



# Phytoplankton uptake and growth rate in the Japanese egg cockle *Fulvia mutica*

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Received: 28 February 2019 / Accepted: 24 October 2019 / Published online: 8 November 2019  
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**Abstract** To clarify the relationship between the quantity of food ingested by and the growth rate of the Japanese egg cockle *Fulvia mutica* (Reeve), we conducted a laboratory breeding experiment for 2 weeks and estimated the chlorophyll *a* (chl-*a*) concentrations in water and the increments in shell length and soft-body weight of this species under five chl-*a* concentrations. Moreover, we compared the relationship between cockle growth (changes in soft-body weight and shell length) and their feeding environment observed in the laboratory experiment with the results of a field investigation conducted at two sites in the Sea of Japan, Kumihama Bay (35°38'5" N, 134°54'00" E) and Kunda Bay (35°33'30" N, 135°15'4" E). The changes in soft-body weight were similar in both laboratory and field investigations, but those in shell length were not. We, therefore, considered shell length changes as unsuitable for evaluating the relationship between growth and feeding in *F. mutica*. Based on the changes in soft-body weight, it was possible to classify the feeding environment of this species into the following three types: (1) < 1.52 μg chl-*a* L<sup>-1</sup>, negative feeding environment for cockle growth; (2) 1.52–5.71 μg chl-*a* L<sup>-1</sup>, neutral feeding environment for cockle growth; (3) > 5.71 μg chl-*a* L<sup>-1</sup>, positive feeding environment for cockle growth (growth increased with increasing chl-*a* concentration up to about 11 μg chl-*a* L<sup>-1</sup>). These results indicate that maintaining chl-*a* concentration in the breeding water within 5.71–11 μg chl-*a* L<sup>-1</sup> is desirable for rearing Japanese egg cockle.

**Keywords** Food demand · Bivalve · Mollusk · Feeding environment · Ingestion rate · Clearance rate

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## Introduction

The Japanese egg cockle, *Fulvia mutica* (Reeve), is a bivalve that inhabits the inner bays along the coast of Japan, excluding those in Hokkaido. This species is also widely distributed across the Korean Peninsula and the coast of China, and imported into Japan (Fujiwara 2009) where it is popular as shellfish sushi. Generally, marketed *F. mutica* are 3–4 g (stripped shellfish) and not treated as a high-class food material. In contrast, the *F. mutica* caught in Kyoto, Japan, are ten times larger (stripped shellfish weight 30–40 g) and their large, thick bodies are appreciated for their good taste. Therefore, *F. mutica* caught in Kyoto are often traded at high prices and have become special products (Fujiwara 2009).

Reports from several regions indicate large annual fluctuations in both catch and stock size of *F. mutica* (Tian and Shimizu 1998; Fujiwara 2009; Yang et al. 2011). Moreover, in Japan, the resources used by this species have collapsed in many areas (Tian and Shimizu 1997). In response, the Kyoto Prefectural Agriculture, Forestry, and Fisheries Technology Center started working on the development of seed production technology in 1976, aiming to ensure a stable supply of this species (Iwao et al. 1993). In addition, the full-scale *F. mutica* farming started by fishermen in 2000 has steadily increased its production since then (Tanimoto et al. 2015). However, in recent years, mass mortality of this organism occurred frequently during the culturing period, resulting in production declines. This mortality is likely related to recent climate changes which have resulted in higher water temperatures in summer, low salinity seawater due to heavy rain, generation of dysoxic water masses, and shortages of food. Currently, we are attempting to use aquaculture techniques to avoid mass mortality resulting from climate change. Specifically, a method for adjusting *F. mutica* to an appropriate water depth, without negatively influencing their survival and growth, has been developed and relies on monitoring the vertical profiles of several environmental factors in the aquaculture area. These factors include water temperature, salinity, dissolved oxygen concentration, and chlorophyll *a* (chl-*a*) concentration, which are used as indicators of the amount of phytoplankton available as food for the bivalves. Hourly measurements of these parameters are automatically performed by a lift-functioning conductivity, temperature, and depth (CTD) sensor installed in the aquaculture raft. To effectively operate such an aquaculture system, it is necessary to clarify the relationship between each environmental factor and the growth and/or survival of cockles. To date, the effects of water temperature, salinity, and dissolved oxygen concentration on the survival of other cockle species have been examined in detail in laboratory experiments (Nogami et al. 1981; Marsden and Bressington 2009; Tanimoto et al. 2015; Taylor et al. 2017; Peteiro et al. 2018). However, there is little information on the relationship between the feeding environment and the growth or survival of *F. mutica* and, as far as we know, no detailed studies examining these relationships have been conducted on this species in laboratory conditions.

The present study aimed to elucidate the relationship between the growth of *F. mutica* and its feeding environment (chl-*a* was used as an indicator of food availability). To achieve this aim, we examined the growth rate of this species in a 2-week laboratory rearing experiment under five different feeding conditions, i.e., different chl-*a* concentrations. Moreover, to ascertain to what extent the results of laboratory experiments represent those in the natural environment, we conducted field observations and compared them with the results of our laboratory experiments.

## Materials and methods

### Experimental individuals

The juvenile *F. mutica* used for the experiment were artificially produced in the Kyoto Prefectural Agriculture, Forestry, and Fisheries Technology Center and then farmed in Kunda Bay, Kyoto, Japan (35°33'30" N, 135°15'4" E) for about 2 months. Before the experiment, the individuals were kept in aerated, algae-free seawater that flowed through a breeding tank, for a period of 2 days. Shell length was  $42.3 \pm 2.4$  mm (mean  $\pm$  SD,  $n = 40$ ) at the start of the experiment. All procedures performed in this study were in strict accordance with the “Guidelines for Animal Experimentation of Seikai National Fisheries Research Institute (SNFRI), Japan Fisheries Research and Education Agency, Japan” and “Guidelines for the Care and Use of Live Fish at SNFRI”.



### Cultivation of the phytoplankton used for the feeding experiment in the laboratory

The cultured diatom *Chaetoceros* sp. (closely related to *C. neogracile*) was used as bivalve feed for the laboratory experiment. This genus of algae is a typical prey organism used in bivalve seedling production and aquaculture, because this alga can be cultivated in large quantities and high growth of bivalves can be obtained. Cultures were maintained for 2 days in 30-L polycarbonate tanks, containing 20 L of seawater medium (Table 1) with a salinity of 33 psu, at 19–20 °C under about 3600 lx of 24 h continuous cool-white fluorescent illumination, and used for the experiment in the logarithmic growth phase when the algal density reached  $4.0\text{--}6.0 \times 10^6$  cells mL<sup>-1</sup>. Cultures were inoculated at  $1.0\text{--}1.8 \times 10^6$  cells mL<sup>-1</sup>.

