Possible effect of a mud snail, *Cipangopaludina chinensis laeta*, and temperature on performance of rice plants and survival, development, and reproduction of other aquatic organisms

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2018

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Chapter 1

General introduction

The expected increase in temperature in the next 30-50 years later due to the climate change is one of the most concerned issues for our life (IPCC 2007). The global warming is possible to alter the biotic and abiotic factors of an ecosystem, as a result development, survival, and interactions of the individuals involved in the ecosystem would be influenced. For example, increased temperature of the water significantly affected the function of an aquatic ecosystem (Walkuska and Wilczek 2010) and the amount of dissolved oxygen as a limiting factor for the survival of aquatic organisms (Matear, 2006). Association between increased temperature and excessive use of inorganic fertilizers in conventional farming led to eutrophication which was detrimental to the environment (Butterwick et al. 2005). In addition, climate warming negatively influenced the performances of zooplankton through the increased abundance of a fish due to rising water temperature (Gutierrez et al. 2016). As a part of global ecosystem, agriculture is also possibly affected by the global warming. Warmer temperature could significantly reduce grain yield in maize by as much as 80-90% from a normal temperature system (Hatfield and Prueger 2015). On the other hand, global warming could bring positive effects to the environment e.g. raised temperature increased the abundance of roach, a zooplanktivorous fish, whose movement disturbed lake sediments resulting nutrients release which was beneficial to the aquatic ecosystem (Jeppesen 2004).

Extensively applied conventional farming relying on inorganic chemicals brings negative consequences to the surroundings. Inefficiency of the use of nitrate fertilizers resulted in groundwater pollution in many developing countries (Bijay-

Singh et al. 1995). In addition to the environmental pollution, many studies revealed the positive correlation between pesticides exposure and cancer diseases in human. The estimated cost for environmental and health care must be compensated due to the residue of pesticide in the United States is about $ 12 billion every year (Pimentel et al. 2005). Currently, the awareness of the society to avoid contaminated food is enhancing as indicated by the increase of global organic products in the market by 43% for three years in 2005 and producers by 31% for only a year in 2009 (Willer and Yusefi 2007; Hamzaoui and Zahaf 2012). In this way, the number of conventional farmers who convert into organic farmers would be more increased in the future. This trend could be a sign that transformation from conventional to environmentally friendly agriculture is a one of possible solutions in reducing the adverse impacts of conventional farming on the environment.

To maintain the yield of rice production similar to conventional farming, environmentally friendly agriculture requires some efforts. Huge amounts of chemical inputs such as inorganic fertilizers should be substituted by the substances which are based on living ecological systems (IFOAM 2005). The nutrients obtained from wetland macrophytes through the process of either grazing by herbivores or decomposing by detritivors usually are deposited extremely high in the sediment (Dodds 2002). Diverse aquatic organisms inhabited in a paddy ecosystem (Fernando 1993) are potential instruments in providing bio-based nutrients positively affecting soil condition with ultimate impacts on the performance of rice plants. Previous study revealed that mud snails affected positively community structure of aquatic organisms and performance of rice plants (Dewi 2017). However, little is known the mechanisms how mud snails positively affected the aquatic organisms. Hence, the study to elucidate the underlying processes is needed.

The change in the one of abiotic factors involved in the process of nutrient cycling in the ecosystem would negatively affect the biotic factors contributing in the production of ecosystem service. Since a balanced ecosystem in rice paddy fields comprising the interactions among aquatic organisms results ecosystem services which are valuable for the members of the ecosystem, the change in temperature as a prominent abiotic factor would bring detrimental effects not only on biotic factors such as the behavior of the organisms but also other abiotic factors e.g. dissolved oxygen in the aquatic ecosystem. The change in the behavior affects their interactions in either population or community levels whose magnitude was determined by the number of species and/or individuals in the ecosystem. Tilman and Downing (1994) suggested that biodiversity of the member involved in the ecosystem showed higher resilience when the ecosystem has faced heavy drought. In order to anticipate the adverse effect possibly caused by the change in temperature in a paddy field ecosystem, understanding the effects and subsequent impacts flowing into the food chain is needed.

The objective of the study in the chapter 2 was to understand the effects of ambient temperature and mud snails, and the interactions of these two factors, on performance of rice plants. Furthermore, in the next chapter, the aim of the study was (1) to reveal the effects of temperature and mud snails on aquatic organisms at individual, population, and community level, and (2) to understand the possible mechanism underlying the process. In general discussion, possible effects of temperature on aquatic organisms through the involvement of biotic and abiotic factors eventually affecting the performance of rice plant were discussed.

Chapter 2

Effects of ambient temperature and the mud snail on performance of rice plant

Introduction

The availability of nutrients plays an important role in the development of cultivated crops. For example, reduced forage production often results from nutrient deficiencies in the soil (Valentine 1980). To maintain forage production, farmers use artificial fertilizers which are immediately available for absorption by plant roots. In addition, as the soil for cultivated crops is highly vulnerable to erosion and nutrient loss, the crops usually must be supplied with additional mineral nutrients (Raven and Johnson 2001). Application of synthetically derived nitrogen, phosphorous, potassium, calcium, magnesium, and micronutrients has significantly increased crop productivity over the past five decades. However, fertilizer remaining in the soil due to inefficiencies in fertilizer application has had negative environmental consequences, such as on the quality of underground water (Killebrew and Wolff 2010).

Organic agriculture is a production system with the goals of sustaining ecosystems and relying on ecological services rather than on the use of artificial chemical inputs, and it may offer a solution for problems associated with modern agriculture (Melece 2010). For example, in organic farming, bio-based plant nutrients obtained from earthworms and beneficial soil microorganisms have promoted the development of several crops (Mc Lean and Parkinson 2000; Scheu 2003).

Recently, global warming gives us a hot issue since it would influence on agricultural production through the development of agricultural crops (Hatfield and Prueger 2015). The important effects of temperature on the physiological processes

influencing the development of plants are well known for many plant species (Haferkamp 1988). Temperature also likely affects the organisms providing bio-based plant nutrients, with possible interactions between temperature and these organisms on plant performance. Since high temperature could give a positive or negative effect on the organisms inhabiting an agricultural ecosystem, the study to reveal the effects of high temperature on the organisms involved in the ecosystem is needed.

Some rice farmers implementing organic farming methods use bio-based nutrients from aquatic organisms instead of chemical or organic fertilizers (Trisnawati et al. 2015). An aquatic ecosystem in rice paddy fields is likely to include many creatures which interact with each other to influence their development and abundance (Salmah et al. 2017). In addition, with increasing biodiversity of an agro-ecosystem, such as a rice paddy and its organisms, the agricultural production of the system also tends to increase (Luo et al. 2014). Collective uptake of nutrients by members of an aquatic community is compensated by remineralization of excreta of these organisms (Dodds 2002). Nutrients contained in the excreta may positively affect rice plant performance. Although there are many aquatic organisms in the paddy fields, the mud snail (*Cipangopaludina chinensis laeta*) may be a key organism as a source of bio-based nutrients (from its excreta) because of its size and abundance (Carlsson et al. 2004). Several studies have suggested that snails positively affect the growth of aquatic organisms. For instance, a field experiment revealed a positive relationship between snail densities and macro algal biomass, and laboratory incubations showed that snail excreta enhanced macro algal growth (Yarrington et al. 2013). In addition, the snail can directly alter the growth of submerged macrophytes (Underwood et al. 1992; Li et al. 2009). Although effects of some freshwater snails on aquatic organisms have received considerable study, the effect of *C. chinensis laeta*, which is a common

species in paddy fields along Japan (Masuda 2007), on rice plant performance, has rarely been investigated.

A paddy field is a system of cultivation enabling various aquatic organisms to grow in the water, thereby creating an aquatic ecosystem. Fluctuations in ambient temperature over time and/or across space within the paddy field possibly affect the development of aquatic organisms. In addition, climatic factors such as temperature influence the composition of the biota as well as the growth of the rice plants (Bambaradeniya and Amerasinghe 2003). As mud snails are larger than other organisms in organic paddy fields, they play an important role in the paddy field ecosystem in influencing community structures of aquatic and terrestrial organisms through the biomass production of rice plants (Dewi et al. 2017). However, the potentially interactive effects of temperature and mud snails on rice plant performance are not fully understood.

To understand the interactive effects of the two factors on the development of rice plants would be important because temperature can represent an important abiotic factor to influence on the development of each species involved in a given ecosystem and mud snails would have a potential effect as a key organism in a paddy field ecosystem. The aim of this study is to understand the effects of temperature and mud snails, and the interactions of these two factors, on performance of rice plants. We conducted a factorial experiment with the two factors and hypothesized that both high temperature and the presence of mud snails would enhance rice plant performance.

Materials and methods

The experiments with and without snails were conducted in both inside and outside a greenhouse at the Faculty of Agriculture, Yamagata University, Tsuruoka, Japan from June 26th until October 3rd in 2015. In order to prepare rice plant seedlings, the seeds (variety Sasanishiki) were soaked in water for 26 days in university farm located in Takasaka which is 5 km south of the Faculty of Agriculture. After that, they were treated with 200 times dilution of fungicide (Ekohopu DJ; Kumiai Chemical Industry) for 24 hours. Seedling trays, 600 mm in length and 300 mm in width, were filled with 5000 ml of akatsuchi soil. The soil in each tray was treated with 15 g of fungicide (Dakoniiru powders; Kumiai Chemical Industry). The seeds were then sowed into the soil and the trays were transferred into a budding machine (Saito SE-361; Kubota) at 32ºC for 48 hours. After the budding procedure, the trays were placed in a plastic greenhouse and kept for 23 days before transplanting. The seedlings were then moved to the green house at the Faculty of Agriculture. Two hills, each containing 6 rice seedlings, were transplanted into a rectangle plastic container (Stack Container #25; Gurinparu), 450 mm in length, 300 mm in width, and 260 mm in height, containing 11 kg of air-dried upland soil. The soil was collected from the field at university farm and sieved using 10 mm mesh to separate out plant remains and other unwanted materials. The soil was then air-dried in fine weather for three days before transplanting. The spacing between the two transplanted hills in each container was 220 mm. Each container had 8000 ml tap water. The depth of water was at maintained 60 - 80 mm from the surface of the soil.

Adult snails of both sexes were randomly chosen and used for the experiment. They were collected from paddy fields at the university farm. The shell height of the adults ranged from 25 mm to 30 mm. The number of snails introduced into each

container for snail treatment and no snail treatment was 6 and 0, respectively. Five replications of each treatment were placed in the same row with no space between containers. To apply two temperature treatments in the experiment, one set of containers was placed in the greenhouse, and a second set of containers was placed outside the greenhouse. Weeding in the containers was conducted first on July 8th in 2015 and continued every two weeks thereafter until no weeds were observed in any of the containers. Weeding was done by evenly scratching by hand the entire surface of the soil to a depth of approximately 10 mm. Uprooted weeds that were floating in the water were then removed by an aquarium fish net.

The temperature of air, water, and soil in the containers located at inside and outside the greenhouse was measured using data logger Elitech RC-4. The data logger sensors for air, water, and soil were positioned at mid-height of the rice plants, at 15 mm above soil surface, and at 15 mm below soil surface, respectively. Mean temperature of water in experimental containers with rice seedlings showed that the temperature inside the greenhouse tended to be higher than outside (Table 1). Rice plant performance was assessed by measuring plant height, tiller number, and leaf color estimating leaf nitrogen through Soil-Plant Analysis Development (SPAD) using SPAD 502 Plus by Konica Minolta. Because it was difficult to see roots on the soil surface when the water was turbid, an empty, transparent glass beaker (250 ml) was placed in the water such that its bottom contacted the soil surface. The circular base of the beaker was divided into eight equal sectors, with each of the eight 45º sectors marked. Root distribution on the soil surface was relatively homogenous, and the number of the roots appearing in each sector was directly counted. To compare food consumption of the snail between the two temperature treatments, we compared the amount of excreta produced by the snails. After 12 hours of daytime exposure in the

two treatments, all snails were removed at 1900 h from the containers and placed individually in small plastic containers, 130 mm in diameter and 60 mm in height. The containers with snails were kept overnight (for about 12 hours) in the greenhouse and then excreta in the containers were collected, filtered using fine woven fabric (No. 50 in mesh size), and dried in an oven (DX 301; Yamato) at 70º C for two days. Dryweight of the excreta was measured using digital scale (Vibra SJ-820JS; JIS JQA). The results of plant performance between temperature and snail treatments were compared using a two-way ANOVA, while the number of roots was done by a generalized linier (mixed) model. The software used for analysis was SPSS (version 15).

Results

The amount of snail excreta

The average dryweight of snail excreta produced in high and normal temperature treatments did not differ significantly ($p = 0.229$ for 26 July; $p = 0.098$ for 20 August), although the trend was that the dryweight at high temperature was lower than at normal temperature treatment on both 26 July and 20 August (Fig. 1).

Rice plant performances

There were two main factors affecting rice plant performances, i.e., mud snails and temperature. Both the presence of mud snails and high temperature resulted in significantly increased plant height ($df = 1$, $p = 0.002$ for mud snails and $df = 1$, $p < 0.001$ for temperature; Table 2 and 3). High temperature negatively affected the number of tillers produced ($df = 1$, $p = 0.016$; Table 2 and 3), while the presence of snails did not significantly affect the number ($df = 1$, $p = 0.471$; Table 2 and 3). On the other hand, SPAD values were higher when snails were present, but were not affected by temperature ($df = 1$, $p = 0.001$ for mud snails and $df = 1$, $p = 0.658$ for temperature; Table 2 and 3). No significant interactions between mud snails and temperature were found in their effects on these three parameters of rice plant performance.

Root distribution on the soil surface

Significantly increase in the number of roots occurred when mud snails were present ($df = 1$, $p < 0.001$; Table 4 and 5).

Discussion

This study clearly showed that the mud snails, as affected by temperature, in turn affected performance of rice plants, although the effects of the snails and temperature differed among different measures of rice plant performance. High temperature resulted in decreased rice performance by reducing the amount of excreta produced by snails. For example, the reduction in tiller number recorded in the high temperature treatment probably resulted from reduced amounts of nutrients derived from snail excreta.

In general, the excreta released from mud snails seem to enhance rice plant performance. For instance, in rice paddy fields, mud snails played an important role by increasing biomass production of rice plants (Dewi et al. 2017). In addition, several studies have suggested that in aquatic ecosystems, the presence of freshwater snails results in increased availability of nutrients to plants and algae (Underwood et al. 1992; Dodds 2002). In this experiment, tiller number was not significantly affected by the mud snails, in contrast to a previous field experiment (Dewi et al. 2017) in which tiller number was significantly higher when snails were present. Lack of significant effect through mud snails on the tiller number may be due to the absence of soil tillage in our experiment, whereas soil tillage was done in the previous field experiment (Dewi et al. 2017), and it would be beneficial for increasing soil nutrients (Zikeli et al. 2013; Zhu et al. 2014).

The relatively small amount of excreta released by mud snails in the high temperature treatment in July and August observed in this study might be due to low activity, as high temperature adversely affects the performance of mud snails (Russell-Hunter 1961; Willmer et al. 2000). For instance, the growth of a snail population was adversely affected by high temperature (van der Schalie and Berry 1973; O'Keeffe

1985) and high water temperature significantly reduced activity of aquatic organisms (Walkuska and Wilczek 2010).

Temperature also directly affected rice plant performance in this experiment. Both plant height and tiller numbers were significantly affected by high temperature, as reported also in previous studies (Kondo and Okamura 1931; Osada et al. 1973; Yoshida 1973). On the other hand, the absence of a significant effect of temperature treatment on SPAD value may indicate that the leaf color tended to be affected by the nutrients instead of the temperature (Dobermann and Cassman 2002; Alam et al. 2005).

The result that significantly higher number of roots occurred on the soil surface when snails were present suggests that production of excreta by mud snails may have affected root movement. Roots are able to find sites in the soil where resources are available, and are capable of morphological responses to obtain the nutrients (Hutchings and de Kroon 1994). The architecture of the root system is also determined by the location of resources in the soil (Rellan-Alvares et al. 2015). This suggests that the rice plants moved their roots in an upward direction towards where the nutrients were accumulated in the soil from deposition of snail excreta.

As there are many aquatic organisms in a paddy field, further studies are needed to understand how the snails and temperature influence other aquatic organisms, with ultimate implications for rice plant performance.

Table 1. Mean temperature of water iin experimental container with rice seedlings placed either outside or inside the greenhouse

Season	Temperature	
	Outside	Inside
July	26.1 (24.3 − 27.1)	27.4 (26.5 − 28.9)[a]
August	25.2 (22.9 − 27.5)	25.7 (24.3 − 27.7)

Student's *t*-test was used; [a] $p = 0.075$

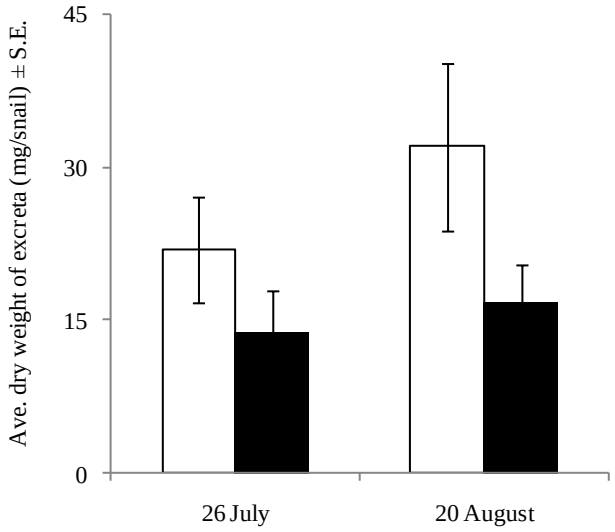

Fig. 1 The amount of excreta produced in 12 hours per individual snail in either high (■) or normal (□) temperature

Table 2. Rice plant performances at the end of the experiment with snails present versus absent, and at either high or normal temperature (mean ± S.E.)

