

博士論文

Paleolimnological analysis of long-term ecological dynamics
of cladocerans in a lake

(湖沼生態系を構成する枝角類の長期生態的動態の古陸水学的解析)

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Abstract

How ecosystems form and change is one of the biggest topics addressed in ecology. Ecosystems consist of multiple hierarchy levels: genetic composition, phenotype, population, community, and ecosystem including abiotic environment. Prior studies found that evolutionary processes occur on the same time scale as ecological processes and that such contemporary evolution can reciprocally affect ecological dynamics, termed as eco-evolutionary dynamics. While interactions between different levels of ecosystems have been studied, simultaneous observations of multiple levels of natural ecosystems and integrations of these data are limited. Although long-term observations to witness responses at each level are important to understand ecosystem changes, especially to measure both genetic and phenotypic data in addition to species data like species composition and abundance of each species, they are rare and challenging. Furthermore, any ecosystem depends on the colonization of multiple species. Thus, the changes such as adaptation which occur during colonization affect later changes and structure of the ecosystem. However, observations of natural ecosystems right from their initial colonization stage are rare, owing to the difficulty related to prior prediction of initial introduction.

In my doctoral thesis, I analyzed long-term changes at multiple levels of a natural lake ecosystem, ranging from the genetic composition of some species to the ecological level including abiotic environment, since the initial stage of the lake ecosystem formation, using the paleolimnological and resurrection ecological method. Paleolimnology allows us to reconstruct past biotic and abiotic changes using lake sediment core samples and subfossils of organisms preserved in the sediment. In addition, resurrection ecology reconstructs long-term genetic and phenotypic changes by collecting living organisms at multiple time points (from past to present) using dormant stages preserved in sediments and ice cores. Combining paleolimnological and resurrection ecological analyses allow to observe the long-term changes from the genetic to ecosystem levels including environment. Paleolimnological and resurrection ecological analysis have previously revealed the long-term changes in ecosystems and how lake ecosystems, communities, and certain species, like *Daphnia*, respond to environmental changes. However, a long-term research of multiple ecological levels using same core samples and integration of paleolimnological observations is still under development. In addition, time-series observations of the early stages of several ecological

processes, such as the colonization of population, are also limited in paleolimnological and resurrection ecological studies. Hence, how lake ecosystems and each ecological level respond to biotic and abiotic changes, and how each level of the ecosystem interacts in the early stages of development, are still poorly understood.

In the present study, I successfully collected lake sediment cores that included the layer indicating lake formation period in Lake Fukami-ike, Nagano prefecture, Japan. I focused on cladoceran and specifically *Daphnia pulex*, which represents a major group and species used in both paleolimnology and resurrection ecology. Japanese *D. pulex* has four lineages that are genetically distant from each other; they are originated in North America. All four lineages are the obligate parthenogenetic lineage, which can produce diapausing eggs by asexual reproduction. Hence, their diapausing eggs are genetically identical to their parent. Thus, I collected genetic information on past generation and reconstructed historical genetic dynamics and phenotypic changes.

In my doctoral thesis, I aimed to reveal the long-term changes in the natural lake ecosystem across different ecological levels, from genetic structure to community composition and the surrounding environment, using lake sediment. I also focused on the changes that occurred from the initial colonization process of the populations, which was lacking in other observation studies. I discuss how multiple ecological levels change and interact with each other during this process, and how such changes affect later ecosystem change. Based on these observations, I gathered knowledge to understand the change of multi-layered ecosystem and reciprocal interactions in natural systems.

In Chapter 2, I focused on the cladoceran community, the population dynamics of each species that constituted the cladoceran community and the related environmental changes. I reconstructed the long-term changes in the cladoceran community and environmental factors that occurred during the lake formation period using lake sediment core samples and subfossils of cladocerans. This was done to test the hypothesis regarding the transition of important control factors affecting the cladoceran community as eutrophication proceeds, which gradually proceed from lake formation and occasionally rapidly proceed by various disturbance like human activity. With these results, I revealed that the major factors controlling the cladoceran community and the relative importance of these factors were temporally changed as eutrophication proceeded. In the initial stage of the cladoceran community assemblage, the bottom-up effect strongly affected the cladoceran community as rapid eutrophication replaced initial benthic

cladoceran community with a pelagic community dominated by small *Bosmina*. After rapid eutrophication occurred, the top-down effect became relatively strong due to an increase of primary production in the pelagic zone, which released the cladoceran from resource restriction and allowed predators at higher trophic levels, such as fish, to establish in the area. These results coincide with each prior study that evaluated the bottom-up and top-down effects in a lake ecosystem, separately.

In Chapters 3 and 4, I focused on the *D. pulex* population and its colonization process during early colonization. In Chapter 3, I tested the genetic dynamics using population genetic analysis based on mitochondrial DNA with diapausing eggs preserved in lake sediments. These results showed the *D. pulex* population colonized and was sustained with greatly limited haplotypes, without turnover nor recovery of genetic diversity, and that a single haplotype (Jpn2C) had dominated throughout the colonization process.

In Chapter 4, I discuss changes in the morphological phenotype related to adaptation to predation pressure during the colonization process by morphological analysis of subfossils and ephippia preserved in lake sediments. While Chapter 3 shows that the genetic diversity of the *D. pulex* population had been limited to a single dominating haplotype, Chapter 4 shows that the morphological phenotype changed during the colonization process. Furthermore, morphological changes were adaptive to the changes in the predator community change: the body size of matured females increased and defensive traits decreased when planktivorous fish decreased and vice versa. Even when genetic diversity decreased in the early stages of colonization as traditional research suggested, biological populations were possibly able to establish and survive with adaptive phenotypic change.

In Chapter 5, I also focused on the *D. pulex* population and the mechanism maintaining genetic diversity. I tested the hypothesis that intraspecific differences in diapause strategy based on photoperiodic response can promote the maintenance of genetic diversity through the storage effect using *D. pulex* lineages originated from diapausing eggs preserved in lake sediments. I used two haplotypes of *D. pulex*, Jpn2C and Jpn1A-C2T2, belonging to distant genetic lineages. Chapter 3 shows that Jpn2C had dominated throughout the colonization process and Jpn1A-C2T2 appeared more recently without replacing Jpn2C, instead maintaining coexistence. I evaluated the competitive ability and photoperiodic response in the diapause of each lineage through laboratory experiments. These experiments

showed that a competitively inferior haplotype, Jpn1A-C2T2, tended to produce diapausing eggs earlier than Jpn2C. The theoretical model analysis suggested that different photoperiod responses and interannual fluctuations of growth conditions can promote the coexistence of the two haplotypes via the storage effect, as the competitively inferior haplotype avoids competition by diapause induction. While germination timing has been investigated as an important factor for the storage effect in plants, I showed the potential importance of diapausing timing induced by photoperiodic changes in promoting the maintenance of genetic diversity. Moreover, this intraspecific difference of diapausing timing dependent on photoperiodic response could have allowed Jpn1A-C2T2 to establish after Jpn2C.