### Feeding procedure in the laboratory experiment

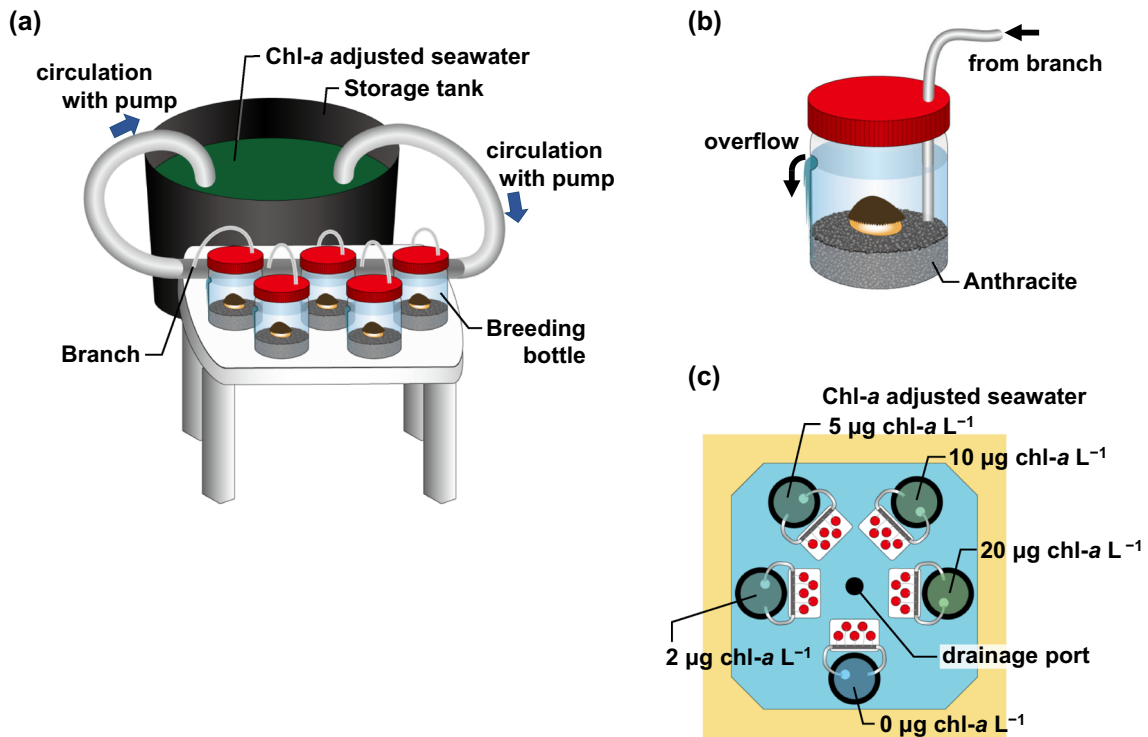
To examine the relationship between the quantity of *Chaetoceros* sp. ingested and the growth rate of juvenile *F. mutica*, the organisms were reared in the equipment illustrated in Fig. 1 for 2 weeks. A gradient for the quantity of ingested *Chaetoceros* sp. was established based on the five different chl-*a* concentrations of the breeding seawater, which was stocked in 500-L polyethylene tanks. The water was renewed every morning (10:00) by adding filtered (1- $\mu$ m pore size, Advantec MFS Inc., Tokyo, Japan) seawater (salinity 33 psu) to each tank, and the chl-*a* levels were adjusted to the five different concentrations (0, 2, 5, 10, and 20  $\mu$ g chl-*a* L<sup>-1</sup>) by adding an appropriate amount of cultured diatoms. Thus, in the present study, the amount of phytoplankton is expressed as the amount of chl-*a*, and this can be converted to number of *Chaetoceros* sp. cells as indicated in Supplementary Figure (Fig. 2). The seawater in the tanks was circulated using electric submersible pumps (CSL-100, Terada pump MFG. Co. Ltd., Nara, Japan), and flowed to the cockle-breeding bottles (outer diameter: 90 mm, height: 118 mm) through the pathways diverging from the center of the circulation pathway (Fig. 1a). Each breeding bottle ( $n = 25$ ), containing a 4-cm thick anthracite layer (grain size: 1.5–2.0 mm, Tohkemy Co. Ltd., Osaka, Japan) and one individual cockle was allowed to overflow with breeding seawater ( $n = 5$  bottles for each chl-*a* concentration, Fig. 1a, b). The flow rate to each breeding bottle (water volume:  $109.6 \pm 16.8$  mL,  $n = 25$ ) was regulated at approximately 2 L h<sup>-1</sup>, and it was calculated daily, because it was slightly altered (see the next section for details). The temperature was maintained at 25 °C ( $\pm 0.3$  °C) by the temperature-controlled water that flowed through a 50,000-L concrete pool (Fig. 1c). The water chl-*a* concentration of each 500-L tank was measured with a fluorometer (model 8000-010, Turner

**Table 1** Components of the seawater medium used for *Chaetoceros* sp. culture

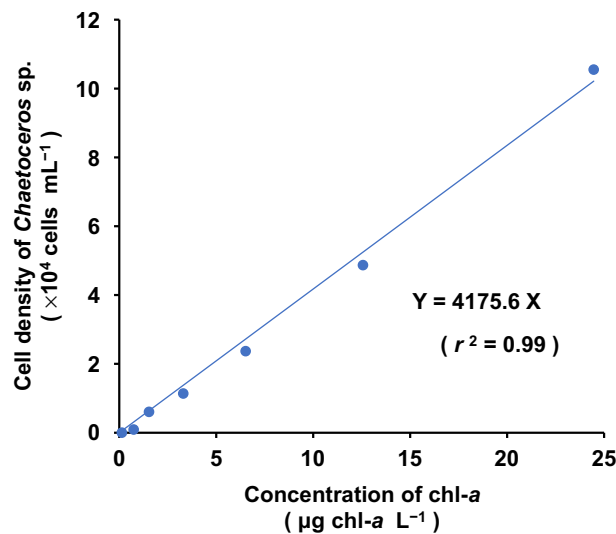
Component	Quantity
Seawater medium	
Sterilized filtered seawater	20 L
NaNO <sub>3</sub>	2.4 g
Na <sub>2</sub> HPO <sub>4</sub>	3.36 g
Na <sub>2</sub> Si <sub>3</sub>	0.24 g
NaHCO <sub>3</sub>	30–40 g
Clewat-32 <sup>a</sup>	0.8–1.2 g
Vitamin mixture solution <sup>b</sup>	24 mL
<sup>b</sup> Vitamin mixture solution	
Distilled water	5 L
Vitamin B <sub>1</sub>	500 mg
Vitamin B <sub>12</sub>	1 mg
Biotin	5 mg

<sup>a</sup>Essential metal additives manufactured by Nagase ChemteX Corporation, Osaka





**Fig. 1** Schematic diagram of the experimental system used for the 2-week feeding experiment on *Fulvia mutica*. **a** Experimental system. The amount of food available to the cockles was adjusted using the chlorophyll *a* (chl-*a*) concentration of seawater in the storage tank. **b** Cockle-breeding bottle. Five bottles were prepared for each experimental system. **c** Placement of each experimental system in the 50,000-L concrete pool for water temperature control (view from above)



**Fig. 2** Relationship between chlorophyll *a* (chl-*a*) concentration and cell density of *Chaetoceros* sp. Dots indicate the measured values. The linear regression line (solid line) and its calculated expression are shown

Designs, Sunnyvale, California, USA) shortly after the water renewal conducted each morning. Twenty-four hours after that, the chl-*a* concentration and the amount of water lost from each 500-L tank was determined. Additionally, the daily chl-*a* concentration in the breeding water was defined as the average value at 0 h and 24 h after water renewal.

### Estimating phytoplankton ingestion quantity in the laboratory experiment

On the 5th and 6th days (48 h) of the 2-week feeding experiment, we examined the fluctuations of chl-*a* concentration in the drainage water of each breeding bottle every 3 h. We estimated the quantity of phytoplankton ingested by each cockle assuming that the chl-*a* concentration in each breeding bottle was maintained at a constant level for the 2 weeks of the feeding experiment through the cockle's water filtration; the constant level of chl-*a* concentration was defined as the average value calculated for the 48 h of observation. The quantity ingested by each individual during the 2 weeks ( $I$ ;  $\mu\text{g chl-}a \text{ individual}^{-1}$ ) was calculated according to the following equations:

$$I = \sum_{n=1}^{14} I_n, \quad (1)$$

$$I_n = F_n \cdot (C_{t_n} - C_b), \quad (2)$$

$$F_n = Q_n/5, \quad (3)$$

where  $I_n$ ,  $F_n$ ,  $C_{t_n}$ , and  $Q_n$  are the ingestion quantity ( $\mu\text{g chl-}a \text{ d}^{-1} \text{ individual}^{-1}$ ), the daily flow for each breeding bottle ( $\text{L d}^{-1}$ ), the chl-*a* concentration in the 500-L tank ( $\mu\text{g chl-}a \text{ L}^{-1}$ ), and the amount of water lost from each 500-L tank ( $\text{L d}^{-1}$ ), respectively, on day  $n$ . The chl-*a* concentration in each breeding bottle ( $C_b$ ,  $\mu\text{g chl-}a \text{ L}^{-1}$ ) was calculated as the average value of the 48 h of observation.