	Treatment	Normal	High
Plant height (cm)	No snail	80.75 ± 1.05	100.90 ± 1.65
	Snail	84.90 ± 0.47	104.70 ± 1.20
Tiller number	No snail	34.10 ± 1.36	32.70 ± 1.16
	Snail	36.50 ± 0.97	32.00 ± 1.14
SPAD (μg cm^{-2})	No snail	29.49 ± 0.77	30.02 ± 0.32
	Snail	32.44 ± 0.45	31.39 ± 0.67

Table 3. Two-way analysis of variance (ANOVA) for measures of rice plant performance

Factor	Source	df	SS	*F*	p
Plant height	Snails	1	158.006	11.537	0.002
	Temperatures	1	3990.006	291.345	<0.001
	Snails x	1	0.306	0.022	0.882
Tiller number	Snails	1	7.225	0.531	0.471
	Temperatures	1	87.025	6.400	0.016
	Snails x	1	24.025	1.767	0.192
SPAD	Snails	1	46.656	13.788	0.001
	Temperatures	1	0.676	0.200	0.658
	Snails x	1	6.241	1.844	0.183

Table 4. Numbers of roots of rice plant as they have appeared on the soil surface with snails present versus absent, and at either high or normal temperature (mean ± S.E.)

Treatment	Normal		High	
No snail	18.2 ±	6.4	18.0 ±	6.2
Snail	55.0 ±	13.3	50.6 ±	13.3

Table 5. A generalized linear (mixed) model with a Poisson error distribution for numbers of roots of rice plant

Factor	Source	df	p
Roots	Snails	1	<0.001
	Temperatures	1	0.338
	Snails x Temperatures	1	0.675

Chapter 3

Effects of temperature and mud snails on survival, development, and reproduction of aquatic organisms: individual, population, and community

Introduction

Temperature is one of the important factors affecting aquatic ecosystems in a paddy field in which many aquatic organisms have been living. Since water is a main habitat for those creatures involved in the ecosystem, change in the temperature occurred in the habitat would give notable effects on reproduction and development of the members of the ecosystem as suggested that oviposition related hormone in snails was majorly affected by the temperature (Wayne 2001). In addition, the phenomenon of global warming in the last couple of decades rising from 2.6 to 4.8°C (IPCC 2013) accelerates the process which could give either positive or negative impacts on the organisms in the agro ecosystem. High temperature positively affected the number of eggs produced and its spawning rates of the freshwater snail *Biomphalaria glabrata* (Pimentel-Souza et al. 1990), while elevated temperature negatively affected fecundity, hatchability, and survival of a limnetic snail *Lymnaea acuminata* (Jigyasu and Singh 2010).

In natural condition, each aquatic organism inhabiting the water body coexists with individuals of same or different species. Intraspecific and interspecific competition would often occur among aquatic organisms because of the limited supply of resources and the same requirements of the organisms for survival, growth, and reproduction (Begon et al. 1996). The behavior responding the competition would

differ in individual, population, and community level. Understanding the factors significantly affecting the performance of organisms in different spatial levels would be important in the implementation of organic farming.

Chemical factors also contribute to understand the components involved in the interactions among the species. Since aquatic organisms like the mud snails cannot directly absorb nutrients dissolved in the water, the presence of other primary producers such as algae, which are able to absorb nutrient in the water, has an important role in the aquatic eco-system (Bronmark and Hansson 2005). The abundance of algae would be influenced by being grazed by snails and temperature (Allan and Castillo 2008). Furthermore, the availability of an abiotic factor such as dissolved oxygen which is substantial to aquatic invertebrate is highly affected by the production process in photosynthesis and consumption in respiration (Dodds 2002). Those processes work in the frame of nutrient cycle providing energy for all creatures involved in the aquatic ecosystem. Understanding the interaction between abiotic and biotic factors in that system would be significant in consideration of survivals, development, and reproduction of each individual involved in an aquatic community which in turn would influence on the structure of the community.

In a paddy field ecosystem, various aquatic organisms coexist with each other and mud snails would play a notable role giving positive and/or negative interactions on the other organisms in that habitat. Dewi et al. (2017) revealed that mud snails have influenced community structures of aquatic and terrestrial organisms through the biomass production of rice plants. Understanding the role of sakamakigai coexisting with mud snails and feeding on algae attached on any structures immersing in the water of paddy field, possibly as an important organism, is needed. In addition, kawanina known as one of the most common freshwater snails found in paddy field

might be important in affecting the other aquatic organisms (Nakanishi et al. 2014). Changes in surrounding temperature over time and across space in the paddy field would probably affect the fate of aquatic organisms in the form of individual, population, and community level. Moreover, temperature has been recognized as a key environmental factor in freshwater, through its role as high heat storage, affecting behavior and metabolic rate of freshwater organisms (Bronmark and Hansson 2005) as well as possibly the growth of the rice plants. However, the potentially interactive effects of temperature and mud snails on aquatic organisms at individual, population, and community level are not fully understood.

The aim of this study was (1) to reveal the effects of temperature and the mud snail on survival, development, and reproduction of aquatic organisms at individual, population, and community level, and (2) to understand the possible mechanism influencing biotic and abiotic interactions. Hypotheses are that the temperature and mud snail would positively affect the aquatic organisms at individual, population, and community level.

Materials and methods

Experimental site and aquatic organisms

The experiments using incubators (MIR-253; Sanyo) and a temperature and humidity controlled room were conducted in the laboratory of Animal Ecology, Faculty of Agriculture, Yamagata University, Tsuruoka, Japan, from August 27th until October 31st 2017. Mud snails and kawanina (*Semisulcospira libertina*) were collected from paddy fields and small ditches, respectively, located in Matsugaoka Farm, Tsuruoka, while populations of sakamakigai (*Physa acuta*) were collected from water canals near paddy fields in Oyama Farm, Tsuruoka, Yamagata prefecture. Three populations of the snails were then separately kept in three containers, 350 mm in length, 600 mm in width, and 300 in height, filled with paddy field soil and evaporated tap water near the green house located in Faculty of Agriculture, Tsuruoka, for approximately 3-5 days to let them acclimate. For the experiment, adult females were chosen for mud snails, adults of both sexes were chosen for kawanina, while adults of sakamakigai were randomly chosen without distinction of sexes. The shell height of adults of the mud snail, sakamakigai, and kawanina ranged from 25 mm to 30 mm, 7.5 mm to 10 mm, and 20 mm to 25 mm, respectively.

Experimental design

Rectangle plastic containers, 140 mm in length, 95 mm in width, and 155 mm in height (Lock pack slim L, D5713; Sanada Seiko), were filled with 1400 ml of tap water which was previously evaporated for three days in the room temperature before the experiments. For individual level experiment, one adult sakamakigai, one adult kawanina, one adult mud snail and sakamakigai or kawanina were introduced into each plastic container (Fig. 3.1). For population level experiment, two adults

sakamakigai, two adults kawanina, one adult mud snail and two adults sakamakigai or two adults kawanina were introduced into each plastic container (Fig. 3.2). As a control, only one mud snail was added into a container at each level of experiments. All treatments were replicated 20 times and maintained inside the incubators with temperatures of 23°C and 29°C equipped with two LED fluorescent tube lights (T8; Ying). In community level experiment, rectangle transparent plastic containers, 225 mm in length, 165 mm in width, and 85 mm in height (Shikkari pack U; Nakaya), filled with 2500 ml evaporated tap water were used and put under the LED lights equipped shelves located in a temperature and humidity controlled room (Fig. 3.3). To preserve the requisite temperature, a heater with thermostat (DX-003; GEX) covered with mesh to prevent direct contact with organisms was placed in the central base of the container (Fig. 3.4). One male and three females of kawanina and four randomly chosen sakamakigai were introduced into each container of all three treatments. The number of the mud snail added in the treatment was 0, 1, and 2 individuals. All treatments were maintained in the water temperatures of 23°C and 29°C. The experiments were replicated 18 times and terminated at 20 days after starting the experiment.

Parameter observed

In individual and population experiments, body weight of each organism was measured before and after the experiments by wiping the snail using tissue paper (Wipers S-200; Kimwiper) for about 2-3 s, and immediately weighing their fresh weight using an analytical balance (AUX120, Shimadzu). The survival was also observed by checking the alive individuals, and dead individuals were removed every two days until termination of the experiment i.e. 14 days after starting the experiments.

The survival rate was calculated by dividing the individuals survived with total individuals. In order to check the required temperature, water temperatures in eight containers per incubator were systematically measured every two days using stick thermometer (formed mercury I72516; Shinwa). In individual experiment, mean temperatures (range) of water in the containers in treatment 29°C, i.e. 29.2°C (28.7-29.7°C), were significantly higher ($p < 0.001$) than in treatment 23° i.e. 23.3°C (22.8-24°C). In population experiment, the mean temperatures in treatment 29°C, i.e. 29.5°C (28.8-30.2°C), were also significantly higher ($p < 0.001$) than in treatment 23° i.e. 23.6°C (23-24.2°C). Chlorophyll content, dissolved oxygen, nitrate (NO_3^-), potassium (K^+), and pH of the water were also measured after experiment using chlorophyll sensor (CHL-30; KRK), portable DO meter (PDO-519; Lutron), nitrate meter (LAQUA Twin B-743, HORIBA), potassium meter (LAQUA Twin B-731, Horiba), and pH meter (Eco pH; Marfied), respectively. At the end of the experiment, organic matters accumulated in the base of each container were filtered using a nylon mesh sheet with opening 308 μm (NB50; Tech Jam), and dried in the drying oven (DVS602; Yamato) at 70°C for 2 days, after that they were weighted using an analytical balance. Total egg clutches of sakamakigai attached on the inner surface of each container were also counted. Additionally, the number of juveniles of kawanina and mud snails found was recorded. Due to the evaporation, the water level of all containers was recorded and added with new water every two days if needed to maintain the volume of 2500 ml.

For community level experiment, body weight of each group of organisms was measured collectively before and after the experiments by the same procedure as previous experiments. The survival was also observed every five days by identical measurement with prior experiments. Temperature inside all containers was checked

by observing water temperature in all containers every two days using stick thermometer. The mean temperatures (range) of water in the containers in treatment 29°C, i.e. 29.2°C (27.9-32°C), were significantly higher ($p < 0.001$) than in treatment 23° i.e. 23.7°C (22.8-25.7°C). Chlorophyll content, dissolved oxygen, nitrate (NO_3^-), potassium (K^+), and pH of the water were measured in the middle and after experiment using the procedures and equipments which were done at individual and population level experiments. Organic matters, egg clutches of sakamakigai, and juveniles of kawanina and mud snails were observed in the end of the experiment using the same procedures as previous experiments.

Statistical analysis

The results of observation of all parameters were compared using a two-way analysis of variance (ANOVA), while survival rates were done by Pearson`s chi-square test. Comparison among treatments in each temperature degree used one-way ANOVA for more than two treatments and Student`s *t*-test for only two treatments. The software used for analysis was SPSS (version 15).

Results

Survival of aquatic organisms

In individual level experiment, high temperature increased the survival of kawanina and decreased the survival of sakamakigai when they coexisted with the mud snail although the difference was not significant ($\chi^2 = 42$, $p > 0.05$ for 23°C and 29°C; Fig. 3.5). In population level experiment, mud snails slightly increased the survival of kawanina at 23°C, although there were no differences in survival at 29°C (Fig. 3.6). In community level experiment, mud snails slightly increased the survival of sakamakigai at both 23°C and 29°C. However, the trend showed that the density of mud snail in high temperature positively affected the survival of sakamakigai, although it was not significantly differed (Fig. 3.7).

Body weight of aquatic organisms

In individual level experiment, temperatures, organisms, and the interaction did not significantly influence the body weight of mud snails, sakamakigai, and kawanina, except the body weight of sakamakigai, was significantly affected by the temperature ($df = 1$, $p = 0.004$; Table 3.1. and Fig. 3.8). In population level experiment, temperatures, organisms, and the interaction significantly influenced the body weight of mud snails. Organisms significantly influenced the body weight of sakamakigai, although it was not significantly influenced by the temperatures and the interaction ($df = 1$, $p = 0.003$; Table 3.2. and Fig. 3.9). Temperatures significantly influenced the body weight of kawanina, although it was not significantly influenced by the organisms and the interaction ($df = 1$, $p = 0.029$; Table 3.2. and Fig. 3.9). In community level experiment, temperatures, organisms, and the interaction did not significantly influence the body weight of mud snails, sakamakigai, and kawanina,

except the body weight of sakamakigai, was significantly affected by the organisms (df = 2, p = 0.019; Table 3.3. and Fig. 3.10).

Egg clutches of sakamakigai

In individual, population, and community level experiment, organisms significantly influenced the number of egg clutches of sakamakigai (df = 1, p = 0.008, Table 3.4. and Fig. 3.11 for individual; df = 1, p < 0.001, Table 3.5. and Fig. 3.12 for population; df = 2, p = 0.005, Table 3.6. and Fig. 3.13 for community).

Juveniles of kawanina

In individual level experiment, temperature significantly influenced the number of juveniles of kawanina (df = 1, p = 0.031; Table 3.7. and Fig. 3.14). In population and community level experiments, no significant differences were found.

Juveniles of mud snail

In individual level experiment, temperatures, organisms, and the interaction significantly influenced the number of juveniles of mud snails (df = 1, p < 0.001 for temperatures, df = 2, p = 0.011 for population, and df = 2, p = 0.042 for interaction between them; Table 3.10. and Fig. 3.17). In population level experiment, temperatures and organisms significantly influenced the number of juveniles of mud snails (df = 1, p = 0.041 for temperatures and df = 2, p = 0.032 for organisms; Table 3.11. and Fig. 3.18). In community level experiment, temperatures significantly influenced the number of juveniles of the mud snail and density of the mud snail significantly influenced the number of the juveniles in 29°C (df = 1, p = 0.026; Table 3.12. and Fig. 3.19). In terms of the effects of other organisms; complexity,

temperatures, and the interaction significantly influenced the number of the juveniles (df = 1, p < 0.001 for complexity, df = 1, p = 0.009 for temperatures, df = 1, p = 0.009 for the interaction; Table 3.13. and Fig. 3.20).

Organic matters from the organisms

In individual level experiment, organisms significantly influenced the organic matters, although the effects of temperature was not significant (df = 4, p < 0.001; Table 3.14. and Fig. 3.21). The interaction between temperatures and organisms affected significantly the organic matters (df = 4, p < 0.001; Table 3.14. and Fig. 3.21). In population level experiment, coexisting with the mud snail treatments resulted in significantly increased organic matters in both low and high temperature (df = 4, p < 0.001; Table 3.15. and Fig. 3.22). In community level experiment, the mud snails and temperature resulted in significantly increased organic matters (df = 2, p = 0.012 for organisms and df = 1, p = 0.002 for temperatures; Table 3.16. and Fig. 3.23).

Chlorophyll content of the water

In individual and population level experiments, temperatures, organisms, and the interaction significantly influenced chlorophyll content (individual: df = 1, p = 0.004 for temperatures, df = 4, p < 0.001 for organisms, df = 4, p < 0.001 for the interaction, Table 3.17. and Fig. 3.24; population: df = 1, p < 0.001 for temperatures, df = 4, p < 0.001 for organisms, df = 4, p < 0.001 for the interaction, Table 3.18. and Fig. 3.25). In community level experiment, temperatures significantly influenced chlorophyll content, although the effects of organisms and the interaction were not significant (df = 1, p < 0.001; Table 3.19. and Fig. 3.26).

Nitrate of the water

In individual level experiment, temperatures, organisms, and the interaction significantly influenced nitrate (df = 1, p = 0.006 for temperatures, df = 4, p < 0.001 for organisms and the interaction; Table 3.20. and Fig. 3.27). In population level experiment, organisms significantly influenced nitrate, although the effects of temperatures and interaction between organisms and temperatures were not significant (df = 4, p < 0.001; Table 3.21. and Fig. 3.28). In community level experiment, organisms and temperatures significantly influenced nitrate (df = 2, p = 0.006 for organisms and df = 1, p < 0.001 for temperatures; Table 3.22. and Fig. 3.29).

Potassium of the water

In individual level experiment, organisms significantly influenced the potassium, although the effects of temperatures and the interaction were not significant (df = 4, p = 0.048; Table 3.23. and Fig. 3.30). In population level experiment, temperatures, organisms, and the interaction did not influence the potassium. In community level experiment, temperatures significantly influence the potassium, although organisms and the interaction were not significant (df = 1, p < 0.001; Table 3.25. and Fig. 3.32).

Dissolved oxygen in the water

In individual level experiment, organisms and temperatures significantly influenced dissolved oxygen (df = 4, p = 0.009 for organisms and df = 1, p < 0.001 for temperatures; Table 3.26. and Fig. 3.33). The interaction between temperatures and organisms also affected significantly dissolved oxygen (df = 4, p = 0.016; Table 3.26. and Fig. 3.33). In population level experiment, organisms, temperatures, and the

interaction significantly influenced dissolved oxygen (df = 4, p = 0.015 for organisms and df = 1, p < 0.001 for temperatures, df = 4, p = 0.036 for the interaction; Table 3.27. and Fig. 3.34). In community level experiment, temperatures significantly influenced dissolved oxygen (df = 1, p = 0.023; Table 3.28. and Fig. 3.35).

pH of the water

In individual level experiment, organisms and the interaction significantly influenced pH (df = 4, p = 0.003 for organisms and df = 4, p < 0.001 for interaction between them; Table 3.29. and Fig. 3.36). In population level experiment, organisms and the interaction between temperatures and organisms significantly influenced pH (df = 4, p = 0.008 for organisms and df = 4, p < 0.001 for the interaction between temperatures and organisms; Table 3.30. and Fig. 3.37). In community level experiment, no significant differences were found.

Water losses in community level experiment

High temperature significantly increased the water losses (df = 1, p < 0.001; Table 3.32. and Fig. 3.39).

