interpretation of the results of the present study.
437 The first potential error is due to the assumption that decreases in prey is caused only by
438 the feeding of polyps in the bottle. If the tentacles of polyps physically caused damage and
439 subsequent destruction of fragile prey such as protists before the feeding process, the feeding
440 activities with respect to fragile prey would be overestimated. However, Kamiyama (2011)

441 reported that polyps can catch aloricate ciliates with their tentacles and transport them into the
442 mouth, and confirmed the assimilation of such prey into their bodies, supporting the
443 presumption that mechanical damage leading to losses of protist prey in the capture process
444 were not consequential in the present study. In addition, high gross growth efficiency of
445 polyps in the field seawater as outlined supports the conclusion that the feeding rates of
446 polyps were not overestimated.

447 A second potential error could be due to “bottle effects” as often pointed out in
448 zooplankton feeding experiments, such effects include changes in predator grazing or prey
449 growth over time due to differing nutrient, light or turbulence regimes, crowding of grazers,
450 interactions with container walls, and trophic cascades of food web effects (Båmstedt et al.
451 2000, Jungbluth et al. 2017). The bottle effects become serious if the effects differentially
452 occur in the experimental treatment compared to the control treatment. In the present study,
453 interaction among taxa of microzooplankton and trophic cascades due to polyp feeding during
454 the two days of incubation are potential factors to cause over- or under-estimation of the
455 feeding activities of polyps in the present study. Further studies are needed to confirm the
456 results of the present study.

457

458 **Prey selectivity**

459

460 Because polyps feed passively, their selectivity probably depends on whether they have
461 the opportunity to encounter prey and can capture them efficiently. The former factor is
462 subject to prey abundance, and the latter factor is influenced by prey features such as prey size
463 and motility, and by the types and sizes of nematocysts on the tentacles of the polyps,
464 assuming the function of nematocysts in prey capture of *Aurelia* polyps is the same as for
465 hydrozoans (Purcell 1984, Purcell and Mills 1988). In the present study, although polyps
466 ingested many prey taxa, negative selectivity for copepods was observed in both feeding
467 experiments. One study on soft coral polyps (*Paramuricera clavate* [Risso, 1826]) has
468 reported highly positive selectivity towards highly abundant, low-motility organisms such as
469 copepod eggs and nauplii and other benthic invertebrate eggs and larvae, and negative
470 selectivity towards copepods throughout the research year (Coma et al. 1994). The negative
471 selectivity may be explained by the swimming characteristics of copepods with their high
472 escape abilities from predators. On the contrary, Ishii and Takahashi (2021) reported high
473 ingestion rates (6.7 µgC $polyp^{-1}$ d^{-1}) of *Aurelia coerulea* polyps on the small copepod *Oithona*
474 *davisae* at high densities (2500 inds L^{-1}) in Tokyo Bay. Although the copepod species in these
475 feeding experiments were dominated by *Oithona* spp., the abundance (ca. 40 inds L^{-1}) was far
476 lower than the highest level in Tokyo Bay and showed a lower contribution (< 1%) in terms

477 of total microzooplankton abundance as well. Seawater passing through a 200 μm plankton
478 net was used for the present feeding experiments, implying that some of the *Oithona* spp.
479 were removed because the body size of this group generally exceeds 300 μm (Uye 1982).
480 Such low abundance and relatively low contribution of copepods in planktonic assemblages
481 might diminish opportunities to capture copepods and have caused the apparent negative
482 selectivity towards copepods observed in this study.
483 Feeding activities of polyps on different size groups of ciliates and dinoflagellates did not
484 show consistent similarities in the two feeding experiments. However, as for aloricate ciliates,
485 lower clearance rates in the first experiment and lower ingestion rates in the second
486 experiment were both observed on the smallest group (<30 μm), suggesting a relatively low
487 availability of the small group of aloricate ciliates as prey for the polyps. This corresponds
488 well with the results of a previous laboratory study reporting no or less feeding responses of
489 *Aurelia* polyps on ciliates less than 30 μm in size (Kamiyama 2011). On the other hand, in
490 both experiments, significantly higher ingestion rates were observed towards the largest group
491 (>50 μm) of aloricate ciliates, rather than towards other size groups, and significantly positive
492 electivity was also observed towards this group in the first experiment. These results suggest
493 that larger aloricate ciliates are an essential prey source for *Aurelia* polyps. It is difficult to
494 clearly explain why the difference of positive and negative electivity towards <50 μm