### Estimating growth rates in the laboratory experiment

In the present study, growth rates were evaluated based on changes in the shell length and dry soft-body weight of juvenile cockles. The shell length of cockles in each breeding bottle was measured at the start and end of the laboratory experiment. Based on these measurements, the average daily extension rate ( $\text{mm d}^{-1}$ ) was calculated for each individual. To determine dry soft-body weight, the juvenile cockles were immediately dissected after the shell length measurement at the end of the breeding experiment, and their body tissues were dried to constant weight for 24–48 h at 60 °C. The soft-body weight of 15 cockles of the same lot as those used in the breeding experiment ( $n = 25$ ) was also measured in the same manner to represent the initial dry weight (DW). Based on these measurements, the average daily weight increase rate ( $\text{mg DW d}^{-1}$ ) was calculated for each individual.

### Estimating the relationship between the chl-*a* concentration in ambient water and the quantity of phytoplankton ingested by cockles

We also intended to examine the effects of chl-*a* concentration in the breeding water (hereafter called 'ambient water') on the growth of *F. mutica* individuals. Unfortunately, we could not generate a chl-*a* concentration gradient in the water contained in the breeding bottles. Although the water in the 500-L storage tanks (Fig. 1) had a chl-*a* concentration gradient (from 0 to 20  $\mu\text{g chl-}a \text{ L}^{-1}$ ), the concentration of chl-*a* in the water within each breeding bottle was low. Therefore, we estimated the relationship between chl-*a* concentration in the ambient water and the quantity of phytoplankton ingested by *F. mutica* as follows. Under high food-particle concentrations, bivalve excreta, called pseudofeces, are observed. Pseudofeces constitute the part of the filtrated feeding particles that is ejected without being taken into the bivalve body through the mouth; filtrated feeding particles excreted as pseudofeces are not related to digestion, absorption, and growth of bivalves. According to our laboratory observations, at 15  $\mu\text{g chl-}a \text{ L}^{-1}$  of ambient water (the chl-*a* concentration in our field study site rarely exceeded this value) *F. mutica* pseudofeces were virtually absent. Thus, we examined the relationship between the chl-*a* concentration of the ambient water and the clearance rate, assuming that the cleared quantity would be equivalent to the ingested quantity. Three glass beakers (outer diameter: 146 mm, height: 216 mm), each containing 3 L of filtered seawater and five *F. mutica* individuals, and one beaker with seawater only (control) were set for this experiment. The cockles were stored under dim light at 25 °C, and,

after being transferred to the beakers, they were left to stand for 30 min before the algal culture (*Chaetoceros* sp.) was inoculated into all four beakers at  $15 \mu\text{g chl-}a \text{ L}^{-1}$ . Before adding the inoculum, a volume of seawater equal to that of the inoculum was drained from each beaker. The seawater in the beakers was gently aerated to ensure the homogeneous distribution of phytoplankton. Measurements of chl-*a* concentrations were conducted every 10 min for 1 h. The rate of chl-*a* clearance by each cockle was calculated according to

$$CI = V \cdot (C_t - C_{t+\Delta t}) / (n \cdot \Delta t), \quad (4)$$

where CI is the clearance rate ( $\mu\text{g chl-}a \text{ min}^{-1} \text{ individual}^{-1}$ ),  $V$  is the volume of water in the beaker (3 L),  $C_t$  is the chl-*a* concentration at the beginning of the experiment ( $\mu\text{g chl-}a \text{ L}^{-1}$ ),  $C_{t+\Delta t}$  is the chl-*a* concentration at the end of the experiment ( $\mu\text{g chl-}a \text{ L}^{-1}$ ),  $n$  is the number of cockles per beaker ( $n = 5$ ), and  $\Delta t$  is the duration of the time interval ( $t, t + \Delta t$ ) (min).

#### Field investigation site and estimation of *F. mutica* growth rate in situ

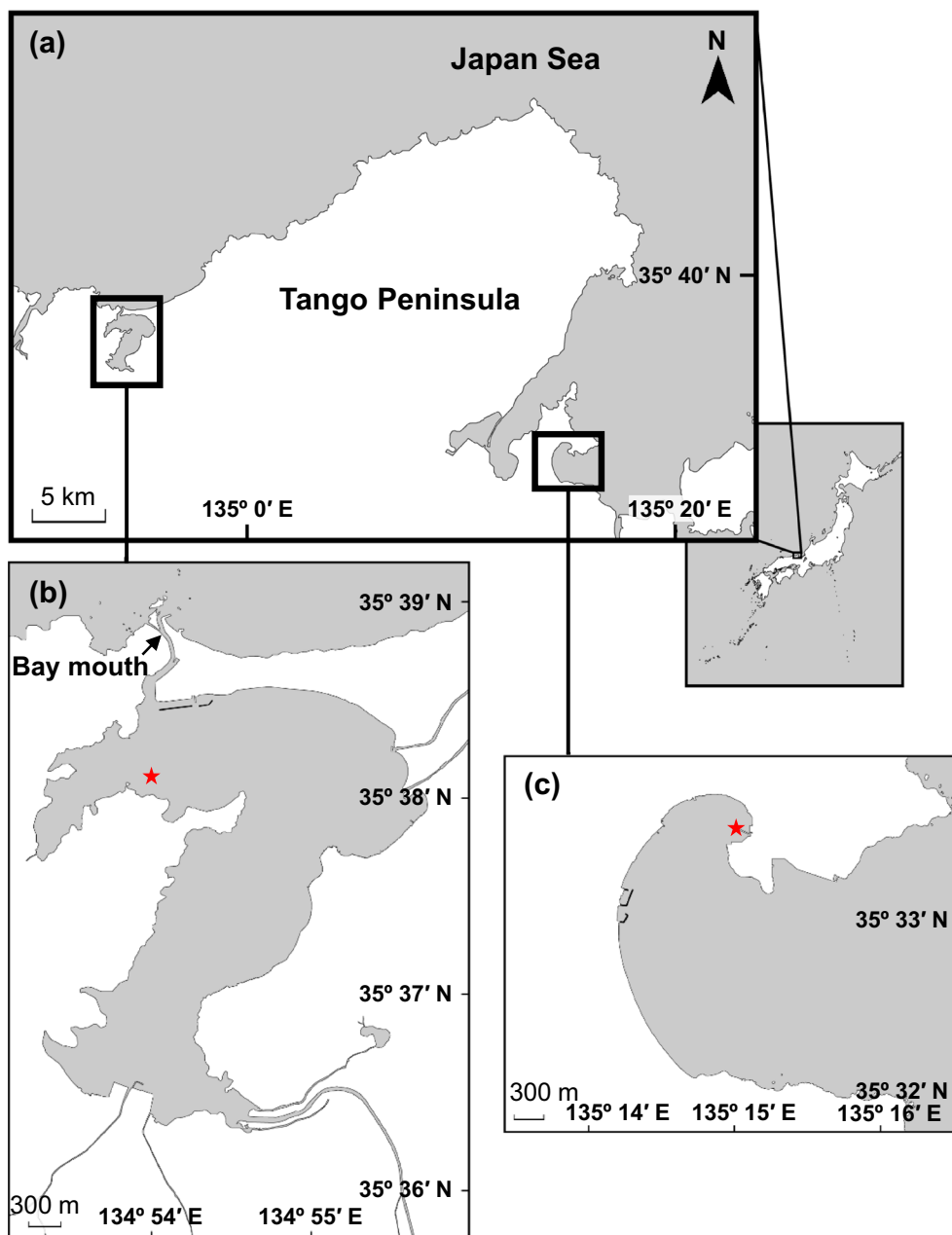
We also conducted a field investigation in 2015 to understand the relationship between the quantity of phytoplankton ingested and the growth rate of juvenile *F. mutica*. Our investigation sites were Kumihama Bay ( $35^\circ 38' 5'' \text{ N}$ ,  $134^\circ 54' 00'' \text{ E}$ ) and Kunda Bay ( $35^\circ 33' 30'' \text{ N}$ ,  $135^\circ 15' 4'' \text{ E}$ ) in the Sea of Japan (Fig. 3). Cockles from the same lot as those used in the laboratory experiment were used in the field investigation. Twenty-five individuals were placed into each polypropylene container ( $508 \times 332 \times 220 \text{ mm}$ ) containing a 10-cm-thick anthracite layer. These containers were suspended at various depths from a culturing raft: at 1, 2, 3.5, 5, and 6 m depths in Kumihama Bay, and at 6 m depth in Kunda Bay. Three containers were prepared for each depth. The periods of cultivation in Kumihama Bay were from August 7 to September 29, at 1 and 2 m depths (53 days), and from August 27 to September 29, at 3.5, 5, and 6 m depths (33 days); in Kunda Bay, cockles were cultivated from August 20 to September 15 (26 days). Growth rates were evaluated based on both shell length and dry soft-body weight. Both measurements were conducted at the start and end of the field cultivation periods. At the start of the cultivation period, 10 individuals from each container at each depth were collected; at the end of the cultivation period, the samples from each depth were pooled, and 9–19 individuals were collected and measured. The daily extension rate ( $\text{mm d}^{-1}$ ) and daily DW increase rate ( $\text{mg DW d}^{-1}$ ) were calculated from the average values of each measurement.