Based on the above analyses, I could simultaneously observe the multiple levels composing the natural lake ecosystem, containing from the genetic structure of *D. pulex* population to the ecosystem including abiotic environment. I found that the multiple interactions between levels that construct the natural lake ecosystem, and found that these interactions may also occur between distant ecological levels, such as between community and phenotype. I also found multiple interactions between different ecological levels during the initial stage of colonization, including interactions that related to successful colonization. These observations suggested that the interactions occurring in colonization affected ecosystem structure and later ecological changes. Furthermore, predator community affected multiple ecological levels while *D. pulex* population was influenced by many levels. These results suggest that some biological groups may facilitate cross-level interactions to occur. In Chapter 2, I report that eutrophication and predator community dynamics change cladoceran community composition and each species' population dynamics. Chapter 3 shows the possible interactions between the predator community and the genotype of the *D. pulex* population, and between the phenotype and genotype within the *D. pulex* population. Chapter 4 illustrates the interaction between the predator community and the phenotype of the *D. pulex* population, and the interaction between phenotype and population dynamics of *D. pulex*. Chapter 5 shows the interactions between the population structure, dynamics, phenotype, and genotype in the *D. pulex* population.

On the contrary, I could not observe sufficient feed-back interactions, including the “evolution to ecology feedback”. To understand the feedback loops occurring in natural systems, it would be necessary to include, a more detailed taxonomic analysis of other biological communities involved, such as of fish community and phytoplankton

that interact with cladocerans and *D. pulex*. Paleolimnological analysis will help us to analyze various biological communities after observation of certain species' population. The recent development in genetic analysis of sediment allows us to reconstruct the dynamics of the taxa that do not leave subfossils in sediments such as fish. To consider evolutionary responses in detail, fine genetic analyses would be effective, such as that of functional genetic regions and/or an association analysis using genome-wide Single Nucleotide Polymorphisms. These analyses on diapausing eggs preserved in lake sediment will elucidate the relative importance of plasticity and genetic evolution, and the history of selection. By integration with long-term observations like the present study, it may be possible to evaluate the "evo-to-eco feedback".

Furthermore, I considered the possibility that certain biological groups possibly facilitate cross-level interactions in ecosystem. During my research, I observed that the change in the predator community affect multiple ecological levels. Several prior studies have shown that the predator community plays a key role in controlling changes in each of the ecological levels. These results of earlier studies and my results have suggested factors that have large impacts on each level of an ecosystem may tend to influence multiple levels simultaneously, which may be caused by indirect effects on other levels. Similarly, I observed that *D. pulex* population was affected by multiple ecological levels. *Daphnia* species are well reported as species sensitive to biotic and abiotic changes: such sensitive biological groups may accumulate impacts from multiple ecological levels and mediate their effects across ecological levels. An analysis of which levels are most affected, and of which levels are more likely to affect multiple levels, may provide the much-needed data for better ecosystem management.

In addition, the present research raised a question regarding how mechanisms that maintain species diversity and those associated with intraspecific genetic diversity interact in a natural system. The storage effect, which the present research suggests as a possible mechanism to maintain genetic diversity in the *D. pulex* population, has also been shown to maintain species diversity. The diapausing egg bank of *Daphnia* could possibly serve as an effective tool to approach this question, because it allows us to reconstruct the chronology of both genetic and species diversity changes in a natural system.

Through the present study, my research suggests that interactions between ecological levels, including the

interactions of distant levels, occur and may have important roles in natural systems. More specifically, during the colonization of natural populations, these interactions possibly affect colonization success, development of the community structure, and ecosystem function. In addition, my research showed that some factors, such changes in the predator community, can affect multiple biological levels at the same time. Furthermore, the present study showed the effectiveness of paleolimnological and resurrection ecological methods to observe the interactions between different ecological levels in natural systems and to provide new insights for future studies to explore such interactions, including feed-back responses and eco-evolutionary dynamics.

Chapter 1 General introduction

The importance of understanding the changes of multiple ecological levels in natural ecosystem

One of the main topics addressed in ecology is how ecosystems form and change, which is instrumental to conserve and manage ecosystem and biological diversity subjected to severe environmental changes driven by human activity (Steffen et al., 2015). Ecosystems consist of multiple levels, containing genetic composition, phenotype, population, community, and ecosystem including abiotic environment (Figure 1-1). Previous studies have revealed that ecological hierarchy levels interact with each other; recently, it was suggested that evolutionary processes occur at the same time scale as ecological processes: such contemporary evolution could reciprocally affect ecological dynamics, termed as eco-evolutionary dynamics (Hairston, Ellner, Geber, Yoshida, & Fox, 2005). Since these findings were reported, the interactions and feedback loops among different ecological levels have been focused upon with special emphasis on related research (Lavergne, Mouquet, Thuiller, & Ronce, 2010; Schoener, 2011; Hendry, 2017; Hendry, 2019). Currently, both theoretical and empirical evidence of eco-evolutionary feedback focused on individual ecological levels are available (reviewed in De Meester et al., 2019; Govaert et al., 2018; e.g. predator-prey dynamics: Meyer et al., 2006; Yoshida et al., 2003; Kasada, Yamamichi, & Yoshida, 2014; Palkovacs et al., 2009; Palkovacs, Wasserman, & Kinnison, 2011, the effect of adaptation to environmental change: Terhorst, Lennon, & Lau, 2014). Recent studies have also revealed that genetic and phenotypic change in a species can have cascading effects, thereby influencing community structure (McGuigan, Nishimura, Currey, Hurwit, & Cresko, 2011; Palkovacs, Wasserman, & Kinnison, 2011) and ecosystem function (Salo, Mattila, & Eklöf, 2019). Furthermore, such intraspecific diversity effects on higher ecological hierarchy levels are comparable to interspecific effects (Des Roches et al., 2018; Raffard, Santoul, Cucherousset, & Blanchet, 2019; Stange, Barrett, & Hendry, 2020; Violle et al., 2012). While interactions across ecological levels have been addressed by the previous studies, adequate knowledge on how cross-level ecological interactions occur and affect ecological change in natural systems is still lacking (De Meester et al., 2019; Hendry, 2019). From these things, to understand change of ecosystem including cross-level interactions in natural ecosystems, we have to address the individual changes at each ecological hierarchy levels in natural ecosystems first.