495 athecate dinoflagellates in Exp. 1 and Exp.2 was observed. This is possibly related to the large
496 difference in abundance of this group in the two feeding experiments.
497
498 **Prey consumption and contribution of each microzooplankton taxon**
499
500 To clarify the contribution of each microzooplankton taxon in the growth experiments, I
501 estimated the carbon contribution of each prey taxon to polyp growth using the daily carbon
502 biomass of each taxon and the clearance rate data obtained in the two feeding experiments.
503 Clearance rates in the first and second experiments were fitted to data from days 0–8 and 9–
504 16, respectively. Extremely high dinoflagellate abundance on day 0 was associated with high
505 rates of consumption of this taxon, and other microzooplankton also contributed as prey
506 sources during the following days (Fig. 8).
507 Furthermore, daily assimilation of prey carbon was estimated by the total carbon
508 consumption an assuming assimilation efficiency of 0.8 (Schneider 1989), and then compared
509 with the daily carbon metabolic demand of a polyp. This metabolic demand was estimated by
510 fitting average temperature (26 ºC) during the field growth experiment into the relationship
511 between carbon weight-specific respiration rate (R; $\mu gO_2\ \mu gC^{-1}\ d^{-1}$) of *Aurelia* polyps and
512 temperature (T; ºC), expressed by $R = 0.0173 \times e^{(0.0657 \times T)}$ (Ikeda et al. 2017) assuming a

513 respiratory quotient of 0.85 due to protein-dominated metabolism (Schneider 1989). As a
514 result, the mean metabolic carbon demand of polyps in the experimental treatments was
515 estimated to be 1.12 μgC $polyp^{-1}$ d^{-1} during the incubation period (Fig. 8). The carbon
516 assimilation of a polyp mostly exceeded daily metabolic carbon demand, but did not exceed
517 this level on day 8 and day 12. This partly supports the observation of stagnation of polyp
518 growth after day 10 of the incubation.
519 In spite of the difference in daily consumption, mean consumption over the whole period
520 indicated that polyps ingested relatively large amounts of carbon from dinoflagellates, ciliates,
521 molluscs, and copepod nauplii, each taxon of which contributed 41, 15, 12 and 12 % of total
522 carbon ingestion and the sum of them collectively accounted for 79% of the total value. This
523 suggests that these organisms played important roles in polyp bud production during the
524 experimental period at the site in the present study. The importance of ciliates has been
525 pointed out in previous studies (Kamiyama 2011, 2013). Although polyps can feed on larger
526 dinoflagellates, they are probably unable to do so with small dinoflagellates (Kamiyama 2011,
527 Huang et al. 2015). The abundance and biomass of dinoflagellates on the first day (23 August)
528 was far higher than during the first feeding experiment, and this difference was mostly
529 accounted for by an abundance of thecate dinoflagellates that are less than 50 μm in size (Fig.
530 4). In general, the clearance rates of filter feeder animals decrease with increasing abundance

of prey organisms above a critical level (Frost 1972), and this response could be similar for polyp feeding. Hence, the estimation of carbon ingestion by the application of clearance rates measured during the feeding experiments to the extremely high abundances of thecate dinoflagellates observed on day 0 may have led to an overestimation of carbon ingestion at that time.

Relationships between growth responses and estimated prey consumption

Relationships between the carbon growth rate of polyps and the cumulative carbon consumption of microzooplankton assemblages were analyzed using two types of linear regression models. These models were fitted to the data collected over the whole incubation period, with the exception of the first and last day of incubation. I assumed that the carbon contents of polyps, including buds produced on each day, reflected the cumulative carbon ingested from prey consumed in the previous two and four days for these models (Fig. 9) because the increase of buds started with a lag period of 2 or 4 days in the growth experiment (Fig 5A). The gross growth efficiency, as indicated by the slope of the regression line, was 75% for a 2 day lag-period and 43% for a 4 day lag-period. Kamiyama (2013) estimated the gross growth efficiency of *A. coerulea* polyps feeding on ciliates in laboratory experiments as

549 42–64% (mean 54%), which is close to the value estimated in this study. The high gross
550 growth efficiency observed during this study period using field microzooplankton
551 assemblages suggests that *A. coerulea* polyps can efficiently utilize energy from
552 microzooplankton prey to fuel growth, even under field conditions.
553
554 **Conclusions and ecological implications**
555
556 Based on the results of the field growth and feeding experiments, *A. coerulea* polyps can
557 utilize a diverse assemblage of common microzooplankton, such as ciliates, dinoflagellates,
558 molluscs, and copepod nauplii, to actively produce new buds when exposed to natural
559 planktonic assemblages of less than 200 μm in size. The specific growth rate of polyps in the
560 growth experiment corresponded to the value reported in previous laboratory experiments,
561 suggesting that polyps can reproduce asexually under field conditions. Whereas polyps
562 showed negative selectivity for copepods in both feeding experiments, they did not
563 consistently show significant selectivity for the main components of microzooplankton such
564 as ciliates and dinoflagellates in the two feeding experiments. Hence, regardless of
565 fluctuations in the main taxonomic components of microzooplankton, polyps could efficiently
566 utilize the energy of microzooplankton prey for growth under field conditions.