#### Field measurement of chl-*a* concentration

During the field study, we measured the chl-*a* concentration around the containers in which cockles were cultivated. At Kumihama Bay, vertical chl-*a* concentration profiles were automatically determined at every hour using the CTD data logger (DS5X; Hydrolab, Loveland, Colorado, USA). The daily chl-*a* concentration was taken as the average value of every 24 h. At Kunda Bay, the chl-*a* concentration at 6 m depth was measured every week using a portable CTD (MS5; Hydrolab).

#### Estimating the quantity of ingested phytoplankton in the field investigation

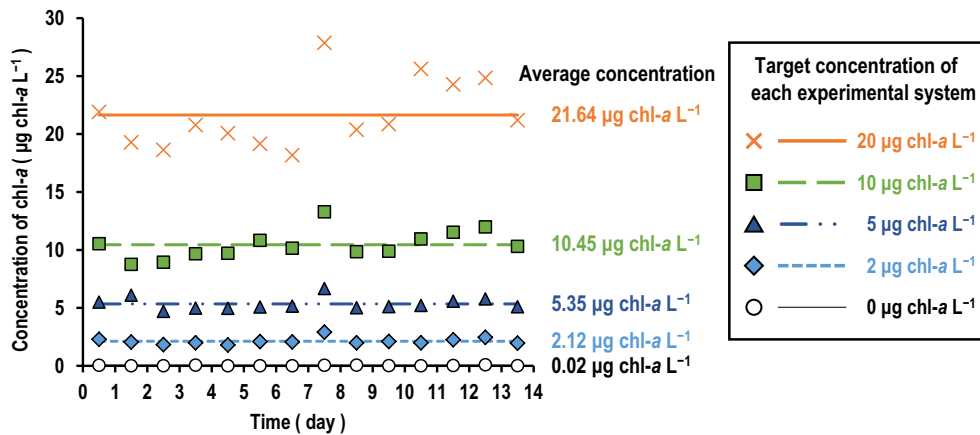
The quantity of phytoplankton ingested by cockles in the field study was estimated as follows. At each water depth at which the containers were suspended, the average chl-*a* concentration during the investigation period was calculated from the daily fluctuations (as described in the previous section), and these average values were regarded as the chl-*a* concentrations of the ambient water to which the cockles were exposed. The quantities of phytoplankton ingested by the field cockles were estimated based on the results of the laboratory experiment obtained for the clearance rate and on the average chl-*a* concentration values measured in the ambient water at the field sites.



**Fig. 3** Location of the field investigation sites. **a** The two sites where the field investigations were conducted, Kumihama Bay and Kunda Bay. **b** Kumihama Bay, a closed bay with a very narrow mouth. **c** Kunda Bay, a bay with a relatively wide mouth. Kunda Bay contains less phytoplankton than Kumihama Bay due to good seawater exchange. The red stars indicate the areas at which the containers were placed

### Statistical analyses

Least squares linear regressions of cockle growth rate (soft-body weight and shell length) against daily quantity of ingested phytoplankton were performed. An  $F$ -distribution was used to calculate the significance of the slope and the 95% prediction intervals for the slope and intercept.

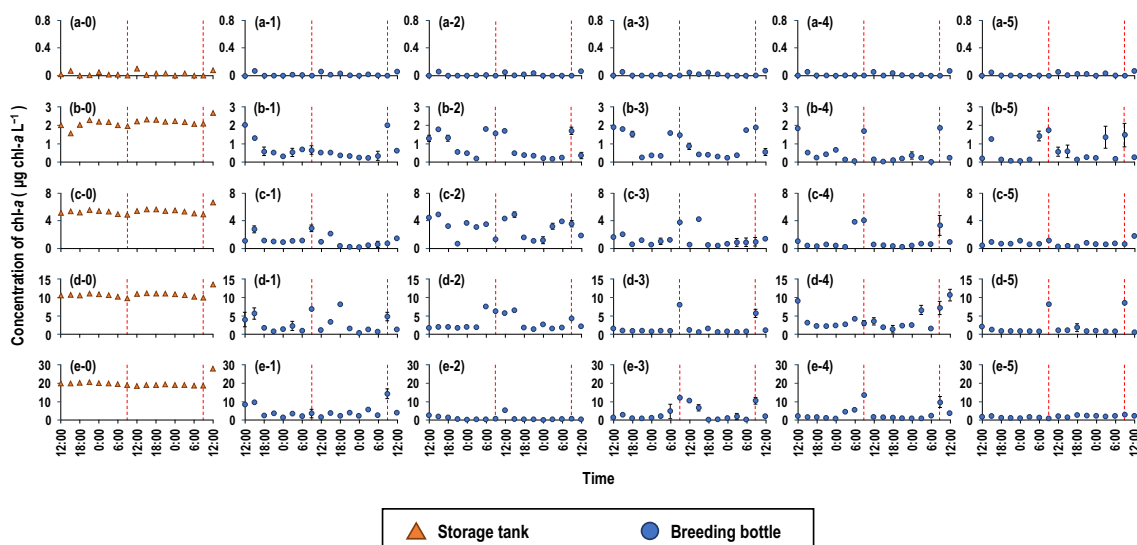


**Fig. 4** Daily chlorophyll *a* (chl-*a*) concentrations in the water of the storage tank of each experimental system (as displayed in Fig. 1a) during the experimental period. Several chl-*a* concentrations were set for the storage tanks of each experimental system (0–20  $\mu\text{g chl-}a \text{ L}^{-1}$ ). The value measured on each day of the experiment is indicated by each symbol, and the lines and numerical values indicate the average chl-*a* concentrations during the experimental period

## Results

### Laboratory feeding experiment

During the 2-week feeding experiment, the chl-*a* concentrations of the breeding seawater (hereafter called the “stocked water”) were adjusted every morning in the five tanks. Although we attempted to adjust the chl-*a* concentrations of the stocked water to 0, 2, 5, 10, and 20  $\mu\text{g chl-}a \text{ L}^{-1}$ , the daily chl-*a* concentrations of the stocked water could only be adjusted to values around these concentrations, and the average chl-*a* concentrations during the experimental period were 0.02, 2.12, 5.35, 10.45, and 21.64  $\mu\text{g chl-}a \text{ L}^{-1}$ , respectively (Fig. 4).