Interactions between ecological hierarchy levels in various ecological processes are also important and one

of the most challenging topics. Since any ecosystem depends on multiple colonization processes by various species that compose its specific community, the adaptive response of each species to the new habitat and its successful colonization can affect dynamics and composition of populations, of the community and ecosystem (Szucs, Melbourne, Tuff, Weiss-Lehman, & Hufbauer, 2017; Szucs et al., 2017; Williams, 2016). Despite this, colonization processes remain scarcely studied and little knowledge exists regarding the interactions occurring during the initial colonization stages between different ecological levels and how they affect later ecosystem changes.

The effectiveness of long-term monitoring of natural ecosystem and its challenges

Long-term and simultaneous monitoring across multiple ecological levels of a natural ecosystem is one of the most effective and direct methods to understand its changes and dynamics in natural system (Barnosky et al., 2012). Such observations need to be made on a sufficiently longer time scale to study responses at each ecological level. However, such long-term monitoring research is restricted by various limitations and requirements, in particular human labor, applications, and funding (Kuebbing et al., 2018; Vucetich, Nelson, & Bruskotter, 2020). Although several long-term monitoring sites across the world belong to the Long-Term Ecological Research (LTER) project (Kuebbing et al., 2018; Mirtl et al., 2018; Vanderbilt & Gaiser, 2017), sites with more than a few decades of monitoring data are still rare (Vanderbilt & Gaiser, 2017). Some ecological processes such as early colonization, are even lesser known as they cannot be predicted in advance, thereby limiting the research setup, in addition to long-term monitoring costs (Cristescu, Constantin, Bock, Caceres, & Crease, 2012). Furthermore, the monitoring projects and sites for quantitative assessment of genetic and phenotypic diversity, in addition to species and abundance, are limited despite the importance of integrating evolutionary and ecological processes in natural systems (Brodersen & Seehausen, 2014).

Paleolimnological analysis allows long-term monitoring of natural ecosystems

Paleolimnological analysis and the resurrection ecological method are effective for observing long-term ecological and evolutionary changes across multiple ecological levels on the same time scale. Paleolimnology allows us to reconstruct past biotic and abiotic changes using lake sediment core samples and subfossils preserved in the sediment,

like carapaces and residues of phytoplankton, invertebrate organisms including zooplankton, and some insects (Smol, 2010; Douglas, 2013). Resurrection ecology reconstructs long-term genetic and phenotypic changes by collecting living organisms at multiple time points (from past to present) using dormant stages preserved in sediments and ice cores. Examples of common dormant stages used in resurrection ecology are diapausing eggs of cladocerans, seeds of plants, or cysts of microorganisms (Burge, Edlund, & Frisch, 2018; Franks, Hamann, & Weis, 2018; Orsini et al., 2013). While resurrection ecology mainly refers to the analysis of organisms hatched or germinated from dormant stages, genetic analysis can be conducted on dormant stages that have not hatched or germinated. Since resurrection ecological analysis can also be used on lake sediment core samples similar to paleolimnology, paleolimnological and resurrection ecological analysis can be conducted simultaneously on the same samples (Burge et al., 2018). Hence, combining these two analyses allows us to observe the long-term changes from the genetic structure to the ecosystem and environment. Furthermore, this approach can help to integrate data from environmental and ecological processes and reveal eco-evolutionary interactions in natural systems (De Meester et al., 2019).

While lake ecosystems have been used to develop several ecological concepts and test multiple theories, they are rarely considered in ecological projects with over ten years monitoring (Duggan et al., 2020). Initially, paleolimnological research analyzed long-term environmental changes using biological subfossils as indicators of lake environment (Smol, 2010). Since the 2000s, the effectiveness of paleolimnological approach has been considered for addressing ecological research questions (Davidson, Bennion, Reid, Sayer, & Whitmore, 2018; Sayer, Davidson, Jones, & Langdon, 2010), such as how biological communities respond to environmental change. For instance, prior paleolimnological studies reported long-term environmental and ecological change using phytoplankton (mainly diatoms; e.g., Adams, Peng, Rose, Shchetnikov, & Mackay, (2019)) and tree communities around catchment areas by pollen analysis (e.g., Parducci et al., 2013).

Cladocerans are one of the representative zooplankton groups, being intermediate consumers in lake food webs and sensitive to environmental and ecosystem changes in whole lake systems (Carpenter & Kitchell, 1993). Some parts of the cladoceran body (e.g., post-abdominal-claw, carapace, and head-shield) are composed of chitin and are well preserved over the long-term from one to several thousand years (Szeroczyńska, 1991; Korhola & Rautio,

2001). Furthermore, most cladoceran species produce diapausing eggs in environments that are unsuitable for population growth, which are also preserved in lake sediment for decades (Miner, De Meester, Pfrender, Lampert, & Hairston, 2012; Morton, Frisch, Jeyasingh, & Weider, 2014) and up to over a thousand years (Frisch et al., 2014). These characteristics make cladocerans ideal organisms to monitor multi-leveled long-term changes, in response to biotic and abiotic variations. Indeed, the response of the cladoceran community to natural and artificial disturbances has been well documented by both paleolimnological analysis and monitoring of the active community (e.g., Hampton, Scheuerell, & Schindler, 2006). Paleolimnological analyses compared the cladoceran community structure based on subfossils dated before and after certain disturbances, thereby revealing its response to various alterations, including eutrophication (e.g., Taylor., 2006; Bennion et al., 2015; Ohtsuki et al., 2015), acidification (e.g., Nevalainen, Sarmaja-Korjonen, Gąsiorowski, & Luoto, 2011), industrial development (e.g., Nevalainen, Luoto, Levine, & Manca, 2011), artificial fish introduction (e.g., Stoks, Govaert, Pauwels, Jansen, & De Meester, 2016), and a reduction of calcium and introduction of copper sulfate (e.g., Korosi & Smol, 2012). Among cladocerans, *Daphnia* species are especially well studied due to several characteristics that make them suitable for both experimental and field studies (reviewed in Miner et al., 2012). For many species of *Daphnia*, genetic markers have been developed and several genomic references are available (Miner et al., 2012). Since *Daphnia* can reproduce in a short-time scale (7 days to maturation, and ability to reproduce 2-3 days after once maturation) clonally, these species are effective to test both evolution and the phenotype of certain genotypes. *Daphnia* species are the most commonly used in resurrection ecology (Burge et al., 2018). They can hatch from diapausing eggs preserved in lake sediments with a high hatching rate within 20 years from being laid, and older eggs have been reported with a lower hatching rate in several lakes (Weider, Lampert, Wessels, Colbourne, & Limburg, 1997). Resurrection ecology has revealed rapid adaptation of *Daphnia* to various factors, like climate change (Cambronero Cuenca, Zeis, & Orsini, 2018), eutrophication (Frisch et al., 2014), predator introduction (Stoks et al., 2016), cyanobacteria blooms (Hairston et al., 1999), and adaptation through parasite interactions (Decaestecker et al., 2007) among others.