Discussion

In general, temperatures did not affect biotic factors such as the survival, body weight, and reproduction of the three aquatic organisms provided, while it influenced on biotic factors at individual, population, and community level experiment (Table. 3.33.). On the other hand, the effects of snails differed from the temperature effects; the snails gave much influence on the biotic factors than abiotic ones. Increased effects of temperature on organic matter from individual and population to community level experiments indicated that the number of species and individuals notably affected the amount of organic matters as suggested by Bronmark and Hansson (2005) i.e. activities of aquatic organisms affected and shaped the abiotic frame. Otherwise, decreased effects of temperature on chlorophyll content, in particular community level experiments, showed that increased temperature would make the organism active since the higher consumption of floating algae the lower amount of chlorophyll content remained. Significantly increased effects of temperature on potassium in community level probably were caused by higher amount of excreta produced by relatively higher activity due to high temperature. The similarity of the effects of temperatures on three levels of experiments was found in dissolved oxygen. The adverse effects of temperatures on dissolved oxygen were caused by the increased metabolic rate of the snails and thus the oxygen demand (Bronmark and Hansson 2005). The absence of effects of temperatures on pH in three levels of experiment was probably due to the weakness of photosynthesis and respiration whose results strongly affected the pH (Reynolds 1984). The positive effects of temperatures on the number of juveniles of mud snail in three levels of experiments were revealed as suggested by Brackenbury and Appleton (1991). In addition, favorable temperatures for oviposition of a freshwater snail were 25-35°C at which mud snail allocated more energy to

offspring production (Parashar and Rao 1985; Haak 2015). The higher reproduction of mud snails would be affected by the favorable temperature regarding the reproduction.

The positive effects of mud snails in individual, population, and community experiments were found in body weight and egg clutches of sakamakigai and organic matters. Positive effects of mud snails on the abundance of sakamakigai were shown as the previous finding by Dewi et al. (2017), however, the mechanism of higher population due to the mud snails was unknown. This study revealed that the high abundance of sakamakigai with mud snails would be caused by higher reproduction of sakamakigai itself. Positive effects of mud snails on organic matters were possibly due to the increased availability of snails' excreta based nutrients (Underwood et al. 1992; Dodds 2002) reinforcing nutrient cycling followed by enhanced organic matters. The absence of the effects of mud snails on the body weight of kawanina was probably owing to the predominance kawanina, which made considerable portion of biomass in freshwater ecosystem, as the most common and quite widespread freshwater snail in Japan (Richardson et al. 1988; Oniwa and Kimura 1986; Miura et al. 2013) leading to be an adequate competitor for mud snails. The lack of the effects of mud snails on the juveniles of kawanina was supporting the predominance of kawanina as suggested by Brackenbury and Appleton (1993).

The difference in the effects of mud snails in individual, population, and community experiments was found in the survival of kawanina, body weight of sakamakigai, and nitrate. It was suggested that the effects of mud snails would be affected by increased number of species and individuals. The change in the effects of mud snails on the survival of kawanina from positive in individual and population to negative in community level showed that the higher number of individuals in community level increased intra specific competition of kawanina resulting in the

decreased survival. The positive effects of mud snails on the body weight of sakamakigai only in population and community levels might relate that mud snails might release chemical substance in excreta which would in turn increase food for sakamakigai. The change in the effects of mud snails on nitrate from negative in individual and population to positive in community level indicated that many individuals and interactions itself in the community level experiments would promote the production of nitrate. In addition, mud snails were possibly responsible for the increased nutrients including nitrate as suggested by Underwood (1992) i.e. nutrients originated from snail such as phosphates and ammonium enhanced growth of macrophyte in pond ecosystem.

This study clearly showed that in the community level mud snails significantly affected the performance of sakamakigai by increasing the survival, bodyweight, and number of egg clutches. The enhanced organic matters and concentration of nitrate in the water were also affected by mud snails and possibly supporting the performances of sakamakigai. In addition, decreased survival of kawanina, as affected by mud snails, in turn probably increased the performances of sakamakigai by reducing the competition.

In general, the sum of positive effects of both temperatures and mud snails on community level seemed to be higher than on either individual or population levels. This was probably because the number of interactions among organisms was getting higher along the gradient of the experiments level. A community composed by individuals and populations tends to have more characteristics comprising not only the sum of its constituent species but also the interactions between them (Begon et al. 1996). Higher number of positive effects of mud snails in community level suggested

that they possibly have potential to be a key species in the aquatic ecosystem as suggested by Carlsson et al. (2014).

The result suggested one of possible mechanisms how mud snails positively influenced the community structure of aquatic organisms as reported by Dewi et al. (2017) i.e. their ability to provide suitable environment via supplying nutrients worthwhile to the performance of other aquatic organisms e.g. sakamakigai.

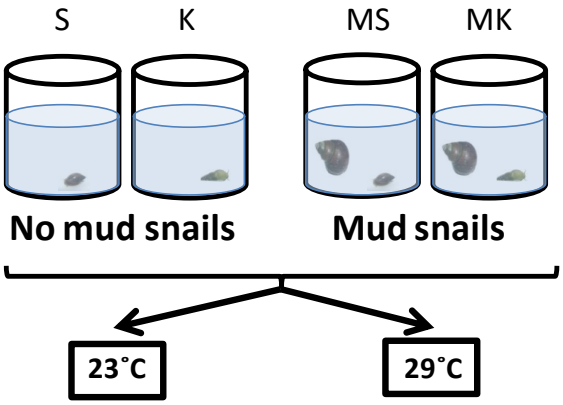

Fig. 3.1 Experimental design of individual level experiment.
S: Sakamakigai, K: Kawanina, M: Mud snail

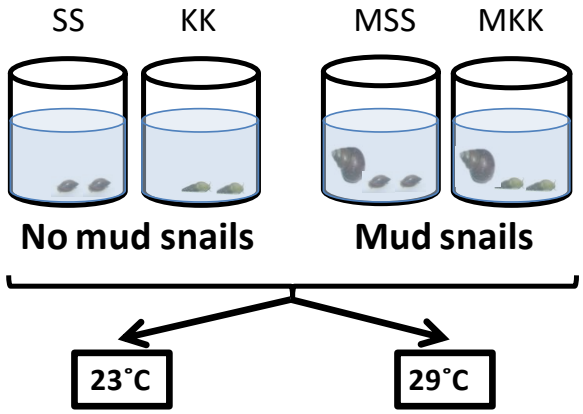

Fig. 3.2 Experimental design of population level experiment.
S: Sakamakigai, K: Kawanina, M: Mud snail

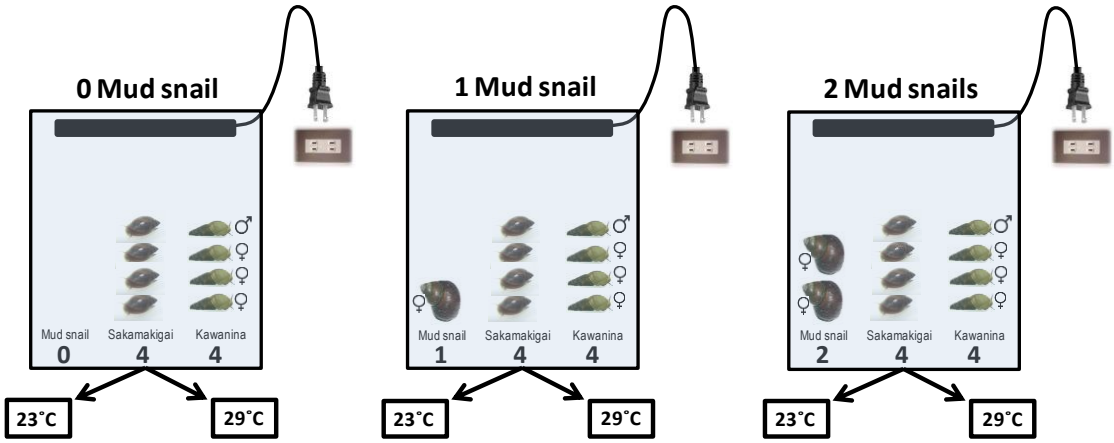

Fig. 3.3 Experimental design of community level experiment.

Fig. 3.4 The heater immersed inside the plastic container used in community level experiment.

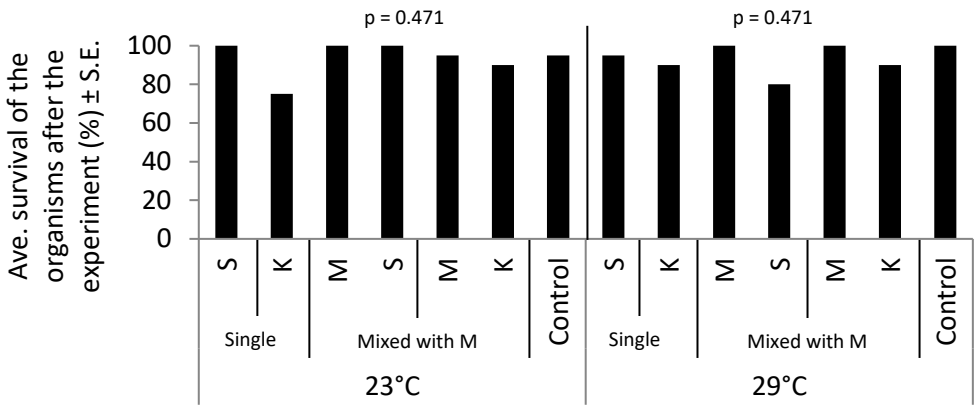

Fig. 3.5 Survival of aquatic organisms in individual level experiment. Abbreviation: S (Sakamakigai), K (Kawanina), M (Mud Snail). Control consists of only one mud snail. Pearson`s chi-square test was used.

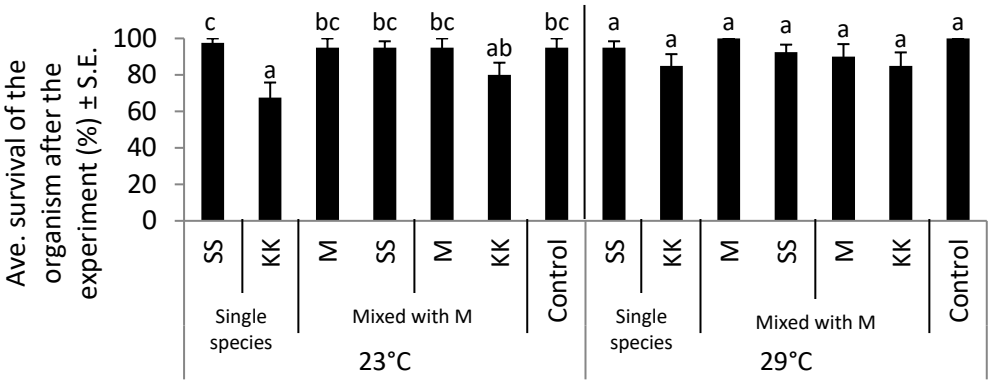

Fig. 3.6 Survival of aquatic organisms in population level experiment. Abbreviation: S (Sakamakigai), K (Kawanina), M (Mud Snail). Control consists of only one mud snail. One-way analysis of variance (ANOVA) was used.

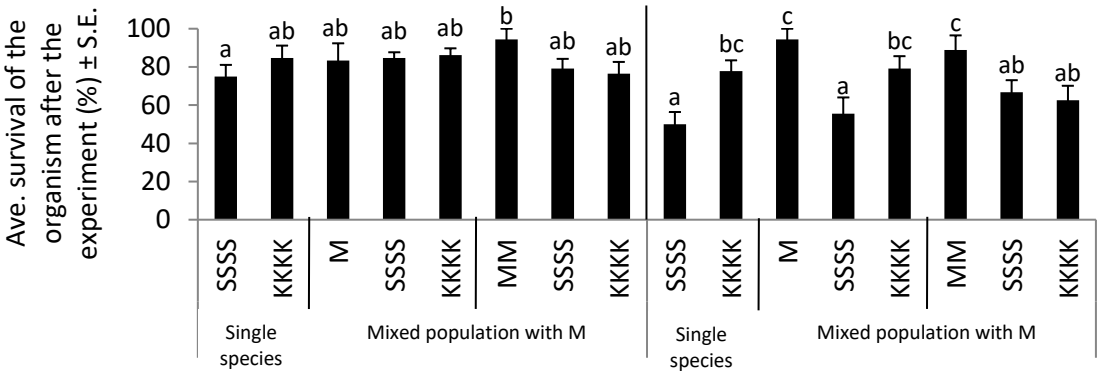

Fig. 3.7 Survival of aquatic organisms in community level experiment. Abbreviation: S (Sakamakigai), K (Kawanina), M (Mud Snail). One-way ANOVA was used.

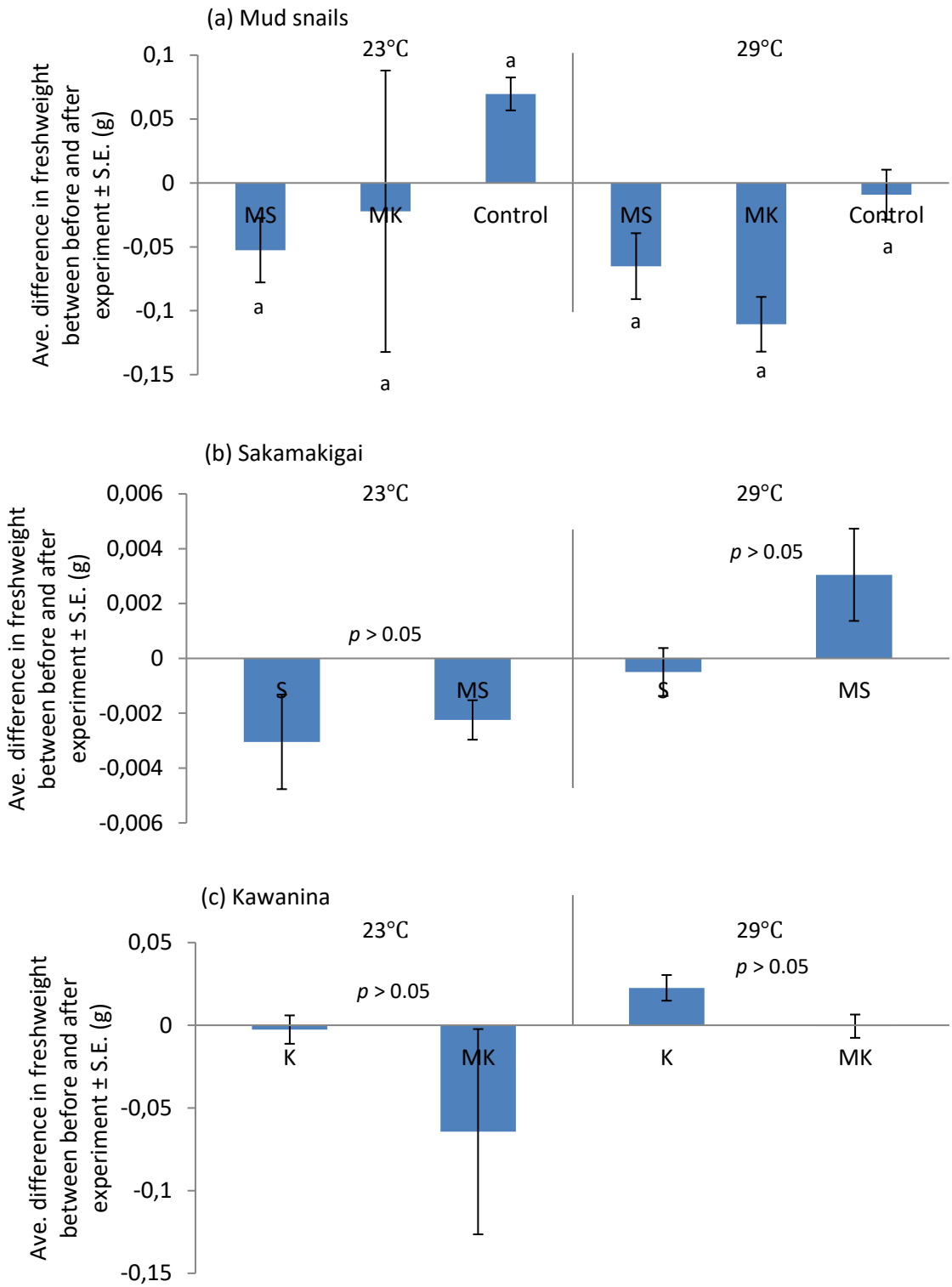

Fig. 3.8 The change in body weight of aquatic organisms in individual level experiment. One-way ANOVA and student`s *t*-test were used.