567 Other than microzooplankton, a variety of larger zooplankton such as copepods,
568 chaetognaths, ctenophores, hydromedusae, molluscs and fish larvae, and planulae can be
569 consumed by *Aurelia* polyps (Gröndahl 1988, Östman 1997), and occasionally they can feed
570 on organisms larger than themselves (Lucas et al. 2012). If they can encounter such large prey
571 they would efficiently get prey energy from them, suggesting that they are more important
572 prey for the polyps from a bioenergetic viewpoint. In fact, results from laboratory experiments
573 and the application of a bioenergetic model to field zooplankton assemblages indicated that
574 small copepods and other mesozooplankton can support potential growth rates of polyps in
575 temperate coastal waters (Ikeda et al. 2017, Ishii and Takahashi 2021). However, abundances
576 of meso- and macrozooplankton in natural seawater are generally one or two orders of
577 magnitude lower than those of microzooplankton (e.g., Uye et al. 1996, Uye & Shimazu
578 1997), possibly indicating that the polyps do not actually have many opportunities to
579 encounter such large prey. Furthermore, it was confirmed that large copepods and other
580 crustaceans often escape from the tentacles of polyps (Östman 1997). At present, quantitative
581 information on the feeding activities of *Aurelia* polyps on microzooplankton, large
582 zooplankton and benthic animals in natural assemblages has not yet sufficiently accumulated.
583 This information, if gathered during further research, will allow us to evaluate the prey

584 importance for each zooplankton group in natural zooplankton assemblages and to clarify the
585 effects of them on the population dynamics of the polyp stage of *A. coerulea.*

586 The results of this study demonstrate that microzooplankton in natural plankton
587 assemblages can serve as prey for *A. coerulea* polyps and promote asexual reproduction. In
588 scyphozoans, the polyp and ephyra stages play a key role in determining population size.
589 However, because these stages have little to no mobility, they are subject to environmental
590 stress and limitations in prey availability. Microzooplankton, which are a numerically
591 abundant subset of zooplankton, can easily be encountered in field seawater and may
592 therefore be important prey sources for *A. coerulea* polyps. In the jellyfish spiral theory (Uye
593 & Ueta 2004), coastal eutrophication and/or changes in nutrient components promote the
594 frequent occurrence of jellyfish blooms and also enhance the dominance of nanoplankton,
595 which in turn are suitable prey for microzooplankton. The results obtained in this study,
596 which indicate that planktonic communities dominated by microzooplankton can also
597 contribute to an increase in *A. coerulea* polyps through asexual reproduction, partly support
598 the jellyfish spiral theory.

599

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601

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716
717

718 **Figure legends**

719

720 Fig. 1. Schematic diagrams of experimental setup. (A) Attachment plates for *Aurelia coerulea*
721 polyps. (B) Plate configuration inside each bottle. (C) Suspended bottles at the sampling site
722 for seawater in the field growth experiment.

723

724 Fig. 2. (A) Environmental parameters and (B) abundance and (C) biomass of
725 microzooplankton in seawater at the study site during the field growth experiment.

726

727 Fig. 3. Ciliates. (A) Total abundance and (B) the contribution of each taxon, and (C) total
728 carbon biomass and (D) the contribution of each taxon during the field growth experiment.

729

730 Fig. 4. Dinoflagellates. (A) Total abundance and (B) the contribution of each taxon, and (C)
731 total carbon biomass and (D) the contribution of each taxon during the field growth
732 experiment.

733

734 Fig. 5. Changes in (A) the cumulative number of buds produced by *Aurelia coerulea* polyps
735 and (B) estimated carbon contents of polyps and their produced buds. Lines labeled EXP1–4

736 show mean values for experimental treatment bottles 1–4 (n = 4 polyps per bottle), and lines
737 labeled Ctr show mean values for the no-prey control treatment (n = 3 polyps). Bars indicate
738 standard errors.
739
740 Fig. 6 Standardized ingestion rates per polyp on each size group of aloricate ciliates (AC),
741 thecate dinoflagellates (TD) and athecate dinoflagellates (ATD) in the two field feeding
742 experiments (Exp. 1: 27 August and Exp.2: 6 September). Bars indicate standard errors (n =
743 4).
744
745 Fig. 7. Changes in the mean calyx diameters of (A) original polyps set into the bottle and (B)
746 buds produced in the bottle. Bars indicate standard errors of calyx diameters of the original
747 polyps (n=4) and the values of produced buds after day 2 (n=4 to 37).
748
749 Fig. 8. Changes in estimated daily microzooplankton consumption and assimilation by
750 *Aurelia coerulea* polyps and the metabolic carbon demand during the field growth
751 experiment. The carbon biomass of consumed microzooplankton was estimated from the
752 biomass of each taxon and the taxon-specific clearance rate observed during 23 August to 29
753 August in the first experiment and 31 August to 6 September in the second experiment.

754

755 Fig. 9. Relationship between the estimated carbon content of *Aurelia coerulea* polyps and
756 buds on each sampling day and the cumulative microzooplankton carbon consumed in the
757 field growth experiment. Two linear regression lines (solid line and dashed line) were
758 calculated using all plotted data, except for data collected on the first and last day of
759 incubation, assuming that polyp carbon content reflected the biomass of microzooplankton
760 carbon consumed 2 days previously (filled circles) and 4 days previously (open circles),
761 respectively.

762

763