While the ecological and evolutionary responses of the cladoceran community and *Daphnia* species have been well studied as discussed above, long-term researches on multiple levels of ecosystem using the same core

samples and integrations of these paleolimnological observations are still being development. For instance, in Lake Constance, long-term changes in several ecological levels have been observed by paleolimnological methods (e.g. Ibrahim et al., 2020; Most et al., 2015; Bjerring et al., 2009; Brede et al., 2009), but an integration of these results is being developed now, and thus there is a lack of systematic implication. In addition, time-series observations of the early stages of several ecological processes, such as colonization, are limited in paleolimnology and resurrection ecology. Hence, it remains poor understand how lake ecosystems, their associated communities, and each species respond to biotic and abiotic changes and how the different ecological levels interact during the early development stages and in several associated ecological processes. One of the major factors restricting the observation of the initial ecological processes is the difficulties in collecting lake-sediment samples that include the sediment layer of lake-formation period. Most of earlier paleolimnological studies on cladocerans observed changes in response to various disturbances over less than a 100-year period. Moreover, most lakes that preserve high-quality sediment cores are ancient, and thus, even those studies investigating changes from over 10,000 years ago did not analyze sediments from the lake formation period. For example, a study assessed the response of a cladoceran community to climate change in the Holocene until 4,500 cal BP (Nevalainen, Rantala, & Luoto, 2015) and another examined the effects of human activity on a cladoceran community more than 10,000 years old, dating back to the prehistoric period (Szerocynska, 1991), but all these studies could not observe changes from lake formation period.

Lake Fukami-ike and Japanese obligate parthenogenetic *Daphnia pulex*

In the present study, I successfully collected lake sediment cores that included the lake formation period in Lake Fukami-ike, Nagano prefecture, Japan (Figure1-2). Lake Fukami-ike is shallow and surrounded by mountains (maximum depth: 7.8 m; Yagi, Kai, Uemura, Nakano, Tanaka & Shimodaira, 2009) with anoxic hypolimnion from April to November (Yagi et al., 2009; Yagi, Shimodaira, Terai, & Saijo, 1983) and is thus little disturbance from winds and benthos. Varved sediment core samples have been collected in prior studies conducted on this lake (Kawakami, Matsuo, Kato, & Fukusawa, 2004). Varved sediments comprise annual coupled layers forming annual lamina and allow us to observe high-resolution temporal changes (Lamoureux, 2001). Therefore, sediments from

Lake Fukami-ike allowed us to analyze the high-resolution temporal changes in the lake ecosystem and the environment from the lake formation period.

In this study, I focused on *Daphnia pulex*. Japanese *D. pulex* is constructed by four lineages, genetically distant from each other, and all of them originated in North America (So et al., 2015). All the four lineages are of the obligate parthenogenetic lineage, which can produce diapausing eggs by asexual reproduction like clonal reproduction during the asexual phase (Lynch, Seyfert, Eads, & Williams, 2008). Hence, their diapausing eggs are genetically identical to their parent, and genetic information of those past individuals could be collected from them and actual genetic dynamics and phenotypic changes could be reconstructed.

Purpose

In my doctoral thesis, I aimed to reveal long-term changes of the natural lake ecosystem across different ecological levels, from genetic structure to community composition and the surrounding environment (Figure 1-1), using lake sediment core samples (Figure 1-3). I also focused on population changes from the initial colonization processes, which have been seldom observed. And I discussed how multiple ecological levels change and interact with each other in this process, thus affecting subsequent ecosystem development. Based on these observations, I aimed to gather data crucial to understand the changes and interactions between multiple ecological levels in natural systems.

In Chapter 2, I focused on the cladoceran community, on each of its species' population dynamics, and on the environmental changes surrounding it (Figure 1-1: red-boxes). I reconstructed the long-term changes in the cladoceran community and the environmental factors that occurred during the lake formation period using lake sediment core samples and cladocerans subfossils. To test whether progressive eutrophication could influence the transition of important control factors affecting the cladoceran community. As eutrophication progresses, the relative importance of the top-down effect was suspected to become more impactful due to the removed resource limitation and the establishment of predators. In Chapters 3 and 4, I focused on the *D. pulex* population and its colonization process during early colonization. I tested the genetic dynamics in Chapter 3 using population genetic analysis on diapausing eggs preserved in lake sediments (Figure 1-1: violet boxes). Moreover, in Chapter 4, I revealed the

morphological phenotypes change that can be related to adaptation to predation pressure during the colonization process, through morphological analysis of subfossils and ephippia preserved in lake sediments (Figure 1-1: blue boxes). In Chapter 5, I also focused on the *D. pulex* population and the mechanism maintaining its genetic diversity (Figure 1-1: green boxes). I tested the hypothesis that intraspecific differences in diapause strategy based on photoperiodic responses can promote the maintenance of genetic diversity through the storage effect by theoretical analysis and laboratory experiments using *D. pulex* lineages originated in diapausing eggs preserved in lake sediments. This maintained diversity mechanism can also relate to the establishment for each genotype.

I conducted the above research on the same lake sediment core samples (Figure 1-3). I collected in Lake Fukami-ike in 2016 and 2017 (Figure 1-2) using a gravity corer (Limnos corer; Kansanen, Jaakkola, Kulmala, & Suutarinen, 1991) with an internal diameter of 93 mm and a Mackereth corer (Mackereth, 1958) with an internal diameter of 65 mm. My sediment core samples were dated (Figure 1-3) based on their correlation with sediment core samples dated in previous studies via ^{14}C dating, annual lamination counting (Ishihara et al., 2003; Kawakami et al., 2004) and sedimentation rate (Kawakami et al., 2004; Yagi et al., 2009).