Table 3.1. Two-way analysis of variance (ANOVA) for measure of the change in body weight of aquatic organisms in individual level experiment

Factor	Source	df	SS	*F*	*P*
Mud snails	Temperatures	1	.002	.906	.343
	Organisms	2	.010	2.638	.076
	Temperatures x Organisms	2	.001	.177	.838
Sakamakigai	Temperatures	1	5.09E-005	8.918	.004
	Organisms	1	1.56E-005	2.738	.102
	Temperatures x Organisms	1	6.03E-006	1.055	.308
Kawanina	Temperatures	1	.003	1.782	.186
	Organisms	1	.002	1.655	.203
	Temperatures x Organisms	1	.001	.586	.447

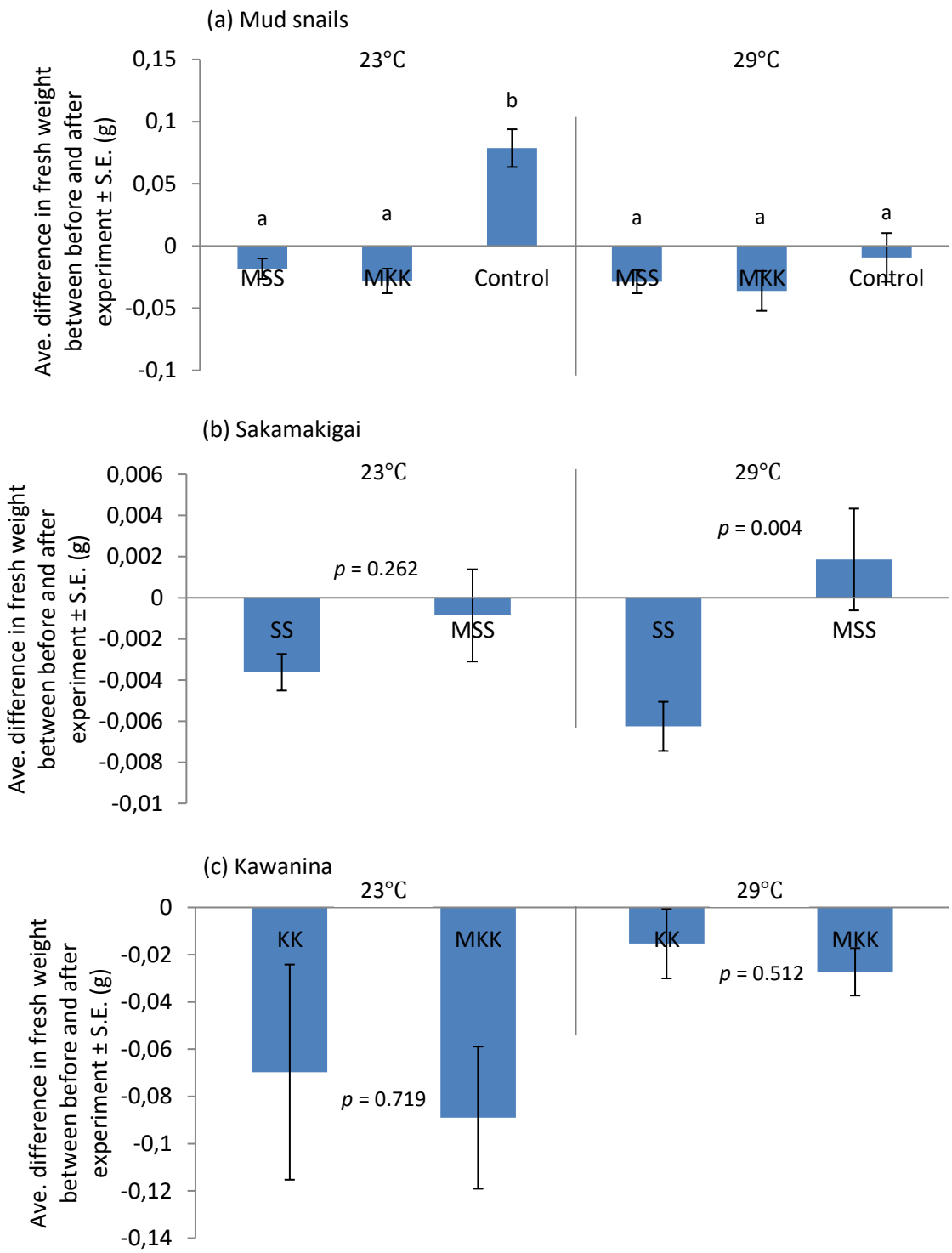

Fig. 3.9 The change in body weight of aquatic organisms in population level experiment. One-way ANOVA and student`s *t*-test were used.

Table 3.2. Two-way ANOVA for measure of the change in body weight of aquatic organisms in population level experiment

Factor	Source	df	SS	*F*	*P*
Mud snails	Temperatures	1	.005	10.114	.002
	Organisms	2	.012	13.034	.000
	Temperatures x Organisms	2	.005	5.226	.007
Sakamakigai	Temperatures	1	4.53E-008	.004	.950
	Organisms	1	.000	9.592	.003
	Temperatures x Organisms	1	2.85E-005	2.474	.120
Kawanina	Temperatures	1	.007	4.952	.029
	Organisms	1	.000	.177	.676
	Temperatures x Organisms	1	2.90E-006	.002	.963

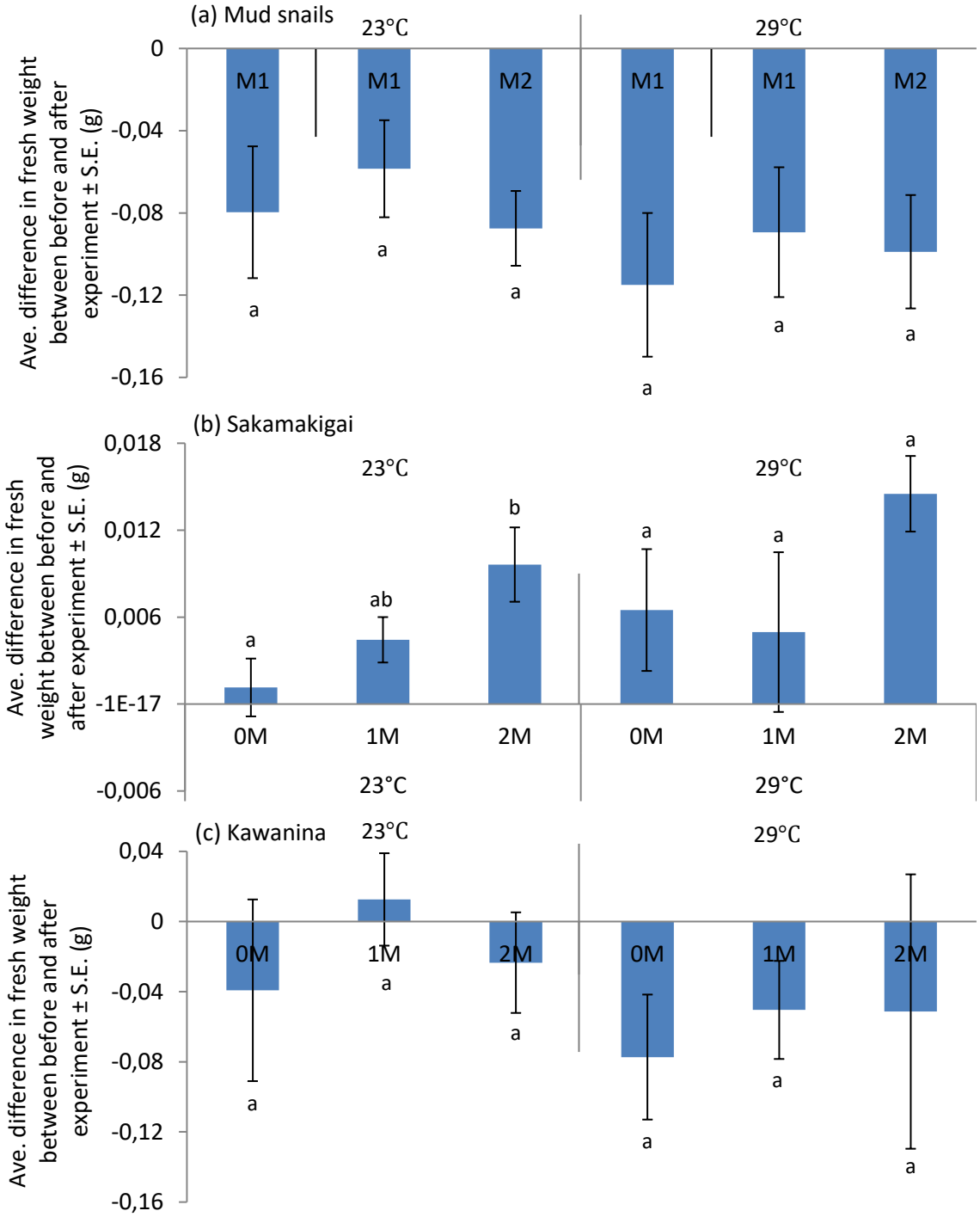

Fig. 3.10 The change in body weight of aquatic organisms in community level experiment. One-way ANOVA was used.

Table 3.3. Two-way ANOVA for measure of the change of bodyweight of aquatic organisms in community level experiment

Factor	Source	df	SS	*F*	*P*
Mud snails	Temperatures	1	.002	1.460	.230
	Organisms	2	.001	.419	.659
	Temperatures x Organisms	2	.000	.100	.905
Sakamakigai	Temperatures	1	5.31E-005	1.785	.185
	Organisms	2	.000	4.108	.019
	Temperatures x Organisms	2	2.13E-005	.358	.700
Kawanina	Temperatures	1	.003	1.568	.214
	Organisms	2	.002	.465	.630
	Temperatures x Organisms	2	.000	.051	.950

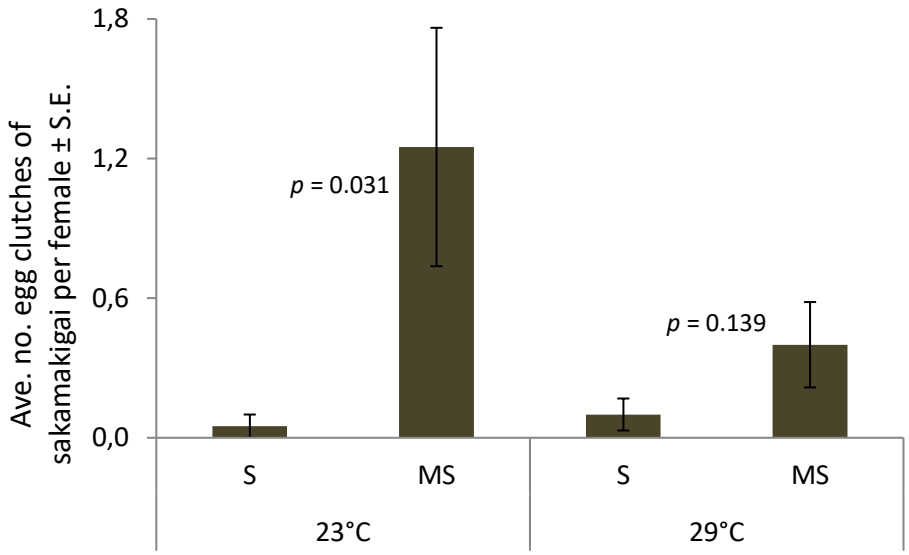

Fig. 3.11 The number of egg clutches of sakamakigai after termination of individual level experiment. Student's *t*-test was used.

Table 3.4. Two-way ANOVA for measure of sakamakigai egg in individual level experiment

Factor	Source	df	SS	*F*	*P*
Sakamakigai egg	Temperatures	1	3.200	2.109	.151
	Organisms	1	11.250	7.415	.008
	Temperatures x Organisms	1	4.050	2.670	.106

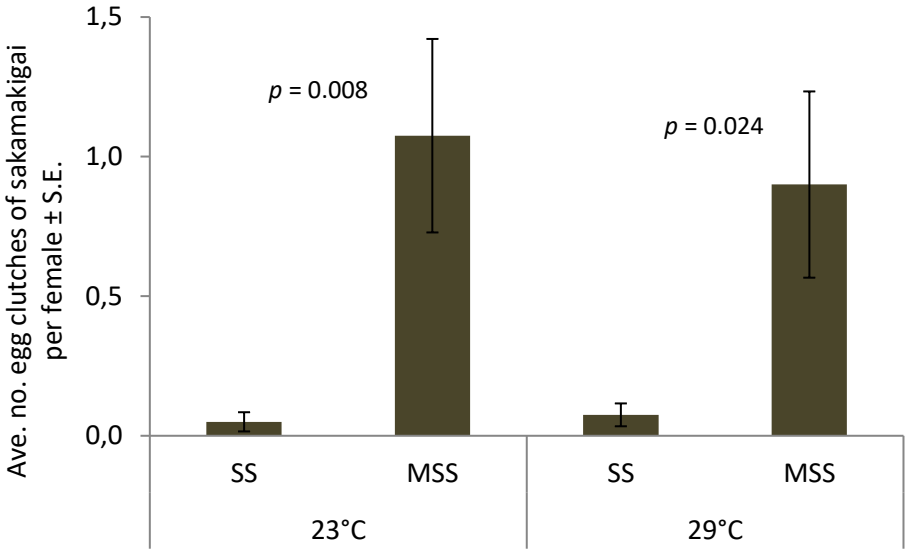

Fig. 3.12 The number of egg clutches of sakamakigai after termination of population level experiment. Student's *t*-test was used.

Table 3.5. Two-way ANOVA for measure of sakamakigai egg in population level experiment

Factor	Source	df	SS	*F*	*P*
Sakamakigai egg	Temperatures	1	.113	.096	.757
	Organisms	1	17.113	14.609	.000
	Temperatures x Organisms	1	.200	.171	.681

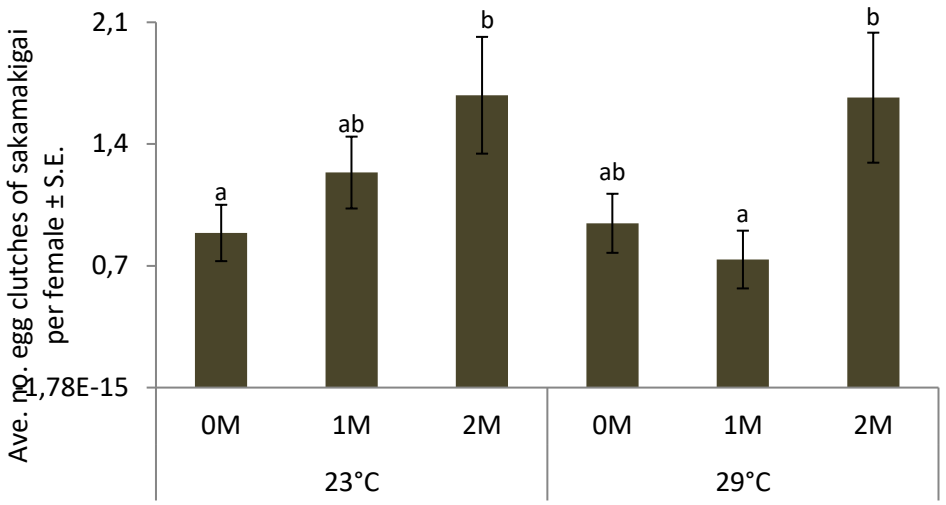

Fig. 3.13 The number of egg clutches of sakamakigai after termination of community level experiment. One-way ANOVA was used.

Table 3.6. Two-way ANOVA for measure of sakamakigai egg in community level experiment

Factor	Source	df	SS	*F*	*P*
Sakamakigai egg	Temperatures	1	10.083	.556	.457
	Organisms	2	201.685	5.563	.005
	Temperatures x Organisms	2	26.389	.728	.485

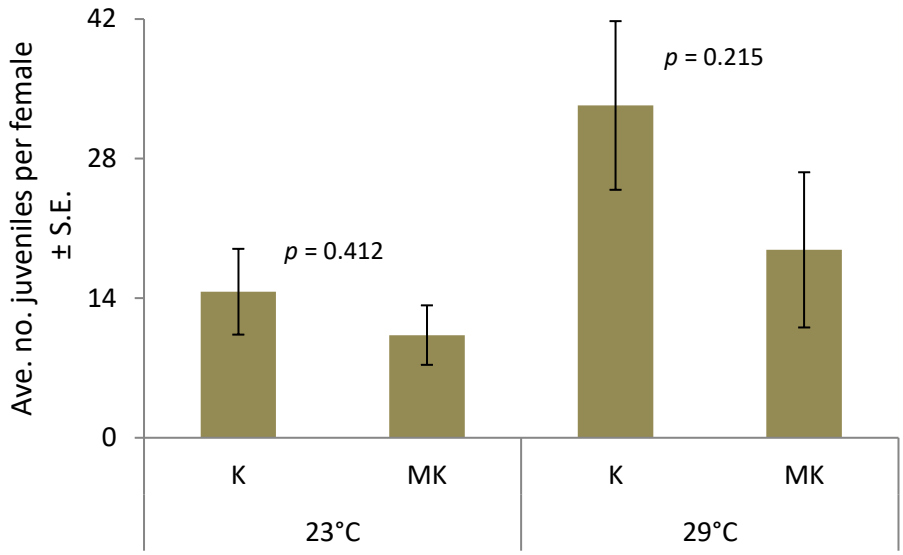

Fig. 3.14 Reproduction of kawanina observed after termination of individual level experiment. Student's *t*-test was used.

Table 3.7. Two-way ANOVA for measure of juveniles of kawanina in individual level experiment

Factor	Source	df	SS	*F*	*P*
Kawanina juvenile	Temperatures	1	3608.049	4.824	.031
	Organisms	1	1725.541	2.307	.133
	Temperatures x Organisms	1	499.546	.668	.416

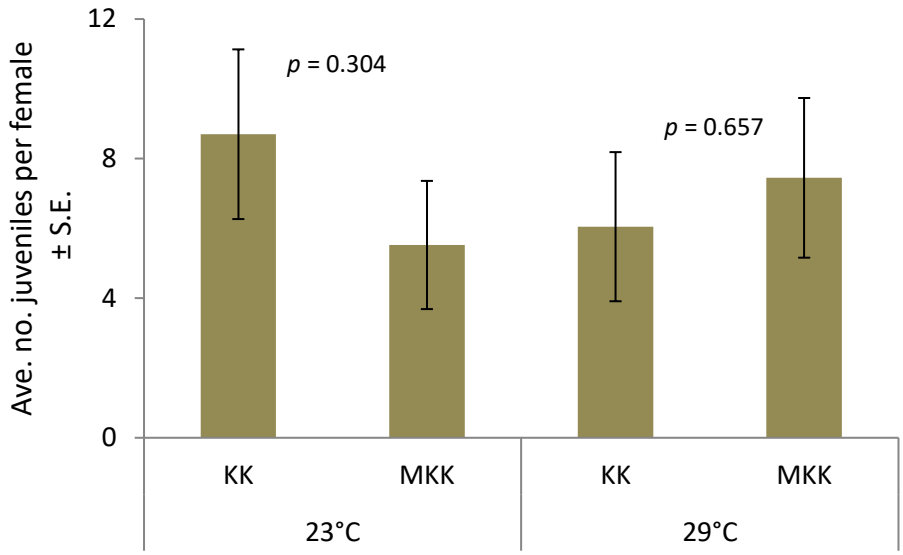

Fig. 3.15 The number of juveniles of kawanina after termination of population level experiment.