**Fig. 5** Fluctuations of chlorophyll *a* (chl-*a*) concentrations registered in the first 48 h of the 2-week laboratory feeding experiment. Measurements were conducted in one storage tank and five breeding bottles of each experimental system comprising a set of five chl-*a* concentrations (a-0–a-5: 0  $\mu\text{g chl-}a \text{ L}^{-1}$ , b-0–b-5: 2  $\mu\text{g chl-}a \text{ L}^{-1}$ , c-0–c-5: 5  $\mu\text{g chl-}a \text{ L}^{-1}$ , d-0–d-5: 10  $\mu\text{g chl-}a \text{ L}^{-1}$ , e-0–e-5: 20  $\mu\text{g chl-}a \text{ L}^{-1}$ ). Dots indicate the measured values in each replicate ( $n = 3$ ). The red broken line indicates the times at which chl-*a* concentration was adjusted, after renewing the seawater in the storage tank



The stocked water flowed into each of the breeding bottles containing a cockle, and the time-dependent changes of the chl-*a* concentration in each bottle were determined (Fig. 5). For the 0.02 µg chl-*a* L<sup>-1</sup> stocked water, chl-*a* concentration in the stocked water and the breeding bottle water were almost identical (Fig. 5a-0–a-5). For the 2.12–21.64 µg chl-*a* L<sup>-1</sup> stocked waters, chl-*a* concentration was lower in breeding bottles than in the corresponding stocked waters. In most bottles, the chl-*a* concentration increased after the renewal of stocked water, (Fig. 5b-1–b-5, c-1, c-3, c-4, d-1–d-5, e-1, e-3, e-4), while in others, it remained consistently low (Fig. 5c-5, e-2, e-5) or decreased slightly (Fig. 5c-2). Although three patterns of decreasing chl-*a* concentration were observed, the fluctuations of chl-*a* concentrations in the same bottle were similar on the first (0–24 h) and second (24–48 h) days of the experiment (Fig. 5). Therefore, these were considered diurnal variation patterns, and the quantity of phytoplankton ingested by the cockle was estimated based on the assumption that these variation patterns would be repeated daily, in each bottle, during the 2-week experimental period (Table 2).

Relationship between the quantity of ingested phytoplankton and *F. mutica* growth

To estimate the necessary concentration of chl-*a* in the habitat for cockles to ingest a certain amount of food, we examined the relationship between chl-*a* concentration in the ambient water and the clearance rate of cockles (Fig. 6). This relationship was expressed by a linear regression equation:

$$Y_{\text{clearancerate}} = 0.0423 \cdot X_{\text{Ambientchl-aconc.}}, \tag{5}$$

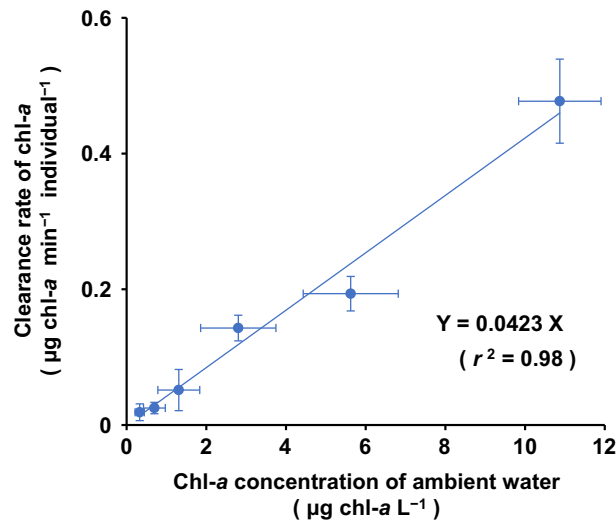
and a high coefficient of determination was obtained ( $r^2 = 0.98$ ,  $F_{1,4} = 204.41$ ,  $P < 0.001$ ).

The 2-week feeding experiment allowed analyzing the relationship between the daily-ingested phytoplankton and daily growth rate (soft-body weight and shell length) (Figs. 7, 8). The growth rates based on soft-

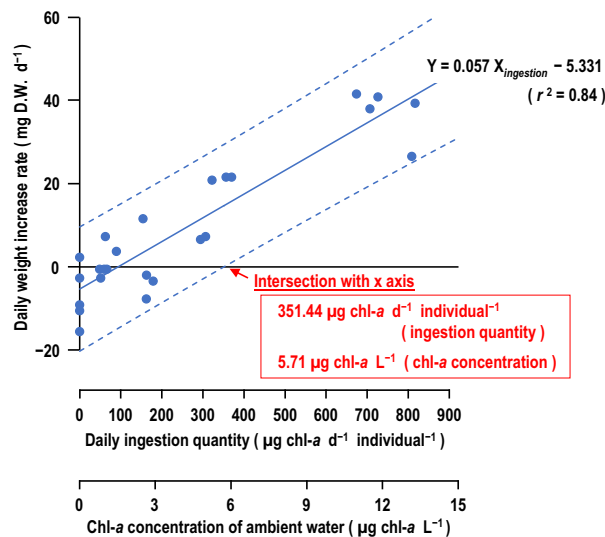
**Table 2** Summary of chlorophyll *a* (chl-*a*) concentrations in the breeding water used for each cockle and estimated quantity of ingested phytoplankton

Average chl- <i>a</i> concentrations in the breeding water of storage tank <sup>a</sup> (µg chl- <i>a</i> L <sup>-1</sup> )	Individual	Chl- <i>a</i> concentrations in breeding bottle <sup>b</sup> (µg chl- <i>a</i> L <sup>-1</sup> )	Total ingested quantity during the 2-week experiment (µg chl- <i>a</i> individual <sup>-1</sup> )	Daily ingested quantity (µg chl- <i>a</i> d <sup>-1</sup> individual <sup>-1</sup> )
2.12	Cockle-1	0.71	834.1	59.6
	Cockle-2	0.91	720.3	51.5
	Cockle-3	0.97	681.4	48.7
	Cockle-4	0.54	928.1	66.3
	Cockle-5	0.63	879.8	62.8
5.35	Cockle-1	1.12	2274.3	162.5
	Cockle-2	3.05	1253.6	89.5
	Cockle-3	1.32	2163.4	154.5
	Cockle-4	1.11	2281.1	162.9
	Cockle-5	0.67	2508.6	179.2
10.45	Cockle-1	2.85	4517.9	322.7
	Cockle-2	3.23	4298.4	307.0
	Cockle-3	1.72	5187.8	370.6
	Cockle-4	3.52	4123.7	294.6
	Cockle-5	2.04	4997.2	356.9
21.64	Cockle-1	4.50	9446.5	674.8
	Cockle-2	1.02	11,334.1	809.6
	Cockle-3	3.66	9905.0	707.5
	Cockle-4	3.16	10,179.1	727.1
	Cockle-5	1.84	9811.8	700.8

<sup>a,b</sup>Refer to Fig. 1



**Fig. 6** Relationship between the clearance rate of chlorophyll *a* (chl-*a*) by *Fulvia mutica* and the chl-*a* concentration in the ambient water. Dots indicate the average value of three replicates. The linear regression line (solid line) and its expression are shown