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Figures

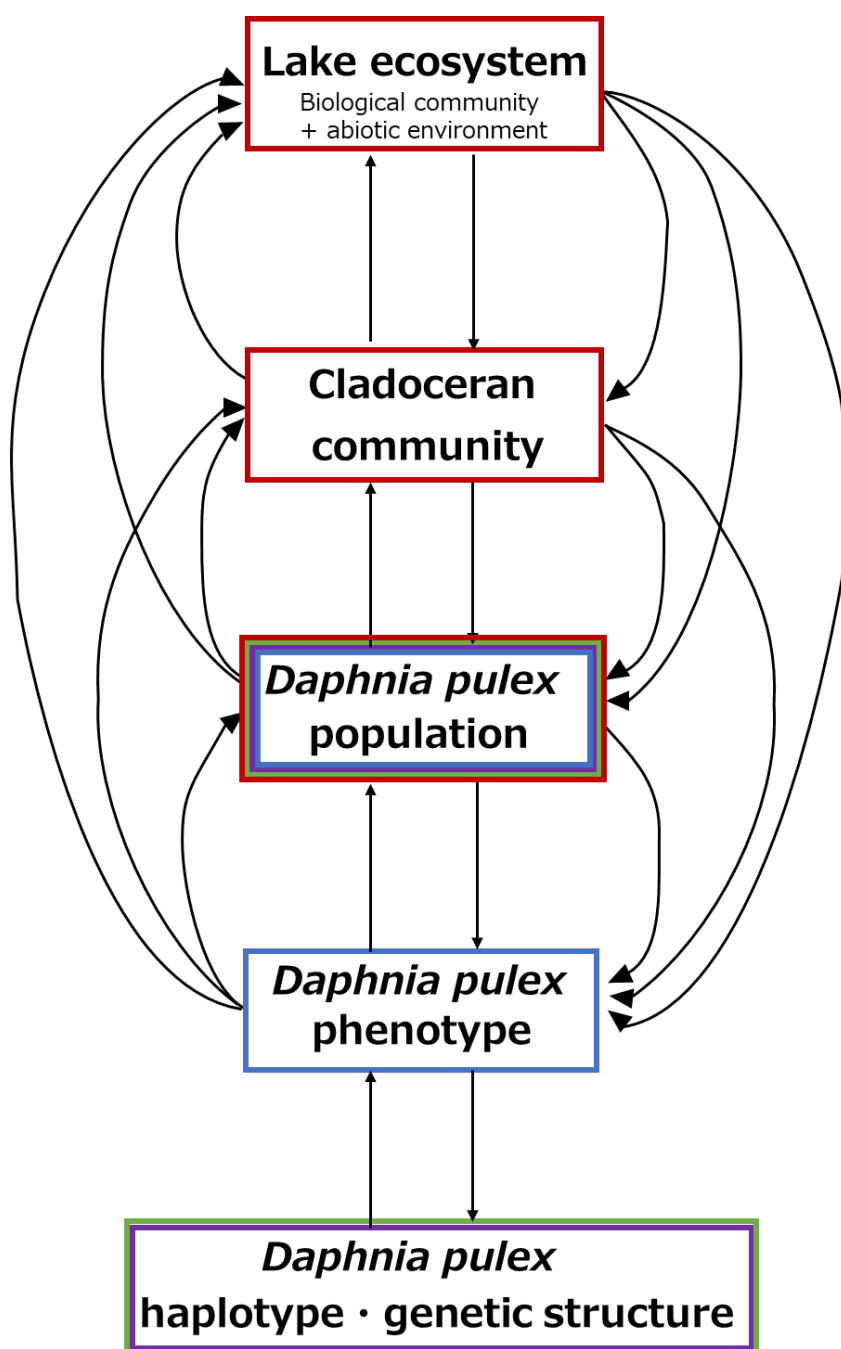


Figure 1-1. Interaction between levels of ecosystems. The colors relate to the chapters explaining the tests and evaluations of the ecological level: red means Chapter 2, which focused on the long-term dynamics of the cladoceran community and on each species' population in addition to environmental changes from lake formation; violet represents Chapter 3, which focused on the genetic dynamics of the *D. pulex* population from the early stage of colonization; blue indicated Chapter 4, which focused on phenotypic change within the *D. pulex* population from the early stage of colonization; green refers Chapter 5, which tested the mechanisms maintaining genetic diversity in the *D. pulex* population.

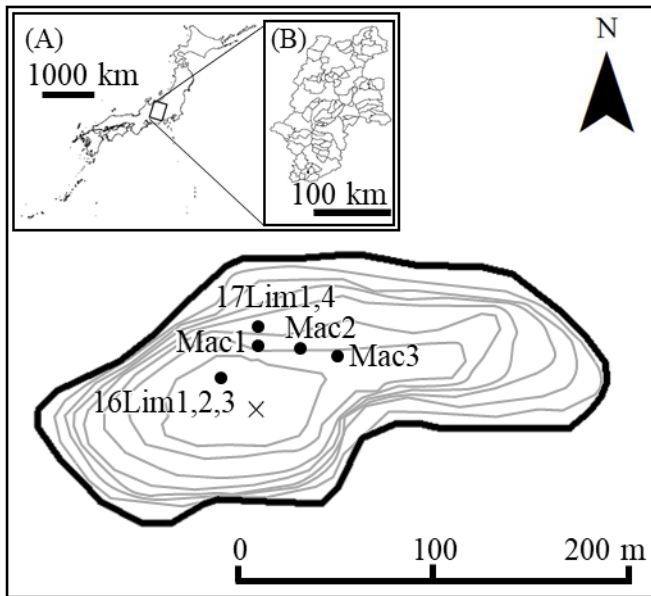


Figure 1-2. Map of Lake Fukami-ike (C, 35°19'N, 137°49') showing the sites from which sediment core samples were taken. The lake is located in Nagano Prefecture (B) in central Japan (A). 16Lim1,2,3 and 17Lim1, 4 indicate the sites where the sediment core samples were taken by a Limnos corer, and Mac1 to Mac3 indicate those taken by a Mackereth corer.