Table 3.8. Two-way ANOVA for measure of juveniles of kawanina in population level experiment

Factor	Source	df	SS	*F*	*P*
Kawanina juvenile	Temperatures	1	10.513	.028	.869
	Organisms	1	63.012	.165	.686
	Temperatures x Organisms	1	418.613	1.096	.298

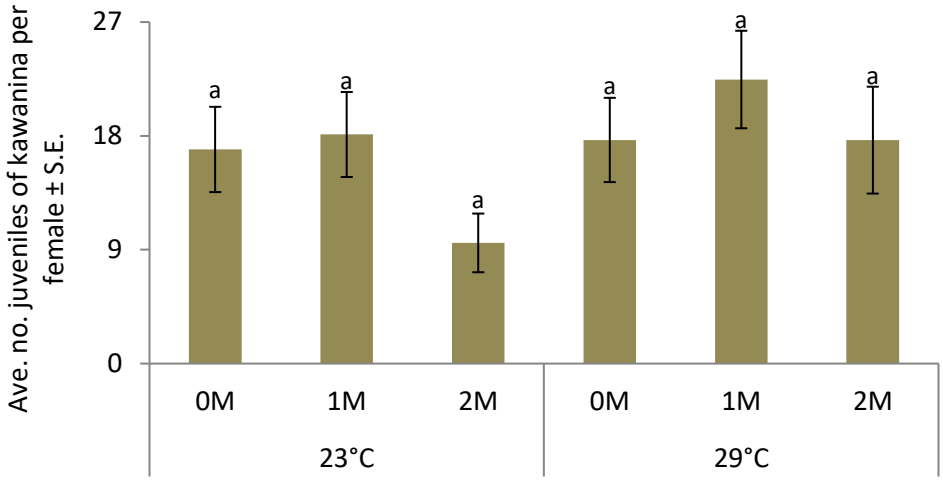

Fig. 3.16 The number of juveniles of kawanina after termination of community level experiment.

Table 3.9. Two-way ANOVA for measure of juveniles of kawanina in community level experiment

Factor	Source	df	SS	*F*	*P*
Kawanina juvenile	Temperatures	1	524.481	2.434	.122
	Organisms	2	806.466	1.871	.159
	Temperatures x Organisms	2	244.807	.568	.568

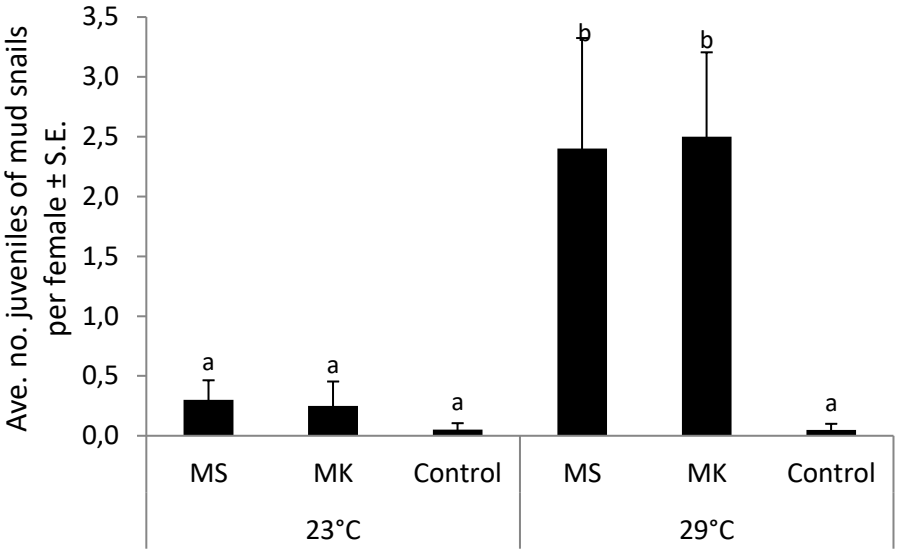

Fig. 3.17 The number of juveniles of mud snail after termination of individual level experiment. One-way ANOVA was used.

Table 3.10. Two-way ANOVA for measure of juveniles of mud snail in individual level experiment

Factor	Source	df	SS	*F*	*P*
Mud snail juveniles	Temperatures	1	62.451	13.029	.000
	Organisms	2	45.068	4.701	.011
	Temperatures x Organisms	2	31.181	3.253	.042

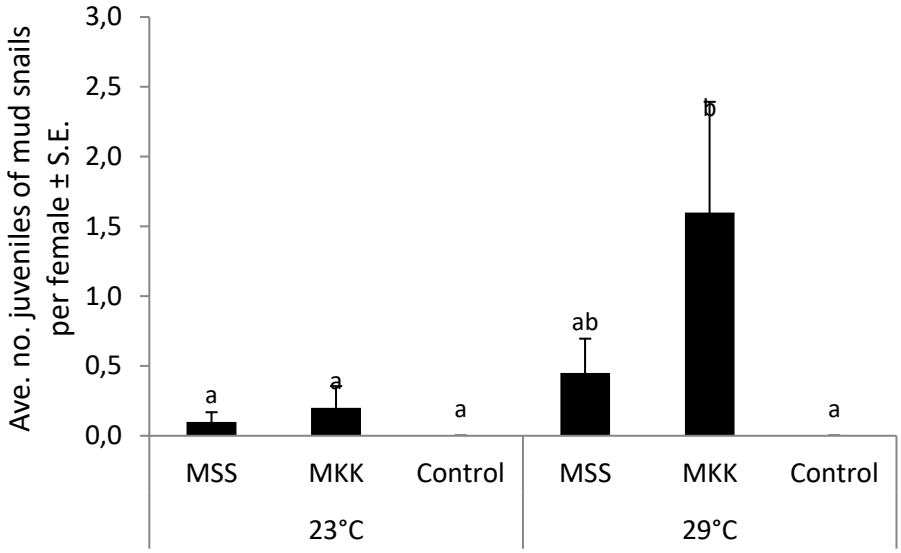

Fig. 3.18 The number of juveniles of mud snail after termination of population level experiment. One-way ANOVA was used.

Table 3.11. Two-way ANOVA for measure of juveniles of mud snail in population level experiment

Factor	Source	df	SS	*F*	*P*
Mud snail juveniles	Temperatures	1	10.208	4.267	.041
	Organisms	2	17.017	3.556	.032
	Temperatures x Organisms	2	10.617	2.219	.113

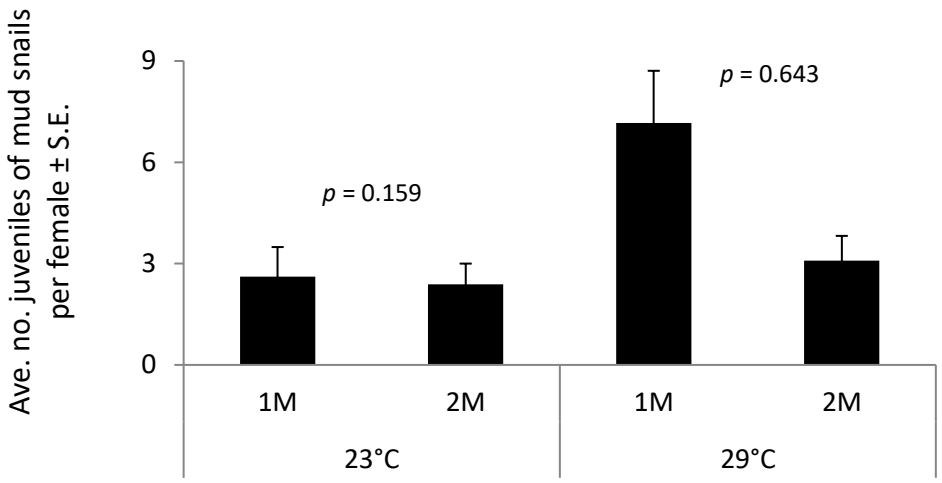

Fig. 3.19 The effects of density of the mud snails on the number of the juveniles. Student`s *t*-test was used.

Table 3.12. Two-way ANOVA for measure of the effects of density of the mud snails on the number of the juveniles

Factor	Source	df	SS	*F*	*P*
Mud snail juveniles	Temperatures	1	159.014	5.178	.026
	Mud snails (density)	1	6.125	.199	.657
	Temperatures x Mud snails	1	45.125	1.469	.230

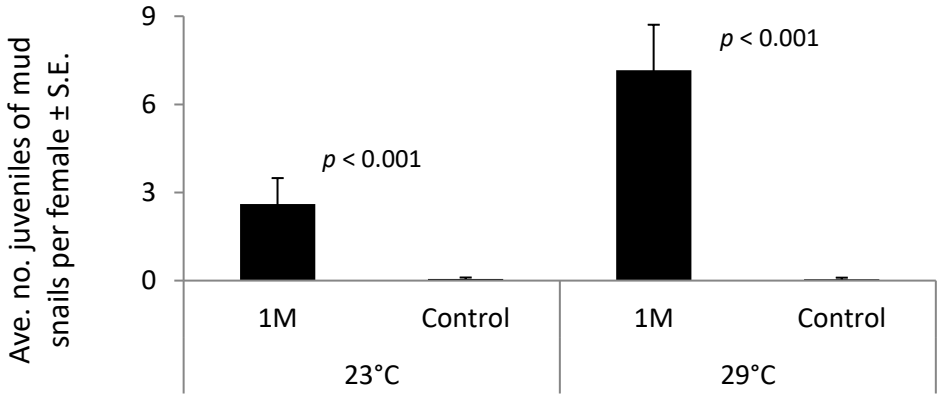

Fig. 3.20 The effects of other organisms on the number of juveniles of the mud snails. Student`s *t*-test was used.

Table 3.13. Two-way ANOVA for measure of effects of other organisms on the number of the juveniles

Factor	Source	df	SS	*F*	*P*
Mud snail juveniles	Temperatures	1	96.982	7.123	.009
	Complexity	1	437.949	32.166	.000
	Temperatures x Complexity	1	97.206	7.140	.009

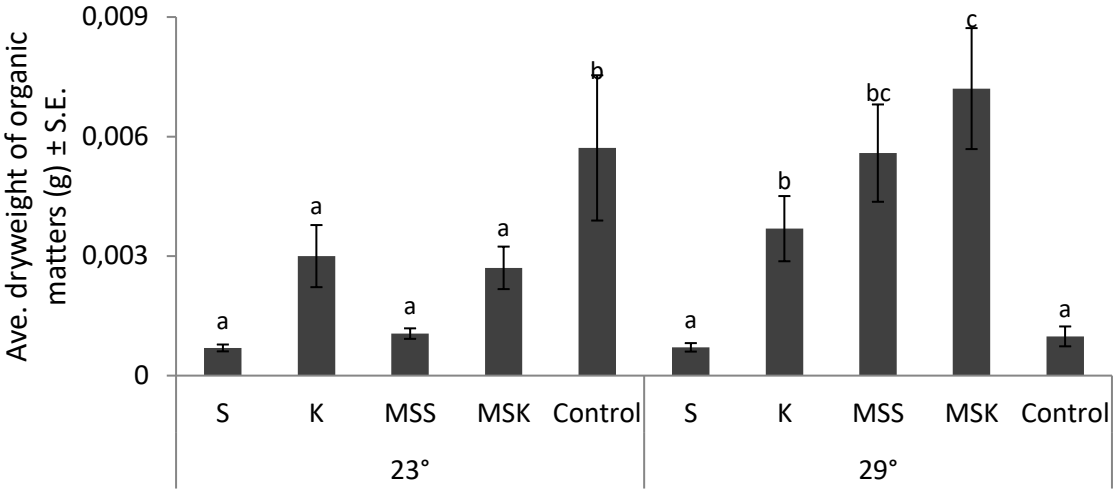

Fig. 3.21 Organic matters accumulated after termination of individual level experiment. One-way ANOVA was used.

Table 3.14. Two-way ANOVA for measure of organic matters in individual level experiment

Factor	Source	df	SS	*F*	*P*
Organic matters	Temperatures	1	4.81E-005	2.860	.093
	Organisms	4	.000	5.560	.000
	Temperatures x Organisms	4	.001	8.590	.000

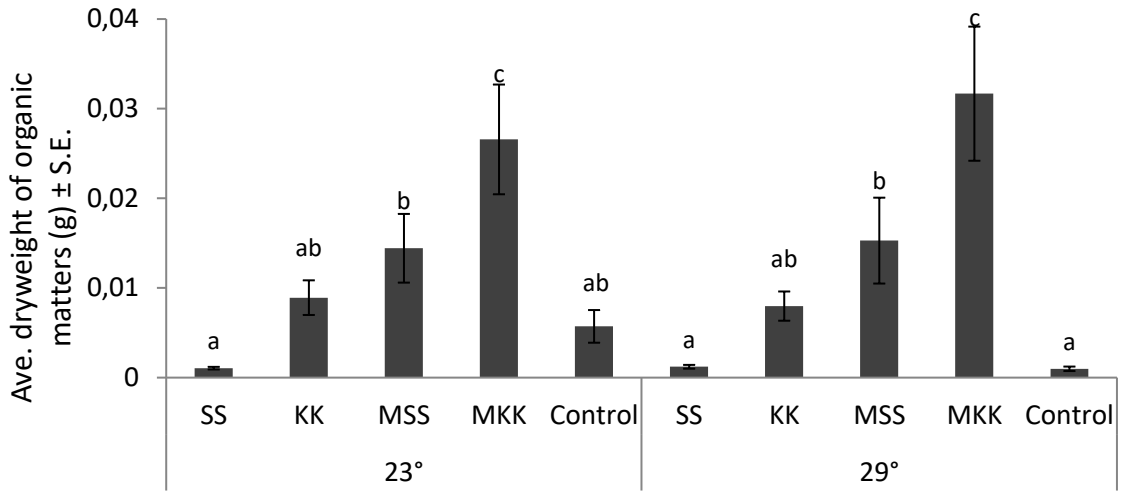

Fig. 3.22 Organic matters accumulated after termination of population level experiment. One-way ANOVA was used.

Table 3.15. Two-way ANOVA for measure of organic matters in population level experiment

Factor	Source	df	SS	*F*	*P*
Organic matters	Temperatures	1	3.67E-007	.001	.971
	Organisms	4	.020	17.908	.000
	Temperatures x Organisms	4	.000	.446	.775

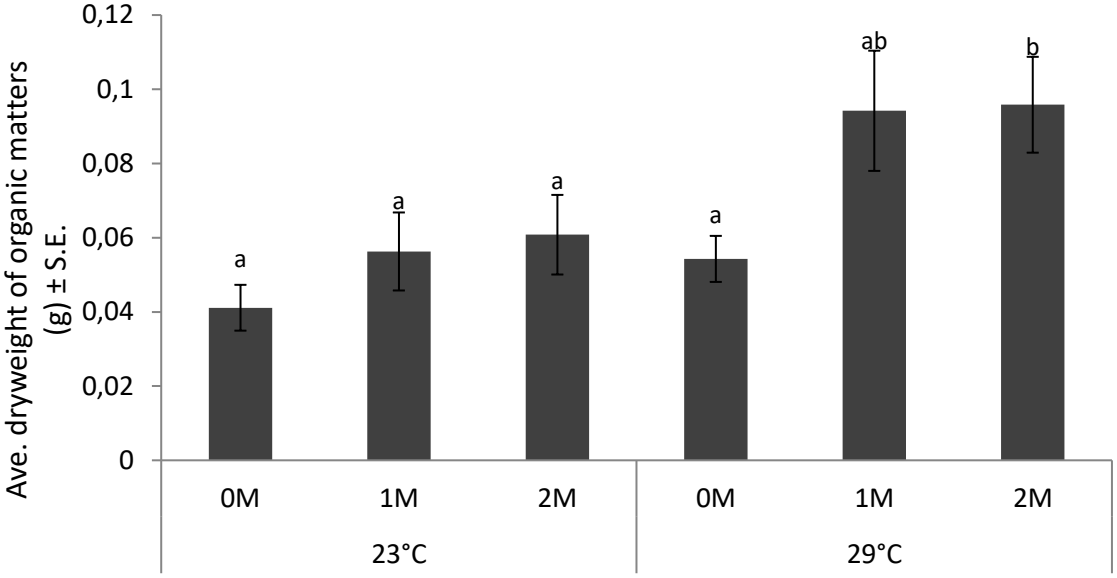

Fig. 3.23 Organic matters accumulated after termination of community level experiment. One-way ANOVA was used.

Table 3.16. Two-way ANOVA for measure of organic matters in community level experiment

Factor	Source	df	SS	*F*	*P*
Organic matters	Temperatures	1	.022	10.136	.002
	Organisms	2	.020	4.663	.012
	Temperatures x Organisms	2	.003	.751	.474

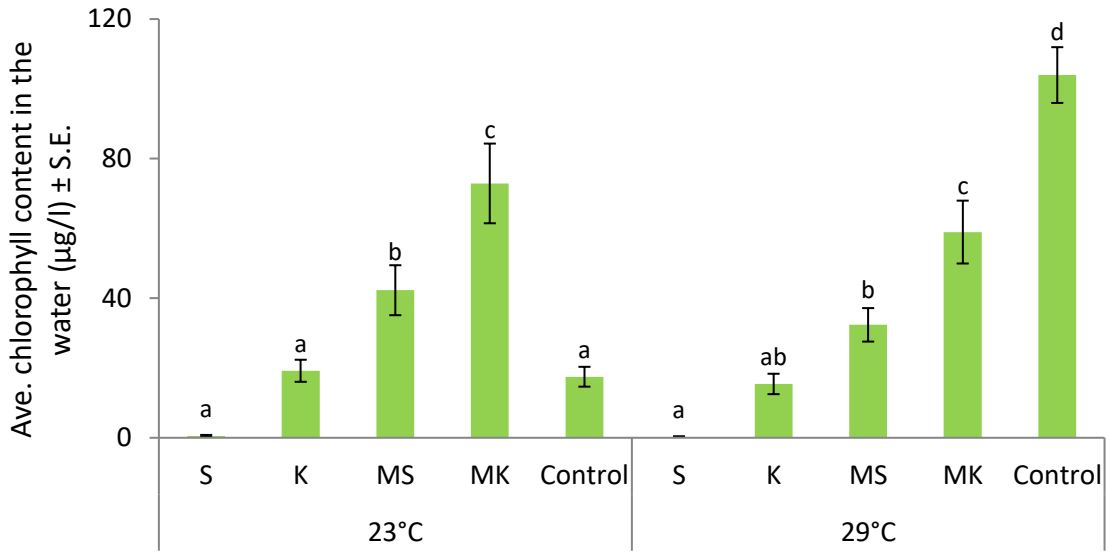

Fig. 3.24 Chlorophyll content in the water after termination of individual level experiment. One-way ANOVA was used.