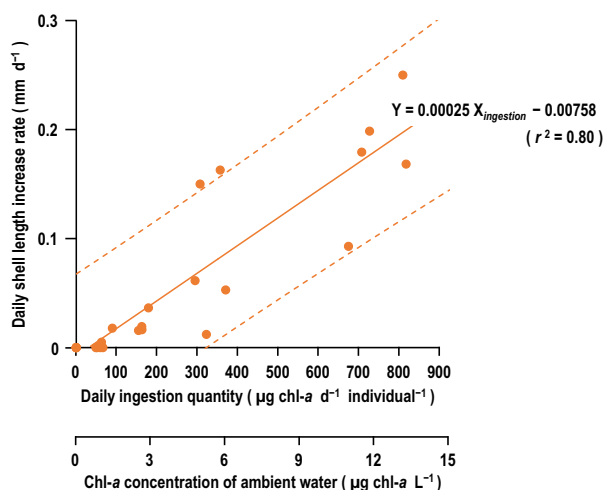


**Fig. 7** Relationship between daily soft-body weight increase of *Fulvia mutica* and the quantity of ingested phytoplankton or concentration of chlorophyll *a* (chl-*a*) in the ambient water. The concentration of chl-*a* in the ambient water (horizontal axis) was estimated from the relationship presented in Fig. 6, assuming that all the chl-*a* removed by the filtration activity was ingested by the cockle. Dots indicate measured values. The linear regression line (solid line) and its calculated expression are shown. The 95% prediction limit for the linear regression is indicated by the broken lines

body weight and shell length showed linear regression relationships with the quantity of ingested phytoplankton:  $r^2 = 0.84$ ,  $F_{1,23} = 123.59$ ,  $P < 0.001$  for soft-body weight growth;  $r^2 = 0.80$ ,  $F_{1,23} = 96.22$ ,  $P < 0.001$  for shell length growth. Equations (6) and (7) describe the soft-body weight ( $Y_{\text{soft body weight}}$ : mg DW  $d^{-1}$ ) and shell length ( $Y_{\text{shell length}}$ : mm  $d^{-1}$ ) of cockles as a function of ingested phytoplankton ( $X_{\text{ingestion}}$ :  $\mu\text{g chl-}a \text{ d}^{-1} \text{ individual}^{-1}$ ):

$$Y_{\text{softbodyweight}} = 0.057 \cdot X_{\text{ingestion}} - 5.331, \quad (6)$$

$$Y_{\text{shelllength}} = 0.00025 \cdot X_{\text{ingestion}} - 0.00758. \quad (7)$$



**Fig. 8** Relationship between the daily shell length increase of *Fulvia mutica* and the daily quantity of ingested phytoplankton or concentration of chlorophyll *a* (chl-*a*) in the ambient water. The concentration of chl-*a* in the ambient water (horizontal axis) was estimated from the relationship presented in Fig. 6, assuming that all the chl-*a* removed by the filtration activity was ingested by the cockle. Dots indicate measured values. The linear regression line (solid line) and its calculated expression are shown. The 95% prediction limit for the linear regression is indicated by the broken lines

**Table 3** Chlorophyll *a* (chl-*a*) concentrations and cockle growth obtained in the field investigation in 2015

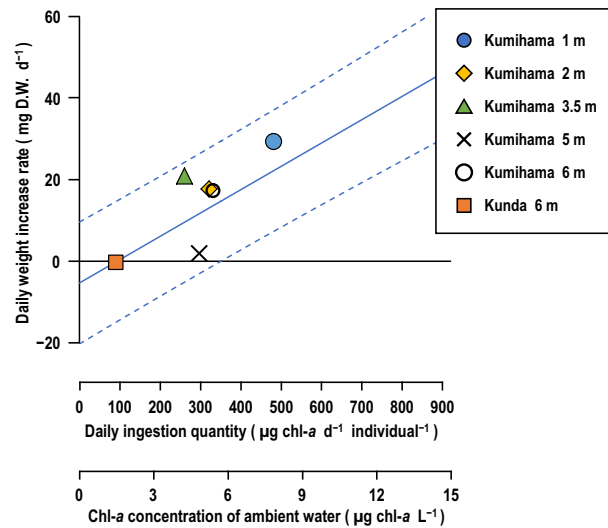
Investigation site	Depth (m)	Study period	Average chl- <i>a</i> concentration (µg chl- <i>a</i> L <sup>-1</sup> )	Average dry soft-body weight (g)		Average shell length (mm)	
				Start	End	Start	End
Kumihama	1	Aug 7–Sep 29 (52 days)	7.83 ± 6.33 (n = 50)	0.15 ± 0.04 (n = 10)	1.70 ± 0.37 (n = 10)	26.8 ± 2.4 (n = 10)	54.0 ± 4.2 (n = 10)
	2	Aug 7–Sep 29 (52 days)	5.23 ± 3.70 (n = 50)	0.18 ± 0.04 (n = 10)	1.12 ± 0.37 (n = 14)	29.5 ± 1.8 (n = 10)	50.7 ± 5.1 (n = 14)
	3.5	Aug 27–Sep 29 (33 days)	4.24 ± 1.98 (n = 33)	0.98 ± 0.15 (n = 10)	1.67 ± 0.23 (n = 19)	49.7 ± 2.5 (n = 10)	59.6 ± 3.3 (n = 19)
	5	Aug 27–Sep 29 (33 days)	4.83 ± 2.22 (n = 33)	1.43 ± 0.22 (n = 10)	1.49 ± 0.20 (n = 10)	50.5 ± 2.5 (n = 10)	56.6 ± 2.2 (n = 10)
	6	Aug 27–Sep 29 (33 days)	5.39 ± 2.66 (n = 33)	1.11 ± 0.19 (n = 10)	1.68 ± 0.36 (n = 9)	46.5 ± 2.4 (n = 10)	58.0 ± 4.5 (n = 9)
Kunda	6	Aug 20–Sep 15 (26 days)	1.47 ± 0.44 (n = 5)	0.67 ± 0.11 (n = 10)	0.66 ± 0.15 (n = 15)	43.5 ± 2.2 (n = 10)	44.1 ± 3.9 (n = 15)

Data are mean ± standard deviation

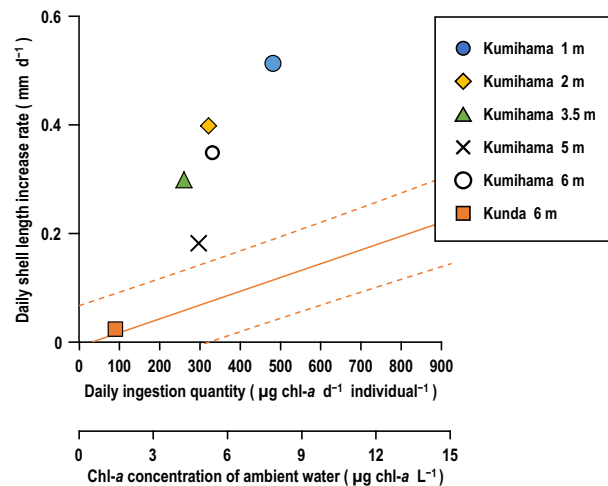
Based on these equations, the minimum amount of ingested phytoplankton required for cockle growth was 93.53 µg chl-*a* d<sup>-1</sup> individual<sup>-1</sup> (estimated chl-*a* concentration of ambient water: 1.52 µg chl-*a* L<sup>-1</sup>) for soft-body weight increase and 30.32 µg chl-*a* d<sup>-1</sup> individual<sup>-1</sup> (0.49 µg chl-*a* L<sup>-1</sup>) for shell length growth.