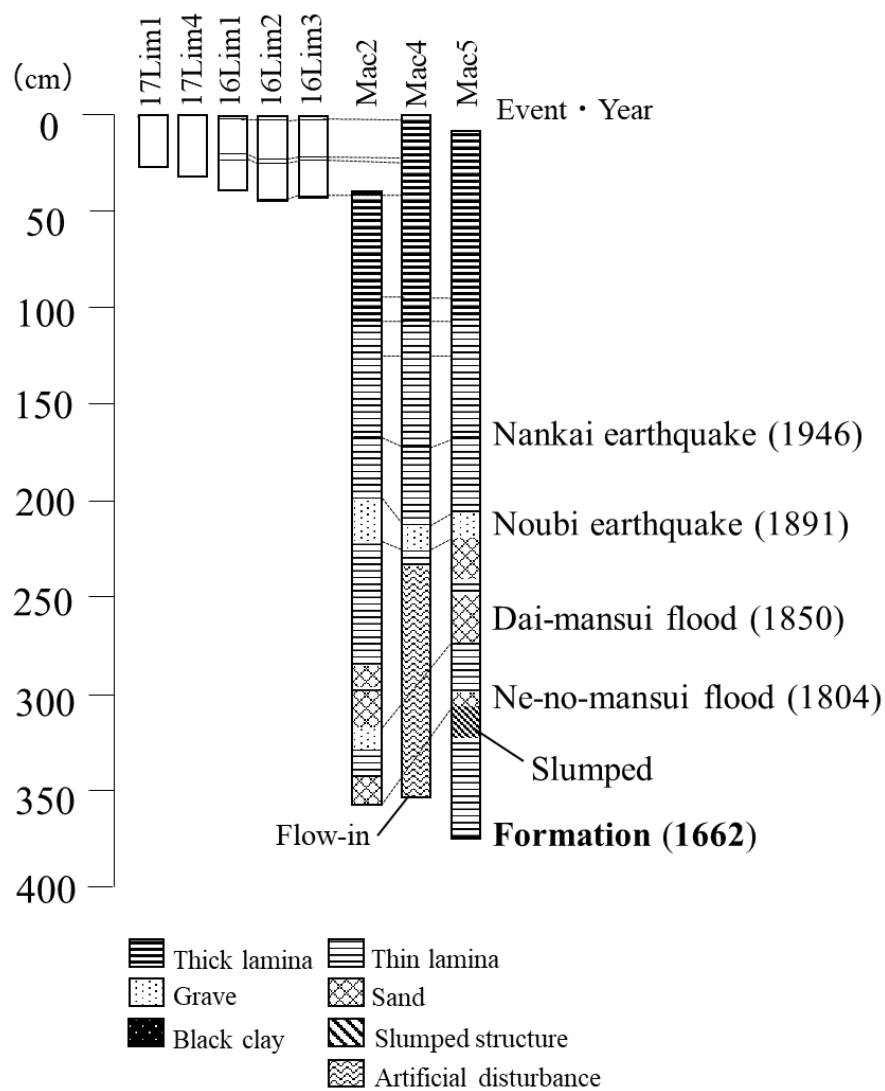


Figure 1-3. Stratigraphic correlation of sediment core samples taken from Lake Fukami-ike.

Chapter 2 Long-term dynamics of a cladoceran community from an early stage of lake formation in Lake Fukami-ike, Japan

Abstract

An increase in nutrient levels due to eutrophication has considerable effects on lake ecosystems. Cladocerans are intermediate consumers in lake ecosystems; thus, they are influenced by both the bottom-up and top-down effects that occur as eutrophication progresses. The long-term community succession of cladocerans and the effects cladocerans experience through the various eutrophication stages have rarely been investigated from the perspective of the early-stage cladoceran community assemblage during lake formation. In my research, long-term cladoceran community succession was examined via paleolimnological analysis in the currently eutrophic Lake Fukami-ike, Japan. I measured the concentration of total phosphorus and phytoplankton pigments and counted cladoceran and other invertebrate subfossils in all layers of collected sediment cores, and then assessed changes in the factors controlling the cladoceran community over a 354-year period from lake formation to the present. The cladoceran community consisted only of benthic taxa at the time of lake formation. When rapid eutrophication occurred and phytoplankton increased, the benthic community was replaced by a pelagic community. After further eutrophication, large *Daphnia* and high-order consumers became established. The statistical analysis suggested that bottom-up effects mainly controlled the cladoceran community in the lake's early stages, and the importance of top-down effects increased after eutrophication occurred. Total phosphorus and phytoplankton pigments had positive effects on pelagic *Bosmina*, leading to the replacement of the benthic cladoceran community by the pelagic one. In contrast, the taxa established post-eutrophication were affected more by predators than by nutrient levels. A decrease in planktivorous fish possibly allowed large *Daphnia* to establish, and the subsequent increase in planktivorous fish reduced the body size of the cladoceran community.

Introduction

Community assembly and long-term dynamics are central topics in ecology. Most lakes are formed under oligotrophic conditions, and eutrophication gradually occurs even without human activity (Sakamoto, 1973; USEPA, 2009). Increasing nutrient levels have important effects on the succession of lakes and their ecosystems. In a lake ecosystem, long-term succession with increasing nutrient levels has been researched through long-term observations comparing multiple lakes (Vadeboncoeur et al., 2003; Vadeboncoeur, Lodge, & Carpenter, 2001) and paleolimnological analysis that reconstruct long-term changes using lake sediments and subfossils (Moss, 1979; reviewed in Davidson & Jeppesen, 2013). These previous studies mainly focused on the changes in lake ecosystems from recent industrial eutrophication, which has been rapidly occurred on a global scale from the 1950s–1960s (Schindler, 2006) and has become a major environmental problem affecting water systems (USEPA, 2009; Smith & Schindler, 2009). Eutrophication can cause the dominant primary producers to change from submerged plants to pelagic algae, leading to shifts in the principal location of primary production from the benthic to the pelagic zone (Moss, 1979; Vadeboncoeur et al., 2001, 2003; Sayer et al., 2010). In addition, excess nutrients cause changes in fish communities, and an increase in total phosphorus (TP) leads to an increased relative abundance of planktivorous fish to piscivorous fish, resulting in the intensification of predation risk to zooplankton (Jeppesen et al., 1997).

Since cladocerans are intermediate consumers in lake food webs, they constitute an important group that links primary producers with high-order consumers, and the cladoceran community is affected by both bottom-up and top-down effects (Carpenter and Kitchell, 1993). Some parts of the cladoceran body (e.g., post-abdominal-claw, carapace, and head-shield) are composed of chitin, and they are well preserved over the long-term from one to several thousand years (Szeroczyńska, 1991; Korhola & Rautio, 2001). Thus, cladoceran community dynamics affected by eutrophication were analyzed by the paleolimnological method comparing the layers of lake sediment before and after eutrophication had occurred (e.g., Taylor, 2006; Bennion et al., 2015; Ohtsuki et al., 2015 and more). This paleolimnological analysis and other long-term observations revealed the following changes in the cladoceran community caused by eutrophication. First, the cladoceran community changed from a benthic to a pelagic community by sensitively responding to the benthic to pelagic shift in primary production (Taylor, 2006; Davidson

et al., 2011; Jeppesen et al., 2011; Bennion et al., 2015). Second, the relative importance of top-down effects increased compared with bottom-up effects on the cladoceran community (McQueen, Post, & Mills, 1986) through predation risk from planktivorous fish, which increased with eutrophication (Jeppesen et al., 1997; Davidson & Jeppesen, 2013). This resulted in the body size of cladocerans decreasing (Jeppesen, Leavitt, De Meester, & Jensen, 2001).

Based on these previous studies, I proposed a hypothesis about sequence cladoceran community dynamics from the early stage of lake formation. In the early stage of lake formation, when the nutrient condition is oligotrophic, benthic cladocerans are introduced and form a community. As eutrophication proceeds, this benthic community is replaced by a pelagic community. Further increases in nutrient levels release the cladoceran community from bottom-up control and allow high-order consumers to become established. As a result, the importance of top-down effects on the cladoceran community increases significantly.