Table 3.17. Two-way ANOVA for measure of chlorophyll content in individual level experiment

Factor	Source	df	SS	*F*	*P*
Chlorophyll content	Temperatures	1	6672.227	8.732	.004
	Organisms	4	122950.664	40.228	.000
	Temperatures x Organisms	4	69297.021	22.673	.000

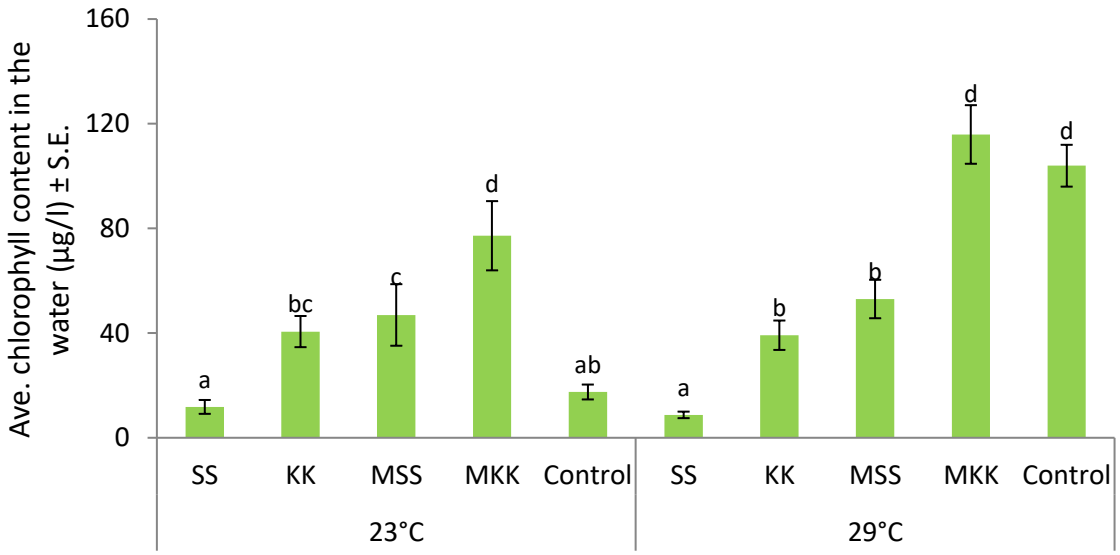

Fig. 3.25 Chlorophyll content in the water after termination of population level experiment. One-way ANOVA was used.

Table 3.18. Two-way ANOVA for measure of chlorophyll content in population level experiment

Factor	Source	df	SS	*F*	*P*
Chlorophyll content	Temperatures	1	31817.026	24.755	.000
	Organisms	4	157845.526	30.702	.000
	Temperatures x Organisms	4	56912.030	11.070	.000

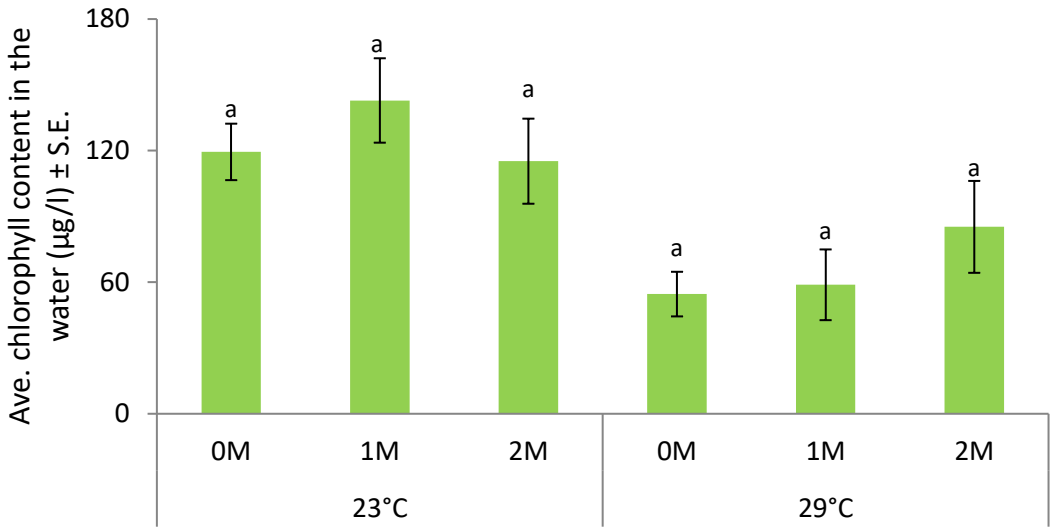

Fig. 3.26 Chlorophyll content in the water after termination of community level experiment. One-way ANOVA was used.

Table 3.19. Two-way ANOVA for measure of chlorophyll content in community level experiment

Factor	Source	df	SS	*F*	*P*
Chlorophyll content	Temperatures	1	95973.891	18.658	.000
	Organisms	2	4398.528	.428	.653
	Temperatures x Organisms	2	13541.778	1.316	.273

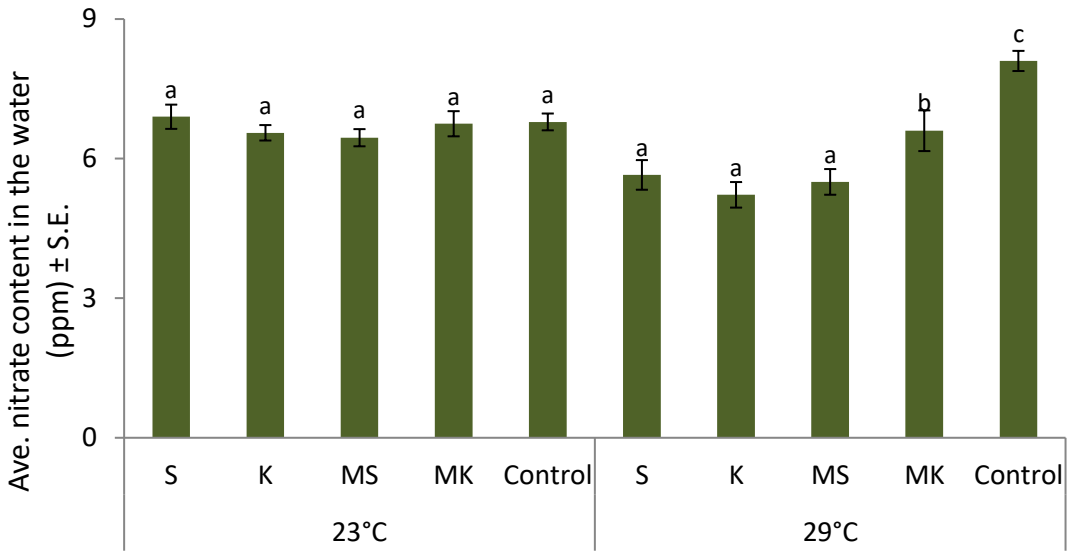

Fig. 3.27 The amount of nitrate in the water after termination of individual level experiment. One-way ANOVA was used.

Table 3.20. Two-way ANOVA for measure of nitrate in individual level experiment

Factor	Source	df	SS	*F*	*P*
Nitrate	Temperatures	1	10.959	7.583	.006
	Organisms	4	62.162	10.753	.000
	Temperatures x Organisms	4	47.009	8.132	.000

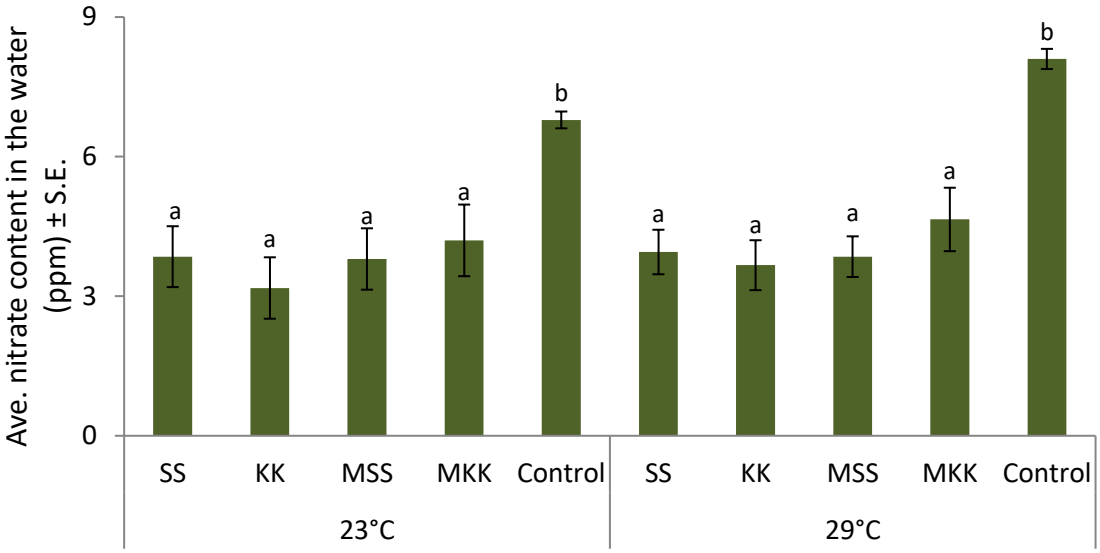

Fig. 3.28 The amount of nitrate in the water after termination of population level experiment. One-way ANOVA was used.

Table 3.21. Two-way ANOVA for measure of nitrate in population level experiment

Factor	Source	df	SS	*F*	*P*
Nitrate	Temperatures	1	10.327	1.682	.196
	Organisms	4	390.367	15.892	.000
	Temperatures x Organisms	4	10.097	.411	.801

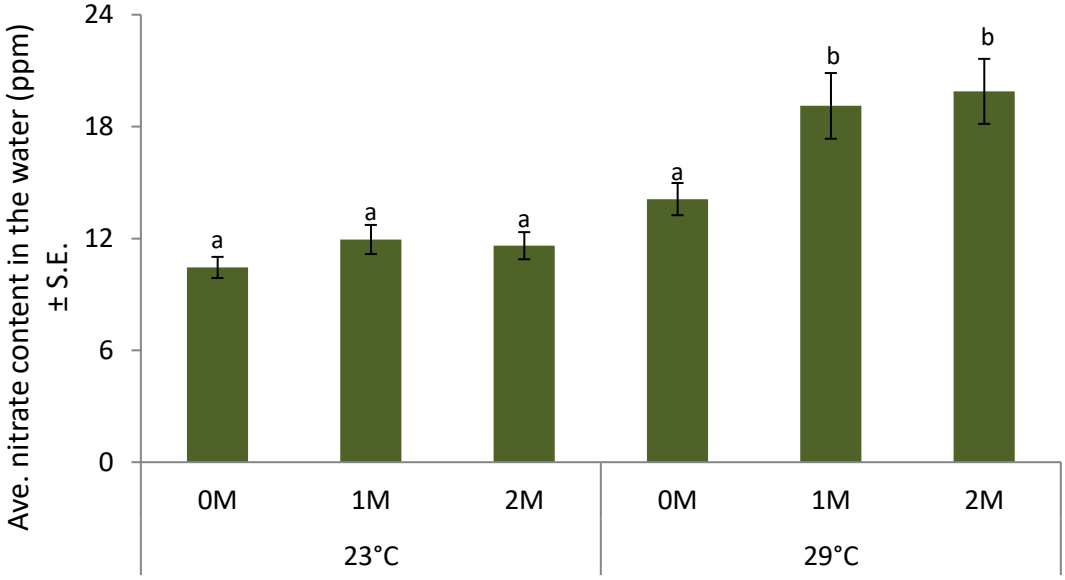

Fig. 3.29 The amount of nitrate in the water at the beginning, middle and end of community level experiment. One-way ANOVA was used.

Table 3.22. Two-way ANOVA for measure of nitrate in community level experiment

Factor	Source	df	SS	*F*	*P*
Nitrate	Temperatures	1	1095.704	43.742	.000
	Organisms	2	272.019	5.430	.006
	Temperatures x Organisms	2	104.241	2.081	.130

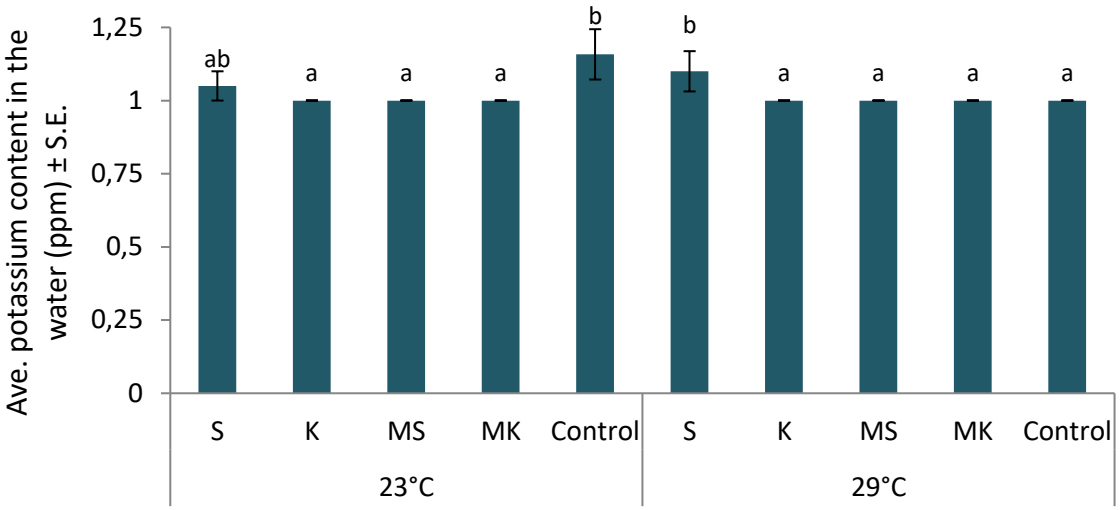

Fig. 3.30 The amount of potassium in the water after termination of individual level experiment. One-way ANOVA was used.

Table 3.23. Two-way ANOVA for measure of potassium in individual level experiment

Factor	Source	df	SS	*F*	*P*
Potassium	Temperatures	1	.023	.795	.374
	Organisms	4	.279	2.441	.048
	Temperatures x Organisms	4	.246	2.155	.076

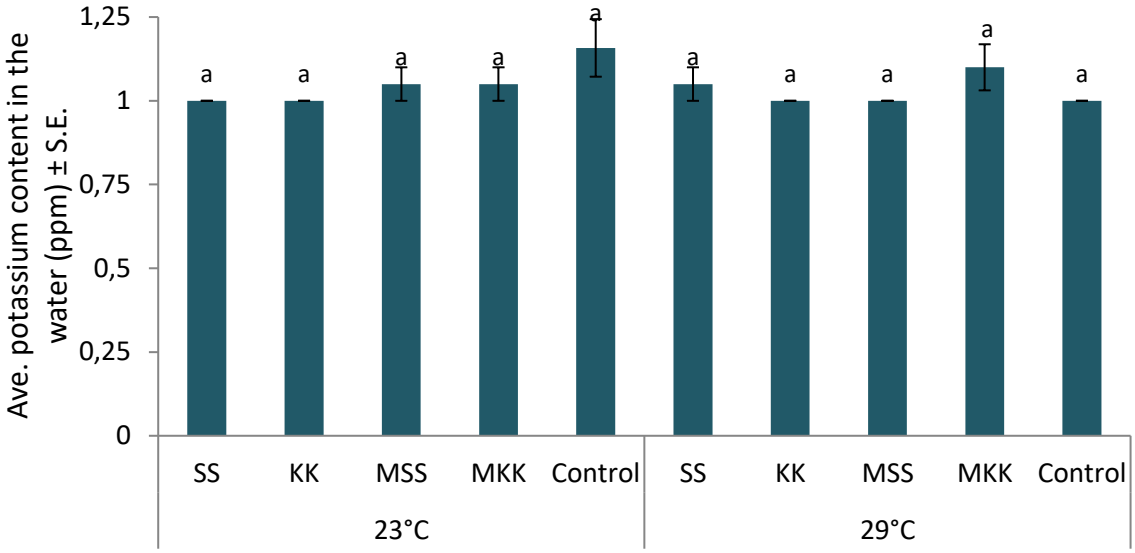

Fig. 3.31 The amount of potassium in the water after termination of population level experiment.