Field investigation

The growth of *F. mutica* and the average chl-*a* concentrations at the various water depths in the two field investigation sites are summarized in Table 3. The average chl-*a* concentration at 1 m depth at Kumihama Bay was the highest (7.83 ± 6.33 µg chl-*a* L<sup>-1</sup>) and that at 6 m depth at Kunda Bay was the lowest (1.47 ± 0.44 µg chl-*a* L<sup>-1</sup>). The field data obtained for the relationship between chl-*a* concentration in the ambient water and *F. mutica* growth (soft-body weight and shell length) were plotted against the results of the 2-week laboratory feeding experiment (Figs. 9, 10). Regarding the increase of soft-body weight, most of the



**Fig. 9** Comparison of the increments of soft-body weight obtained in the laboratory experiment and in the field investigation. Symbols indicate the values measured in the field investigation. Solid and broken lines indicate the regression line and its 95% prediction limit, respectively, obtained in the laboratory experiment, as displayed in Fig. 7. The concentration of chlorophyll *a* (chl-*a*) in the ambient water (horizontal axis) was estimated as indicated in Fig. 6, assuming that all the chl-*a* removed by the filtration activity was ingested by the cockle



**Fig. 10** Comparison of the increments in shell length obtained in the laboratory experiment and in the field investigation. Symbols indicate the values measured in the field investigation. Solid and broken lines indicate the regression line and its 95% prediction limit, respectively, obtained in the laboratory experiment, as displayed in Fig. 8. The concentration of chlorophyll *a* (chl-*a*) in the ambient water (horizontal axis) was estimated as indicated in Fig. 6, assuming that all the chl-*a* removed by the filtration activity was ingested by the cockle

data obtained from the field investigation was included in the 95% prediction interval obtained for the laboratory experiment (Fig. 9). In contrast, for the increase of shell length, the results of the field investigation and that of the laboratory experiment were remarkably different; the growth rates of the cockles kept in the field were higher than those of the cockles kept in the laboratory, under the same chl-*a* concentrations (Fig. 10). For reference, the temperature, salinity, and dissolved oxygen concentration during the field investigation are shown in Table 4. There were no remarkable differences in the average water temperature between investigation sites or water depths (24.4–26.4 °C). The lowest average salinity was 22.5 psu and this was registered at 1 m depth at Kumihama Bay. Moreover, no long-term occurrence of dysoxic water masses

**Table 4** Ambient water characteristics measured in the field investigation in 2015

Investigation site	Depth (m)	Study period	Temperature (°C)	Salinity (psu)	Dissolved oxygen (mg L <sup>-1</sup> )
Kumihama	1	Aug 7–Sep 29 (52 days)	25.7 ± 2.4 (n = 50)	22.5 ± 7.4 (n = 50)	7.5 ± 1.2 (n = 50)
	2	Aug 7–Sep 29 (52 days)	26.4 ± 1.8 (n = 50)	29.5 ± 2.4 (n = 50)	7.0 ± 1.0 (n = 50)
	3.5	Aug 27–Sep 29 (33 days)	24.8 ± 1.0 (n = 33)	32.0 ± 0.4 (n = 33)	5.8 ± 0.5 (n = 33)
	5	Aug 27–Sep 29 (33 days)	24.6 ± 0.8 (n = 33)	32.8 ± 0.2 (n = 33)	5.1 ± 0.8 (n = 33)
	6	Aug 27–Sep 29 (33 days)	24.4 ± 0.6 (n = 33)	33.1 ± 0.1 (n = 33)	4.5 ± 1.0 (n = 33)
Kunda	6	Aug 20–Sep 15 (26 days)	25.6 ± 1.6 (n = 5)	33.7 ± 0.6 (n = 5)	6.5 ± 0.2 (n = 5)

Data are mean ± standard deviation

was observed during the field investigation period, and the average dissolved oxygen concentrations were  $\geq 4.5$  mg L<sup>-1</sup> at all depths and sites.

## Discussion

In the aquaculture industry, it is important for fishery products to grow fast and systematically. In other words, it is necessary to understand the relationship between food consumption and growth. This is especially true for bivalves, as this basic information is necessary when considering sea area selection or production density. However, when examining the growth of bivalves, two indicators are generally used: body weight increment and shell size growth (shell length, shell height, and shell width) (e.g., Franz 1993; Alunno-Bruscia et al. 2001; Larson et al. 2014). In the present study, we examined the growth of *F. mutica* based on soft-body weight and shell length increments, and compared the results of the laboratory experiment with that of the field investigation. Although for the relationship between the soft-body weight increment and feed consumption, the measurements from the field investigation fell almost entirely within the range of the 95% prediction interval of the regression analysis based on the laboratory experiment (Fig. 9), for the increment of shell length, the measurements from the field investigation were remarkably different from that of the laboratory experiment (Fig. 10). Many studies have reported that the increase in bivalves' soft-body weight and shell length are affected by various factors in their habitat (Malone and Dodd 1967; Seed 1976; Bayne and Worrall 1980; Kautsky 1982; Franz 1993; Alunno-Bruscia et al. 2001; Wong and Levinton 2004; Berge et al. 2006; Hiebenthal et al. 2012). In particular, it has been reported that food quantity affects the growth of bivalves differently when considering soft-body weight increase or shell-size increase (Franz 1993; Alunno-Bruscia et al. 2001). Franz (1993) reported that the mussel *Geukensia demissa* conserves shell growth at the expense of body weight increase in the case of food depletion. Using *Mytilus edulis*, Alunno-Bruscia et al. (2001) demonstrated that the effects of reducing or ceasing soft-body weight increase due to food depletion were stronger than the effects of reducing shell growth. However, the authors pointed out that the materials used for the production of bivalve shells and soft tissues partly originated from different sources. It is known that the shell is formed largely through the deposition of ions, mostly calcium from seawater (Wilbur and Saleuddin 1983), and that the organic matter content of the shell is below 5% (Jørgensen 1976; Price et al. 1976). In addition, many studies have reported that shell growth still occurs in starved or undernourished mollusks (e.g. Orton 1925; Palmer 1981; Lewis and Cerrato 1997). Thus, bivalves may not necessarily require nutritional supplementation by feeding to form shells, while nutrient supply via feeding seems indispensable for soft-body growth as it contains many organic components. Still, habitat conditions (e.g. crowding due to population density, properties of sediments, etc.) directly and physically affect bivalve shell formation (Seed 1968; Brown et al. 1976; Newell and Hidu 1982). Although we cannot ascertain the cause, in the present research,



the differences in shell length increment between the laboratory experiment and the field investigation might have been influenced by factors other than the food quantity ingested. In summary, it is considered that the best indicator to directly evaluate the influence of the quantity of ingested food on bivalve growth would be to estimate the increment in soft-body weight. Furthermore, because aquaculture is a protein-providing industry, information on efficiently increasing bivalve soft-body weight is important. Thus, the results of the present study are discussed below focusing on the increment of soft-body weight.