However, continuous community succession and changes in the relative effect of bottom-up and top-down effects from an early stage of cladoceran community assembly during a lake formation period have rarely been studied; therefore, few previous studies have tested this hypothesis. Furthermore, only a few previous studies have observed the early stage of cladoceran community assembly in newly formed lakes (e.g., Allen, Thum, Vandyke, & Cáceres, 2012). This scarcity is due to difficulties in long-term observations of lake systems. Paleolimnology has solved such difficulties by allowing the reconstruction of past change (Smol, 2010; Douglas 2013). Prior paleolimnological studies that analyzed long-term cladoceran community change and related factors can broadly be divided into two types based on their observation periods. One major type of study compared cladoceran communities between before and after a marked environmental change (e.g., eutrophication: Davidson et al., 2011; acidification: Nevalainen, Sarmaja-Korjonen, Gąsiorowski, & Luoto, 2011; industrial development: Nevalainen, Luoto, Levine, & Manca, 2011; artificial fish introduction: Strock, Saros, Simon, McGowan, & Kinnison, 2013; reduction of Ca and introduction of copper sulfate: Korosi & Smol, 2012). Most of these studies observed changes over less than a 100-year period. Second, some studies have examined changes over thousands of years. For example, a study examined the response of a cladoceran community to climate change in the Holocene until 4,500 cal BP (Nevalainen, Rantala, & Luoto, 2015); another study examined the effects of human activity on a cladoceran community over more than

10,000 years, dating back to the prehistoric period (Szerocynska, 1991). However, lakes from which sediment core samples including the lake formation period can be taken are very rare, and most lakes that preserve high-quality sediment cores are ancient. Thus, even those studies investigating changes over 10,000 years have not analyzed sediments from the lake formation period.

Previous studies analyzed cladoceran community formation by observing the cladoceran community in a water column for three years (Louette, De Meester, & Declerck, 2008) and by examining sediment core samples from a new artificial lake which formed only decades ago (e.g., Allen et al., 2011). However, these studies could not investigate the long-term changes in the controlling factors of environmental effects due to the short research periods. On the other hand, other studies have compared cladoceran communities at several time points over a long-term eutrophication process (e.g., Straile & Geller, 1998). Such studies can evaluate the cladoceran community response to eutrophication but cannot assess changes that occurred in the early stage of community formation. In addition, although some previous studies have examined the temporal transition from bottom-up to top-down effects on cladoceran communities using a paleolimnological method, these studies did not examine effects on entire cladoceran communities. For example, Perga, Desmet, Enters, and Reyss (2010) examined bottom-up and top-down effects on only *Daphnia* and *Bosmina*, and Nevalainen, Luoto, and Pfrender (2017) assessed the effects on only one functional group of cladoceran.

In this study, I successfully collected lake sediment cores that included the lake formation period and analyzed changes within a cladoceran community from the early stage of lake formation via paleolimnological analysis. Our study site was Lake Fukami-ike in Nagano Prefecture, Japan. Lake Fukami-ike is shallow and surrounded by mountains (maximum depth: 7.8 m; Yagi et al., 2009) with anoxic hypolimnion from April to November (Yagi, Shimodaira, Terai, & Saijo, 1983); thus, there is little disturbance from winds and benthos. Varved sediment core samples had been collected in prior studies on this lake (Kawakami, Matsuo, Kato, & Fukusawa, 2004). Varved sediments comprise annual coupled layers forming annual lamina and allowed us to observe high-resolution temporal changes (Lamoureux 2001). Therefore, sediments from Lake Fukami-ike allowed us to analyze the high-resolution temporal changes from the lake formation period. Thus, in the present study, I attempted to reconstruct the

continuous succession process of a cladoceran community from lake formation to test whether the assumed change in bottom-up and top-down effects had occurred.

Materials and Methods

Sediment core sampling

Lake Fukami-ike (35°19'N, 137°49'E) is located in Anan town, Nagano Prefecture, Japan (Figure1-2). The lake is naturally formed and currently eutrophic with a maximum depth of 7.8 m and a surface area of 2.2 ha (Yagi et al., 2009). The lake was formed by a landslide triggered by an earthquake in 1662 (Table 2-1, Ueno 1952), and environmental archaeologists have succeeded in collecting sediment core samples that included the layer indicating the 1662 earthquake (Yamada et al., unpublished). A total of five lake sediment cores were collected from around the center of the lake on October 6 and 7, 2016 (Figure1-2). Two short sediment cores (Figure1-3), each ~35–42 cm long, were collected using a gravity corer (Limnos corer; Kansanen, Jaakkola, Kulmala, & Suutarinen, 1991) with an internal diameter of 93 mm. Three long sediment cores (Figure1-3), each ~306–360 cm long, were collected using a Mackereth corer (Mackereth, 1958) with an internal diameter of 65 mm. The two short and three long cores were sliced at 3-cm and 10-cm intervals, respectively. Then, these sliced samples were stored at 4 °C in the dark. Each sample was mixed well, and then 1 cm³ was measured for the wet weight (WW). Then, 1 cm³ was dried at 60 °C for 48 h to measure dry weight (DW). The ratio of WW to DW was calculated for each sample.

Our sediment core samples were dated (Figure1-3) based on their correlation with sediment core samples dated via ¹⁴C dating and annual lamination counting in previous studies (Ishihara et al., 2003; Kawakami et al., 2004) and the sedimentation rate calculated in previous studies (Kawakami et al., 2004; Yagi et al., 2009). The previously analyzed sediment cores and my working cores were correlated using lithological tie points, and the layers that represent the different events that were dated in previous studies (Table 2-1, Ishihara et al., 2003; Kawakami et al., 2004). I decided the correlation between long-cores and short-cores based on the shared layers (19 cm of Lim1, 21 cm of Lim2 and 25 cm of Mac2; 23 cm of Lim1, 25 cm of Lim2 and 28 cm of Mac2; 37 cm of Lim2, and 45 cm of Mac2; Figure1-3). The sedimentation rate (cm year⁻¹) was calculated based on the dates estimated for the sediment layers and the thickness of the sediments between the dated layers. I integrated the information from subfossils,

nutrients, and fossil pigments of each core sample by smoothing (see below).