Table 3.24. Two-way ANOVA for measure of potassium in population level experiment

Factor	Source	df	SS	*F*	*P*
Potassium	Temperatures	1	.023	.604	.438
	Organisms	4	.188	1.234	.298
	Temperatures x Organisms	4	.296	1.939	.106

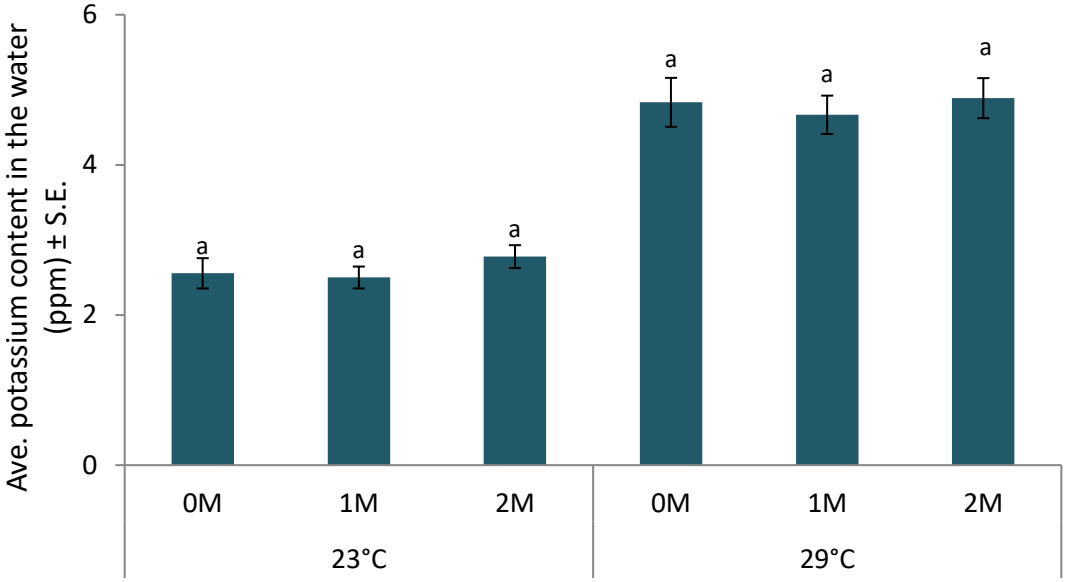

Fig. 3.32 The amount of potassium in the water at the beginning, middle and end of community level experiment. One-way ANOVA was used.

Table 3.25. Two-way ANOVA for measure of potassium in community level experiment

Factor	Source	df	SS	*F*	*P*
Potassium	Temperatures	1	128.926	131.068	.000
	Organisms	2	1.130	.574	.565
	Temperatures x Organisms	2	.130	.066	.936

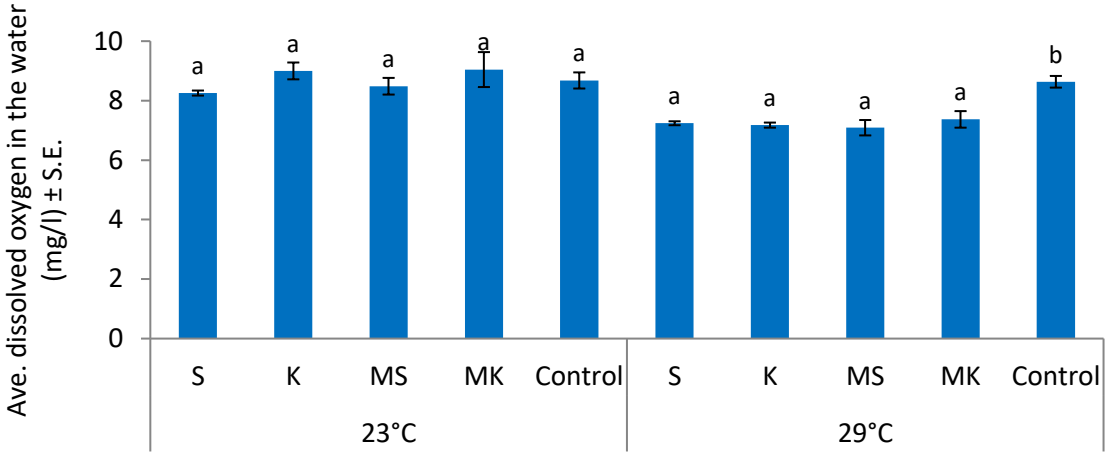

Fig. 3.33 The amount of dissolved oxygen in the water after termination of individual level experiment. One-way ANOVA was used.

Table 3.26. Two-way ANOVA for measure of dissolved oxygen in individual level experiment

Factor	Source	df	SS	*F*	*P*
Dissolved oxygen	Temperatures	1	68.938	44.475	.000
	Organisms	4	21.429	3.456	.009
	Temperatures x Organisms	4	19.471	3.140	.016

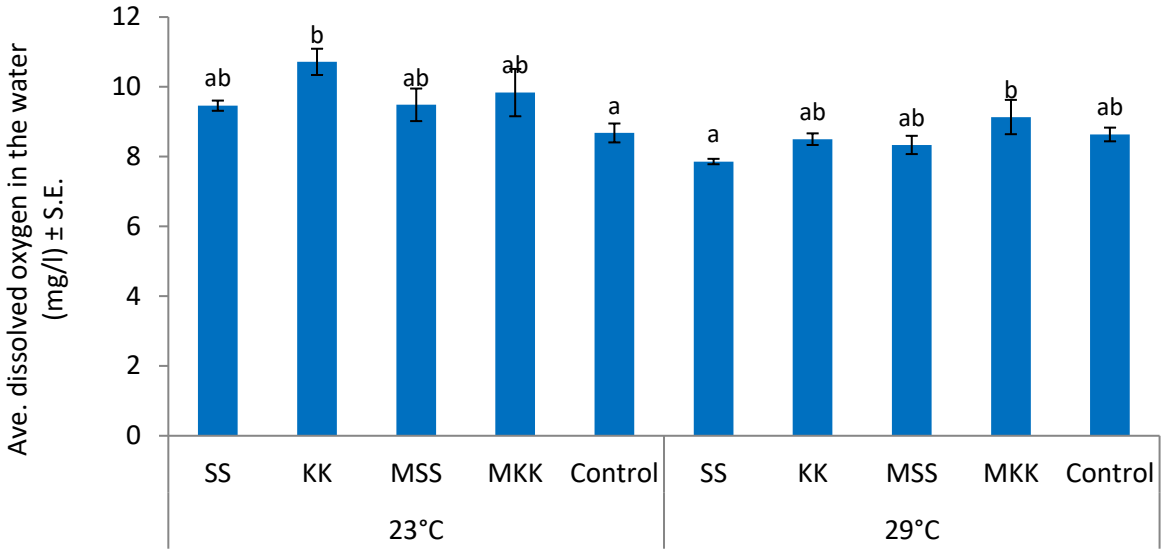

Fig. 3.34 The amount of dissolved oxygen in the water after termination of population level experiment. One-way ANOVA was used.

Table 3.27. Two-way ANOVA for measure of dissolved oxygen in population level experiment

Factor	Source	df	SS	*F*	*P*
Dissolved oxygen	Temperatures	1	64.523	25.050	.000
	Organisms	4	32.553	3.160	.015
	Temperatures x Organisms	4	27.035	2.624	.036

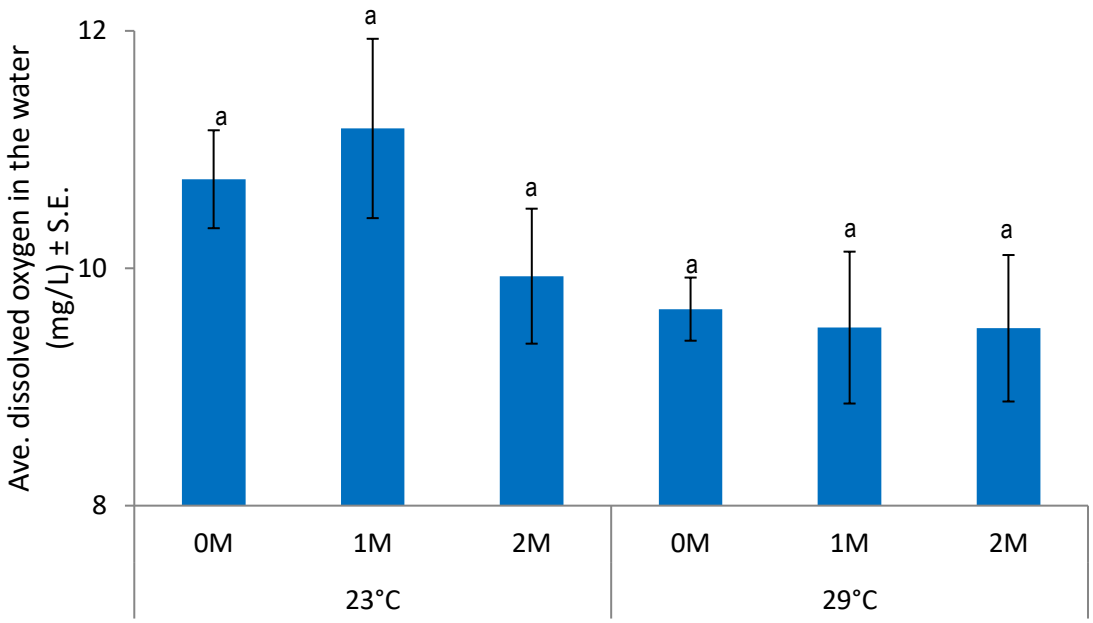

Fig. 3.35 The amount of dissolved oxygen in the water at the beginning, middle and end of community level experiment. One-way ANOVA was used.

Table 3.28. Two-way ANOVA for measure of dissolved oxygen in community level experiment

Factor	Source	df	SS	*F*	*P*
Dissolved oxygen	Temperatures	1	30.934	5.357	.023
	Organisms	2	7.778	.673	.512
	Temperatures x Organisms	2	6.915	.599	.551

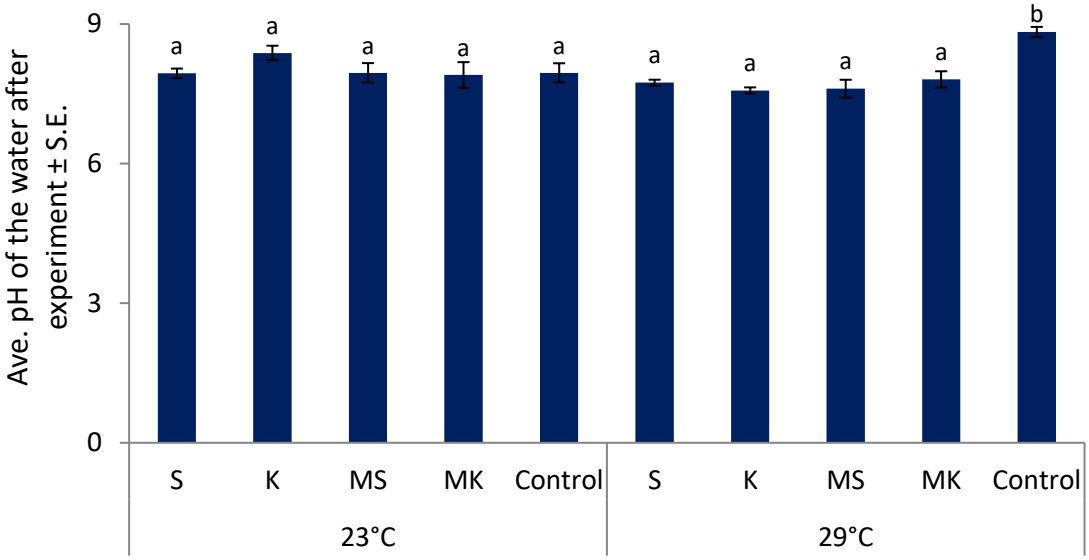

Fig. 3.36 The amount of pH in the water after termination of individual level experiment. One-way ANOVA was used.

Table 3.29. Two-way ANOVA for measure of pH in individual level experiment

Factor	Source	df	SS	*F*	*P*
pH	Temperatures	1	.617	1.087	.298
	Organisms	4	9.543	4.201	.003
	Temperatures x Organisms	4	14.463	6.367	.000

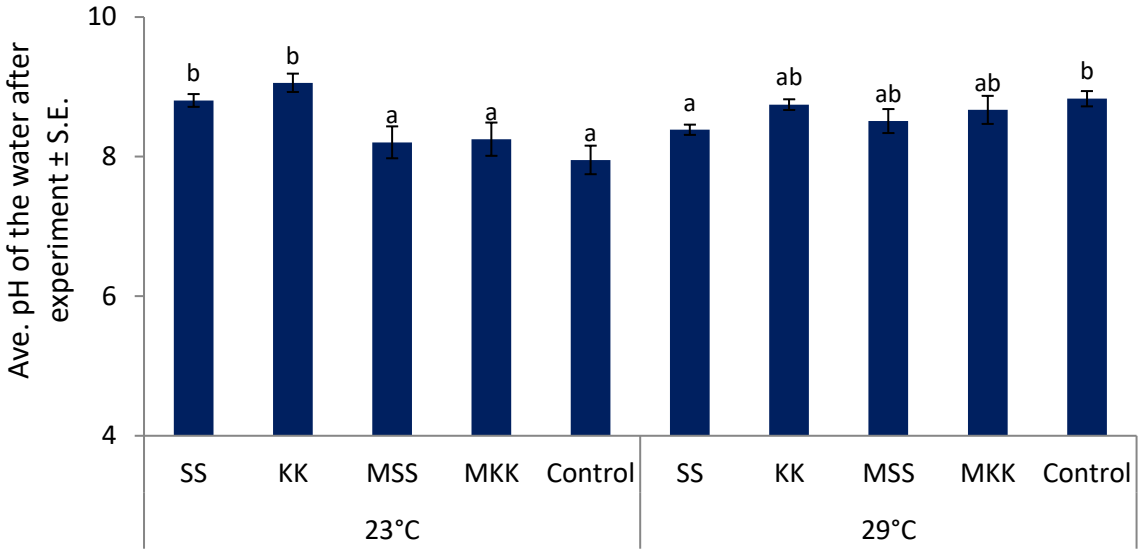

Fig. 3.37 The amount of pH in the water after termination of population level experiment. One-way ANOVA was used.

Table 3.30. Two-way ANOVA for measure of pH in population level experiment

Factor	Source	df	SS	*F*	*P*
pH	Temperatures	1	1.496	2.806	.096
	Organisms	4	7.663	3.592	.008
	Temperatures x Organisms	4	11.435	5.361	.000

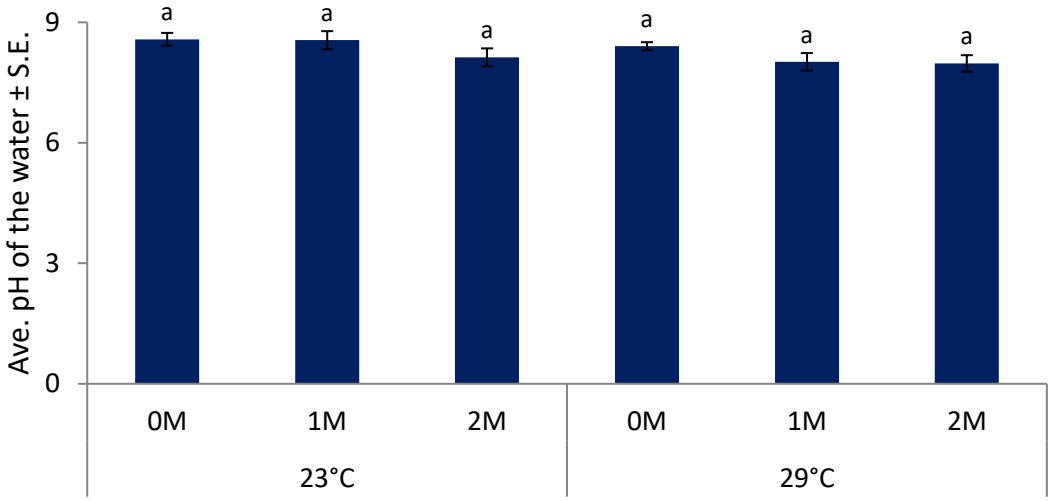

Fig. 3.38 The amount of pH in the water at the beginning, middle and end of community level experiment. One-way ANOVA was used.

Table 3.31. Two-way ANOVA for measure of pH in community level experiment

Factor	Source	df	SS	*F*	*P*
pH	Temperatures	1	2.225	3.278	.073
	Organisms	2	3.472	2.558	.082
	Temperatures x Organisms	2	.859	.633	.533

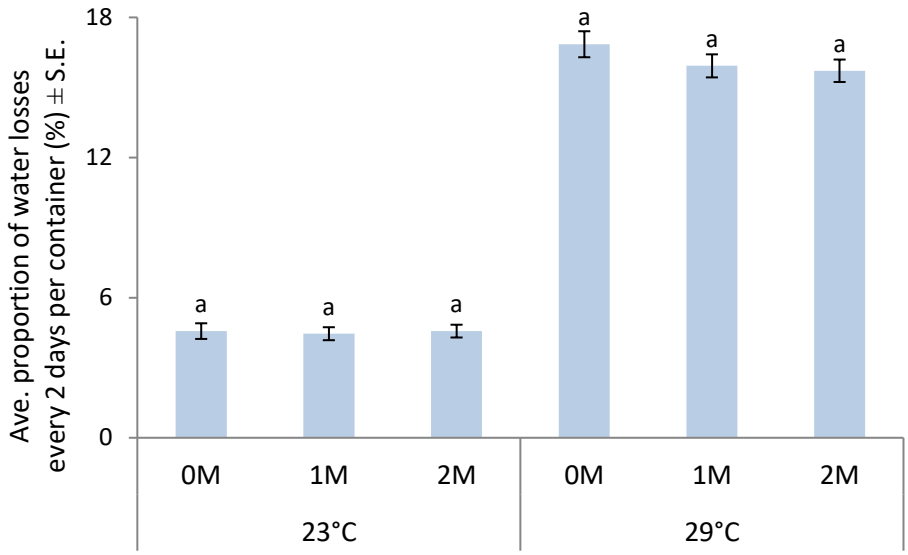

Fig. 3.39 Water losses caused by evaporation in community level experiment. One-way ANOVA was used.