In the present study, the relationship between ingested phytoplankton quantity and soft-body weight increment was represented by a linear regression (Fig. 7). According to this equation, the amount of ingested food required for *F. mutica* soft-body weight increase was at least  $93.53 \mu\text{g chl-}a \text{ d}^{-1} \text{ individual}^{-1}$  (estimated chl-*a* concentration in ambient water:  $1.52 \mu\text{g chl-}a \text{ L}^{-1}$ ). When a similar ingested food quantity (estimated chl-*a* concentration in ambient water) was calculated from the lower limit of the 95% prediction interval, the amount of ingested food required for *F. mutica* soft-body weight increase was  $351.44 \mu\text{g chl-}a \text{ d}^{-1} \text{ individual}^{-1}$  ( $5.71 \mu\text{g chl-}a \text{ L}^{-1}$ ) (Fig. 7). Up to about  $700 \mu\text{g chl-}a \text{ d}^{-1} \text{ individual}^{-1}$  ( $11.37 \mu\text{g chl-}a \text{ L}^{-1}$ ), which was the highest quantity of food ingested under the experimental conditions, the soft-body weight of *F. mutica* increased as the amount of food increased. Based on these results, it is possible to divide the feeding environment for breeding *F. mutica* into three categories according to ambient chl-*a* concentration: (1)  $< 1.52 \mu\text{g chl-}a \text{ L}^{-1}$ , negative feeding environment for cockle growth; (2)  $1.52\text{--}5.71 \mu\text{g chl-}a \text{ L}^{-1}$ , neutral feeding environment for cockle growth (individuals either grow or not); (3)  $> 5.71 \mu\text{g chl-}a \text{ L}^{-1}$ , positive feeding environment for cockle growth (soft-body weight increases with increasing chl-*a* concentrations up to about  $11 \mu\text{g chl-}a \text{ L}^{-1}$ ). Saito et al. (2007) reported the relationship between the resource characteristics of *Ruditapes philippinarum* and their habitat environment, based on field surveys. The authors divided the habitat chl-*a* concentrations into four stages [(1): bad—(4): good] depending on the clams' resource situation: (1)  $< 1 \mu\text{g chl-}a \text{ L}^{-1}$ , (2)  $2\text{--}4 \mu\text{g chl-}a \text{ L}^{-1}$ , (3)  $4\text{--}6 \mu\text{g chl-}a \text{ L}^{-1}$ , (4)  $\geq 6 \mu\text{g chl-}a \text{ L}^{-1}$ . Comparing the results obtained here with that of Saito et al. (2007), and although the studied species and research methods were different, a similar tendency can be recognized regarding the classification of chl-*a* concentrations in the ambient water for bivalve growth. In the future, it will be necessary to clarify the relationship between bivalves and their feeding environment by examining and comparing results for other bivalve species. Notably, however, our laboratory experiments were carried out at  $25 \text{ }^\circ\text{C}$ , in filtered seawater with salinity 33 psu, and normoxic conditions. It is known that the filtration rate of bivalves is influenced by factors such as temperature, salinity, and dissolved oxygen concentration of the ambient water (e.g. Sará et al. 2008; Enríquez-Ocaña et al. 2012; Nieves-Soto et al. 2013; Riisgård et al. 2013; Kang et al. 2016; Tang and Riisgård 2018). Because bivalves are filter feeders, changes in filtration rates are directly related to changes in the quantities of ingested food. In addition, temperature and salinity also affect the metabolism of bivalves, and therefore change in these factors result in change in the growth rate of bivalves (e.g. Liu et al. 2018; Zhang et al. 2018; Sanders et al. 2018; Haider et al. 2019); for example, low water temperatures might reduce energy requirements, and as a result, less food would be required for growth; under high water temperature, the opposite phenomenon might be observed. So far, the biological effects of several environmental factors, such as ambient water temperature, salinity, and dissolved oxygen concentration, on *F. mutica* have been investigated in the laboratory (Nogami et al. 1981; Tanimoto et al. 2015). Regarding the influence of water temperature, the oxygen consumption of cockles increased with increasing water temperature in the range of  $15\text{--}24 \text{ }^\circ\text{C}$ , and reached a maximum value of around  $25 \text{ }^\circ\text{C}$ , before decreasing gradually from  $26$  to  $28 \text{ }^\circ\text{C}$  (Nogami et al. 1981). In addition, it was reported that survival rate on the 5th day of breeding would be about 60% in normal seawater (salinity 34 psu) at  $28 \text{ }^\circ\text{C}$  (Tanimoto et al. 2015). With respect to salinity, it was reported that salinity levels which would not affect the survival of cockles were 22 psu or more, in water temperatures of  $20\text{--}26 \text{ }^\circ\text{C}$  (Tanimoto et al., 2015). As for the influence of dissolved oxygen concentration, under conditions of less than  $2 \text{ mL L}^{-1}$ , decrease in oxygen consumption, the appearance of abnormal open-shell or mortality individuals were observed (Nogami et al. 1981). Based on the present results, the laboratory experimental conditions used here are desirable conditions for the high physiological activity of *F. mutica*. Thus, depending on the conditions of the ambient water (including temperature, salinity, and dissolved oxygen), the minimum food consumption required for cockle growth (and the minimum chl-*a* concentration required in the ambient water to ensure minimal food intake) could be overestimated using the regression line obtained in the present study.



It is known that bivalves change their filtration rates according to food-particle concentrations (Winter 1978; Navarro and Winter 1982), and highly concentrated feeding environments, in particular, inhibit bivalve filtration activities (Toba and Miyama 1993; Pérez-Camacho et al. 1994; Nagasoe et al. 2011). In addition, under high food-particle concentrations, bivalves filter a quantity of food particles exceeding the amount that could be ingested per unit time, and excess particles must be excreted as pseudofeces (Widdows et al. 1979). Because, as mentioned above, a highly concentrated food-particle environment might cause bivalve feeding inhibition, it has been emphasized that maintaining food-particle concentration within an appropriate range is necessary for efficient bivalve rearing (Thompson and Bayne 1974; Toba and Miyama 1993). Thus, determining food concentration thresholds that inhibit bivalve feeding activity and growth is as important as grasping the minimum amount of food required for growth. Unfortunately, the phytoplankton concentrations within the cockle-breeding bottles used in the present study were all equally low, despite the chl-*a* concentration gradient generated in the water of storage tanks. Consequently, we could not clarify the food concentration threshold that would inhibit cockle growth. Widdows et al. (1979) mentioned that the amount of material filtered per hour would be equivalent to the maximum ingestion rate at the threshold concentration of pseudofeces production (before any rejection occurs). This could be interpreted to imply that feeding inhibition occurs above the threshold concentration at which bivalves excrete pseudofeces. The results of the laboratory experiment performed here revealed an average chl-*a* concentration of about  $11 \mu\text{g chl-}a \text{ L}^{-1}$  as the upper limit of food concentration (Figs. 6, 7) above which no excretion of pseudofeces was observed. Thus, food concentrations above  $11 \mu\text{g chl-}a \text{ L}^{-1}$  inhibit the growth of *F. mutica*, but these should be clarified by detailed experimentation. To examine the growth of *F. mutica* under a highly concentrated feeding environment using the experimental system of the present study (Fig. 1), it will be necessary to adjust chl-*a* concentrations in the water of stock tanks to considerably higher levels than used here; in other words, it will be necessary to keep a certain amount of food in the ambient water inside the breeding bottles even after bivalve filtration.

## Conclusions

The comparison of the results of the laboratory experiment and field investigation implies that soft-body weight increment is a better evaluation index than shell length increment to illustrate the effects of bivalve feeding on growth. In addition, the feeding environment (chl-*a* concentration in ambient water) could be classified into three stages based on the soft-body weight increment of cockles: (1)  $< 1.52 \mu\text{g chl-}a \text{ L}^{-1}$ , negative feeding environment for cockle growth; (2)  $1.52\text{--}5.71 \mu\text{g chl-}a \text{ L}^{-1}$ , neutral feeding environment for cockle growth (individuals either grow or not); (3)  $> 5.71 \mu\text{g chl-}a \text{ L}^{-1}$ , positive feeding environment for cockle growth (growth increases with increasing chl-*a* concentration up to about  $11 \mu\text{g chl-}a \text{ L}^{-1}$ ). For the reasons stated above, it would be desirable to cultivate *F. mutica* at chl-*a* concentrations between 5.71 and  $11 \mu\text{g chl-}a \text{ L}^{-1}$ . The information obtained in the present study can contribute to the efficient breeding and farming of *F. mutica* and in seedling production; for example, in the selection of sea areas and depths with suitable food environments, in the estimation of breeding cockle density to maintain a suitable food environment, and in the consideration of feed volumes for indoor breeding. However, we could not observe growth inhibition of *F. mutica* due to high food concentration, which must be clarified by further detailed experiments in the near future.

**Acknowledgements** This study was part of the results obtained with the support of the NARO Bio-oriented Technology Research Advancement Institution [Grant number 14526938]. We kindly thank K. Tarutani, Y. Matsuyama, T. Kurihara and the anonymous referees for their constructive comments, which have improved our manuscript.

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