Table 3.32. Two-way ANOVA for water losses in community level experiment

Factor	Source	df	SS	*F*	*P*
Sakamakigai egg	Temperatures	1	32888.597	1161.954	.000
	Organisms	2	64.132	1.133	.323
	Temperatures x Organisms	2	55.539	.981	.375

Table 3.33. Summary of the effects of temperature and the mud snails on survival, development, and reproduction of other aquatic organisms and abiotic factors at individual, population, and community level experiments (-: no effect, ○: positive effect, ×: negative effect, n.a.: not available; NE: generally no effects, O: strong positive effect, X: strong negative effect).

Parameters	Effects of temperature			Remarks	Effects of mud snails			Remarks
	Individual	Population	Community		Individual	Population	Community	
Biotic factors								
Survival								
All organisms	-	○	-	NE	n.a	n.a	n.a	
Sakamakigai	n.a	n.a	n.a		×	-	○	NE
Kawanina	n.a	n.a	n.a		○	○	×	NE
Body weight								
Mud snail	-	×	-	NE	n.a	n.a	n.a	
Sakamakigai	○	-	-	NE	-	○	○	O
Kawanina	-	○	-	NE	-	-	-	NE
Reproduction								
Egg of sakamakigai	-	-	-	NE	○	○	○	O
Juveniles of kawanina	○	-	-	NE	-	-	-	NE
Juveniles of mud snails	○	○	○	O	n.a	n.a	n.a	
Abiotic factors								
Organic matters	-	-	○	O	○	○	○	O
Chlorophyll content	○	○	×	O	○	○	-	NE
Nitrate	×	-	○	NE	×	×	○	O
Potassium	-	-	○	O	×	-	-	NE
Dissolved oxygen	×	×	×	X	×	○	-	NE
pH	-	-	-	NE	×	×	-	NE
Water losses	n.a.	n.a.	○		n.a	n.a	-	

Chapter 4

General discussion

Irrespective of the varied opinions about the existence of the global warming, alterations in the earth showed by some climate parameters and natural phenomenon indicated that the atmosphere has been changing. The changes affecting also other abiotic factors followed by not only positive but also negative responses from the biotic factors in the ecosystem would influence the development, survival, and reproduction of organisms in addition to interactions among organisms involved in the system. Consequently, the knowledge related to these effects and other factors involved is needed to understand the effects of global warming.

Agriculture which is responsible for feeding to dramatically raised human population has been facing many obstacles including not only insufficiency in food production but also safety issues of the agricultural products. Increased quality of life in most countries is followed by their awareness in accessing the safety food from chemical contaminants such as pesticide residues due to conventional farming. In this way, strengthening the transformation from conventional to alternative farming more friendly agriculture to the environment and less harmful products to the human health is necessary to have. Organic farming which offers principles of productivity achievement of healthy products by implementing methods based on living ecological systems and cycles would be a possible solution for the concerned issues.

The cultivation of rice paddy fields, as accountable for providing main staple food in many countries, using organic farming had been initiated in the past several decades. Some studies were also conducted during that time to find out the effects of temperature on the performance of aquatic organisms. The higher temperature is

followed by the lower density of particle of water stimulating profound effects on the spatial and temporal distribution of heat, and therefore on the aquatic organisms (Bronmark and Hanson 2005). Different responses of some aquatic organisms along the temperature gradient of water were recorded and implied as an expression of their adaptation to fluctuations in temperature (Yentsch 1974; Magnuson et al. 1979; Madsen and Adams 1989; Slusarczyk 1995; Dodds2002).

Considering the variation of the degree of the suitable temperature affecting the performance of aquatic organisms, manipulation of the environment serving the suitable temperature for a given key species is necessary. Naturally occurred biotic factor such as weed in an organic paddy field facilitates aquatic animals susceptible to high temperature to temporarily discover refuge. In addition, the use of organic fertilizers helps to soften the soil at which some species of freshwater snails are easy to burrow escaping from high temperature.

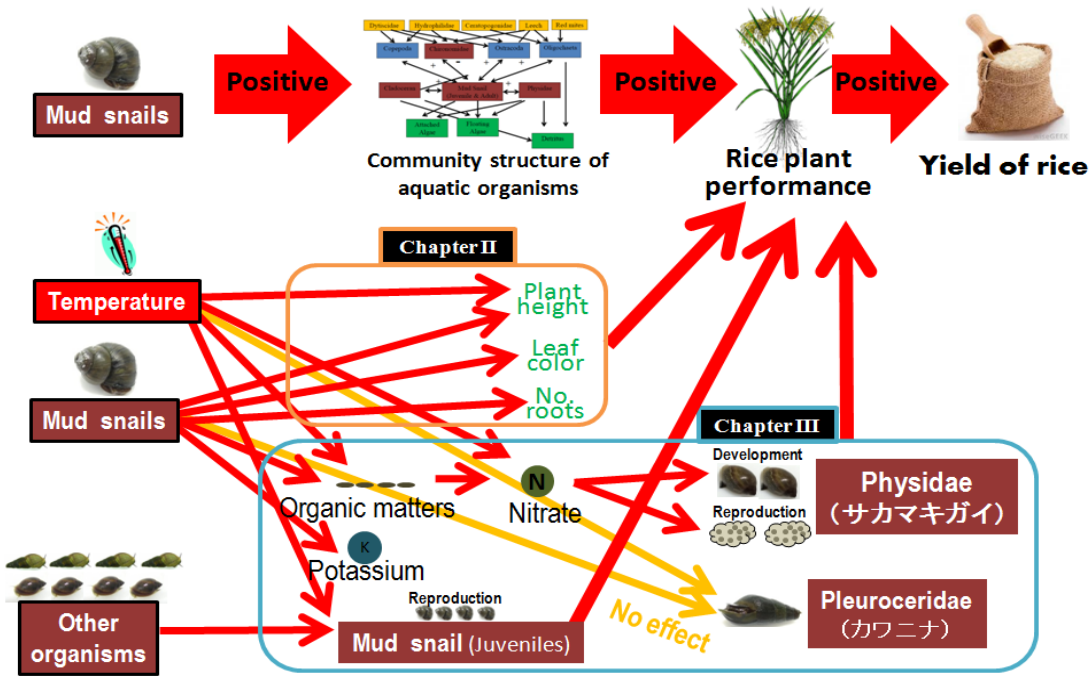

Fig. 4.1 The contributions of the results from chapter II and III in responding the questions from previous study conducted by Dewi (2017).

Dewi et al. (2017) revealed that mud snails positively influenced both the community structure of aquatic organisms in the paddy field ecosystem and rice plant development (Fig. 4.1). However, the actual effects of mud snails influencing some aquatic organisms were still unknown. In chapter II, positive influences of temperature and mud snails on height, leaf color, and number of the roots of rice plants were revealed. In addition, positive effects of high temperature which increased the development of rice plants especially plant height had been reported by earlier study (Yoshida 1973). Otherwise, global warming through large increase of the temperature is expected to decrease the yield of agricultural crops by 2080s (Parry et al. 2004).

Positive influences of mud snails on abiotic factors such i.e. organic matters and nitrate, and biotic factors i.e. reproduction and development of sakamakigai resulted in the chapter III would give positive effects on rice production through the development of rice plant i.e. plant height and leaf color as suggested by chapter II. In addition, the positive effects of mud snails on aquatic community structure and rice production in the paddy field have also been revealed by previous study (Dewi, 2017).

Biodiversity of aquatic organisms, however, positively correlated with the beneficial effects obtained by the ecosystem. The presence of other coexisting organisms was proved supporting the reproduction of given species followed by the incorporation of increased bio-based nutrients eventually worthwhile for the performance of cultivated plants.

The absence of abiotic factors, possibly affecting the process of temperature and mud snails influencing rice plant performance, investigated in chapter 2 was confirmed in chapter 3 i.e. the enhanced organic matters and concentration of nitrate

in the water were also affected by mud snails and possibly supporting the performances of other aquatic organisms.

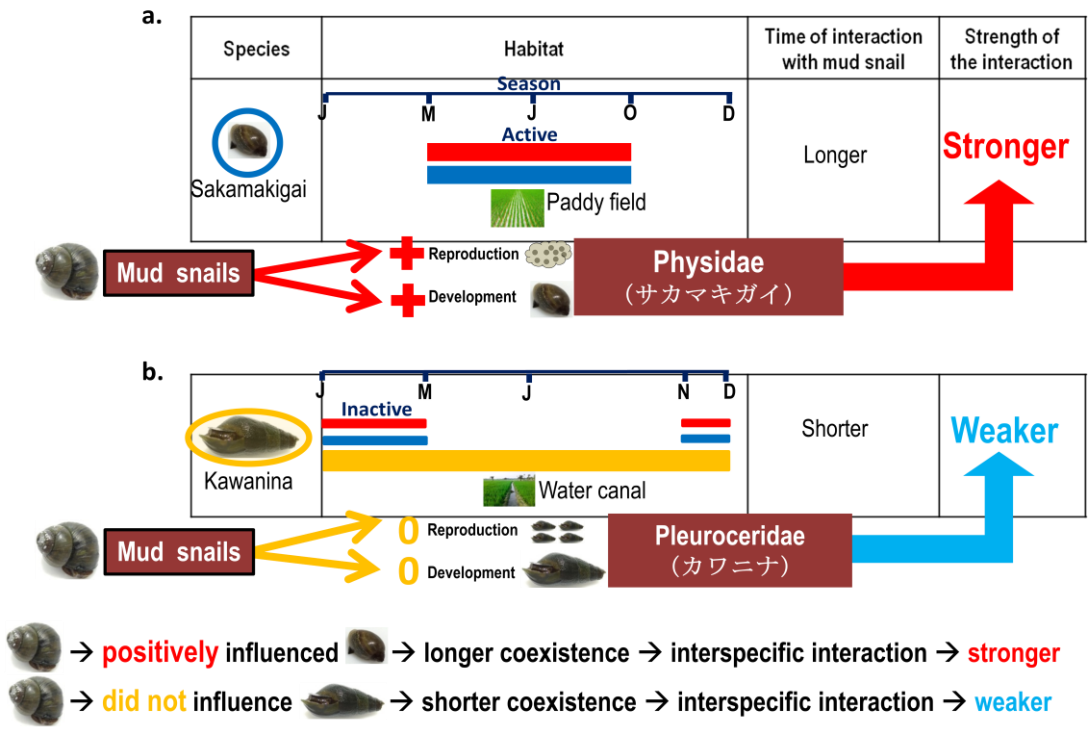

Fig. 4.2 Expected interspecific interactions from the point of view of habitats: a. mud snails and sakamakigai, b. mud snails and kawanina.

This study also elucidated interspecific interactions between the mud snails and two other freshwater snails according to their different habitats. Since mud snails and sakamakigai share the same habitat in a paddy field and spend relatively the same period, the interactions between the two species would be strong. This is supported by the higher development and reproduction of sakamakigai due to the presence of the mud snails (Fig. 4.2a). Mud snails were possibly responsible for the increased nutrients including nitrate as suggested by Underwood (1992) i.e. nutrients originated from the snail such as phosphates and ammonium enhanced growth of macrophyte in pond ecosystem. In the current study, the higher nitrate possibly had positive influence

on the performance of sakamakigai. On the other hand, considering the different habitat between mud snails and kawanina and the short coexistence between the two species, their interactions would be weak. This is promoted by the absence of the effects of the presence of the mud snails on the development and reproduction of kawanina (Fig. 4.2b).

This study revealed that the effects of temperature and mud snails were mostly significant in abiotic and biotic factors, respectively. The magnitude of those effects was different across the spatial levels suggesting that the number of species and individuals plays an important role in the aquatic ecosystem.

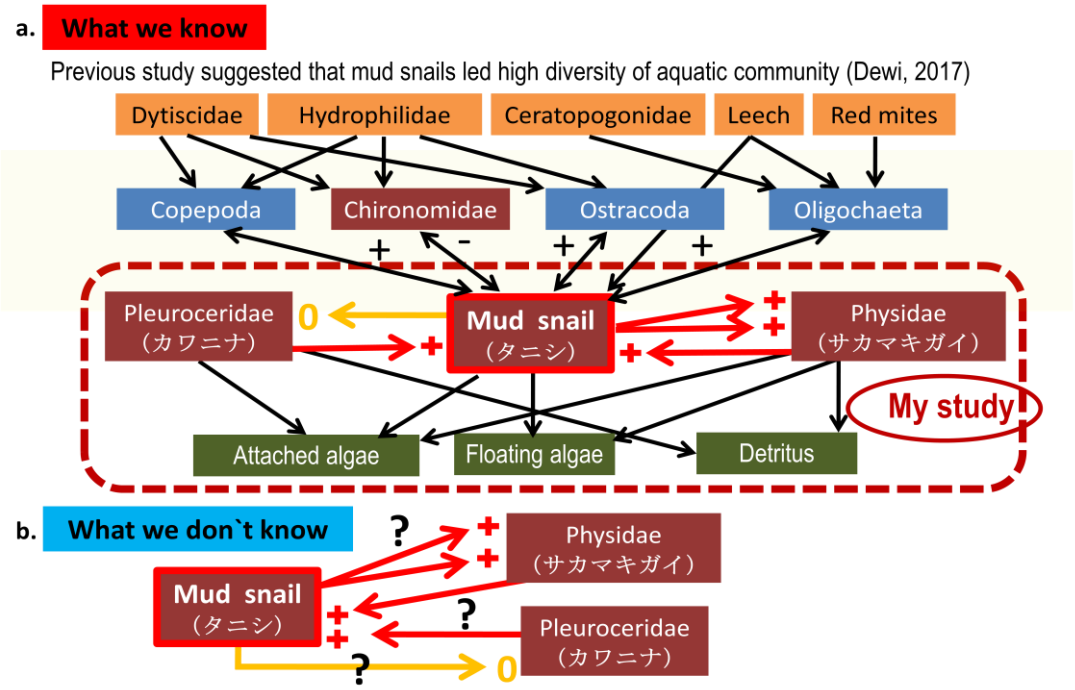

Fig. 4.3 Foregoing results about the effects of mud snails on community structure of aquatic organisms suggested by Dewi (2017) and the future studies related to the mechanisms underlying the interaction among three freshwater snails.

The current studies stating the findings that the presence of mud snails increased the development and reproduction of sakamakigai and oppositely, other organisms consisting of sakamakigai and kawanina increased the reproduction of mud

snails which might be a possible mechanism shown in the previous results by Dewi (2017) that mud snails had beneficial effects on aquatic community structure (Fig. 4.3a). Thereby, the study to understand the mechanisms of how mud snails positively influenced sakamakigai and did not increase kawanina, and also how other organisms had positive effect in mud snails is needed (Fig. 4.3b).

Summary

The aim of this study was to understand the mechanism of how the mud snail and temperature affect the survival, development, and reproduction of other aquatic organisms and performance of rice plant in a paddy field. In order to understand these effects, two experiments were carried out at outside and in the laboratory. Firstly, the effects of ambient temperature and the mud snail on rice plant performance were examined in 2015 using a factorial experiment. The presence of mud snails resulted in significantly increased plant height and SPAD value (reflecting leaf color) of the rice plants, although the tiller number was not significantly affected. The effects of temperature (high versus normal) on rice plant performance were assessed by the experiments inside and outside greenhouse. The results suggested that the effects of temperature differed among plant performances; plants grew taller but had fewer tillers when the rice was grown at high temperature, while SPAD was not significantly affected. Significantly more roots appeared on the soil surface when snails were present. In general, the mud snails resulted less excreta at high temperature than at normal temperature. The results of this study indicated that ambient temperature influenced rice plant performance both directly, and indirectly through the activity of the mud snails.

Secondly, in order to understand the effects of temperature and mud snails on the survival, development, and reproduction of other aquatic organisms at individual, population, and community level, laboratory experiments were conducted. The aim of this study was (1) to reveal the effects of temperature and the mud snails on the biotic parameters mentioned above of aquatic organisms at individual, population, and community level, and (2) to understand the possible mechanism influencing biotic and

abiotic interactions. Other aquatic organisms except the mud snails used for the experiments were sakamakigai and kawanina. To clarify the effects of temperature and mud snails, biotic and abiotic parameters were measured in the experimental containers.

In general, temperature did not affect biotic factors such as the survival, development, and reproduction of other aquatic organisms, while it influenced on abiotic factors at individual, population, and community experiments. On the other hand, the effects of snails differed from the temperature effects; the snails gave much influence on the biotic factors than abiotic ones. The similarity of the effects of temperature at individual, population, and community experiments was found in dissolved oxygen, pH, egg clutches of sakamakigai, and juveniles of mud snails. It was suggested that the effects of temperature on those parameters were not affected by increased number of species and individuals. The difference in the effects of temperatures in individual, population, and community experiments was found in the organic matters, chlorophyll content, and potassium. In addition, the effects of temperatures on those parameters were affected by increased number of species and individuals. In terms of the effects of mud snails, the similarity was found in body weight of kawanina, organic matters, egg clutches of sakamakigai, and juveniles of kawanina suggesting that the effects of mud snails on those parameters were not affected by increased number of species and individuals. The difference was found in the survival of kawanina, body weight of sakamakigai, and nitrate, suggesting that the effects of mud snails on those parameters were affected by increased number of species and individuals. In general, the sum of positive effects of both temperatures and mud snails on community level seemed to be higher than on either individual or population levels. This was probably because the number of interactions among

organisms affected by the number of both species and individuals was getting higher along the gradient of the experimental level. In addition, this study clearly showed that in the community level mud snails significantly affected the performance of sakamakigai by increasing the survival, bodyweight, and number of its egg clutches. The enhanced organic matters and concentration of nitrate in the water was also affected by mud snails and possibly supporting the performances of sakamakigai. Furthermore, decreased survival of kawanina, as affected by mud snails, in turn probably increased the performances of sakamakigai by reducing the competition.

This study suggested that one of possible mechanisms how mud snails positively influenced the community structure of aquatic organisms previously studied i.e mud snails provide suitable environment through supplying nutrients which are advantageous to the performance of other aquatic organisms e.g. sakamakigai.

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