Nutrient and fossil pigment determination

I measured the concentration of total phosphorus (TP) and fossil pigment, chlorophyll-a (Chl.a) and its derivative pheophytin-a (Pheo.a), in every layer to estimate the nutrient increase process and test the bottom-up effects on the cladoceran community. While testing the Chl.a, as it might possibly be degraded in the sediment, I also measured Pheo.a. Both Chl.a and Pheo.a are commonly found in all algal taxa and were preserved in the sediments; therefore, I used them as proxies of phytoplankton abundance (Leavitt & Hodgson, 2002). The concentration of TP was measured using the molybdenum blue method (Murphy & Riley, 1962) after oxidization. Briefly, I weighed 0.5 g (WW) of sediment from each layer and dissolved it in 10 ml distilled water, then oxidized it with persulfate at 120 °C for 60 min. After centrifugation (2000 rpm, 5 min), I separated the supernatant and measured absorbance at 880 nm to determine the concentration of TP with the calibration curve. The concentrations of Chl.a and Pheo.a were measured using the Lorenzen method (Lorenzen, 1967). I weighed 0.5 g (WW) of sediment from each layer and added 10 ml acetone; then I mixed them in an ultrasonic bath. After leaving the samples for 24 h at room temperature in the dark, I centrifuged them (2000 rpm, 10 min). Then, I separated the supernatant and measured absorbance at 750 nm and 665 nm. I measured absorbance in the same way after the addition of two drops of 1 N hydrochloric acid. The concentration of Chl.a and Pheo.a was then calculated based on these absorbances. Concentration fluxes of TP, Chl.a, and Pheo.a ($\mu\text{g cm}^{-2} \text{ year}^{-1}$) were calculated from the concentration of TP, Chl.a, and Pheo.a ($\mu\text{g g WW}^{-1}$) according to Kerfoot, Robbins, & Weider (1999). I used the sum of Chl.a and Pheo.a fluxes as an indicator of phytoplankton abundance. As Chl.a flux was high when a peak of TP was observed (between period I and period II, Figure 2-1), the degradation of fossil pigments was assumed to be sufficiently negligible to reconstruct the long-term changes.

Sediment P concentration may not reflect the P concentration in water due to sedimentary P mobility, causing sediment P to migrate to the sediment core surface influenced by redox chemistry and dissolved oxygen (Ginn et al., 2012). However, I observed the peak of TP not only near the surface but also in the middle of the sediment core

(Figure 2-1). In addition, Chl.a + Pheo.a concentration, a proxy of phytoplankton abundance, showed similar peaks to TP (Figure 2-1). These peaks indicated that sedimentary TP concentration in this study probably exhibit a long-term trend in bio-available TP, even if they do not reflect the absolute concentration of TP in the water column.

Subfossils

I counted the cladoceran subfossils preserved in each sediment layer to examine the structure of the cladoceran community. I also counted chironomid subfossils as the major benthic invertebrates. In addition, I counted subfossils of *Chaoborus* larvae, selectively predated by fish, as a proxy for the abundance of planktivorous fish (Sweetman & Smol, 2006; Palm et al., 2011) to test top-down effects on cladocerans. The *Chaoborus* specimens found in my samples were likely *C. flavicans* based on their morphology (Sweetman & Smol, 2006) and previous research on the studied lake (Nagano, Yagi, & Yoshida, 2014). Although *C. flavicans* can coexist with planktivorous fish as they exhibit diel-vertical migration as a defensive strategy against fish predation, a previous study reported that *C. flavicans* tended to be more abundant in a fish-less lake (Sweetman & Smol, 2006), suggesting that the abundance of *C. flavicans* could reflect the degree of predation by planktivorous fish. Subfossils were counted according to the method of Korhola and Rautio (2001). Briefly, 1 g WW of sediment from each layer of all core samples was extracted and dissolved in 50 ml distilled water. Inorganic particles did not prevent observation, so I did not remove them. Then, 1-ml of the 50-ml sample was put into a 1-ml Sedwick-Rafter chamber, and subfossils were counted twice (i.e., 2 ml in total) at a 200× magnification using an Olympus CX41 microscope. I counted and identified first 200 subfossils per layer and then confirmed whether other species were present in the rest of the 2-ml subsamples. If there were < 200, then I counted all subfossils in the 2-ml subsamples. The cladoceran subfossils were identified with reference to the literature (Sweetman & Smol, 2006; Korosi & Smol, 2012a, b; Tanaka & Makita, 2017), as were *Chaoborus* (Walker 2001; Sweetman & Smol, 2006).

For the cladocerans identified as *Daphnia*, I further identified species based on the morphology of the post-abdominal-claw. I detected two types of post-abdominal-claws: one with distinct teeth and one without pectens. I also found two morphological types of ephippia that originated from *Daphnia pulex* and *Daphnia*

ambigua in the same sediment core samples, and I confirmed that ephippia of *D. pulex* have mitochondrial DNA of *D. pulex* (Otake in prep). Furthermore, previous studies have reported *D. pulex* and *D. ambigua* in Lake Fukami-ike (So et al., 2015, Nagano & Yoshida, 2020). Thus, I identified the post abdominal-claw with distinct teeth as the *D. pulex* and those without pectens as the *D. ambigua*. If multiple body parts of one species were observed (i.e., headshield and carapace of *Bosmina longirostris*), I counted these separately, and then the body part that was observed the most was used. The number of subfossils in each layer was converted to the number per g DW using the ratio of WW to DW of the sediment measured separately. Then, the flux of sedimented subfossils per year (number cm⁻² year⁻¹) was calculated as an index of the abundance of each species in each year using the sediment mass flux (g DW cm⁻² year⁻¹) following Kerfoot et al., (1999). For statistical analysis, I used only the species that accounted for >1% of the total number of cladocerans in at least one layer, as in Korhola (1999). In addition, I calculated the ratio of pelagic to benthic species subfossils in each layer to determine the succession from a benthic to a pelagic cladoceran community.

I attempted to assess the abundance of cyclopoid copepods that were not well preserved in the lake sediments owing to their soft carapace using a method based on the ratio of defensive head-carapace morphology of *Bosmina* according to Korosi, Kurek, and Smol (2013). The specimens of *Bosmina* found in my samples were most likely to be identified as *B. longirostris* based on their morphology and the previous research on the studied lake (Ueno, 1952; Tanaka, 1992; Suda, Tanaka, Nobori, & Yagi, 2016). *Bosmina longirostris* can exhibit morphological defenses against cyclopoid copepods. When cyclopoid copepods are present, *B. longirostris* exhibits long antennules (pellucida-type), but when the copepods are absent, the antennules are curved (cornuta-type) (Sakamoto, Chang, & Hanazato, 2007). Thus, I can estimate the abundance of copepods from the subfossils of *B. longirostris*. I counted the subfossils of *Bosmina* by identifying the antennule, defensive pellucida, or non-defensive cornuta types. Then, I calculated the ratio of defensive pellucida-type antennules to the total *B. longirostris* antennules for each layer of three sediment core samples (Lim1, Mac1, and Mac2).

Post-abdominal-claw length (PCL) of *Daphnia* species reflects body size (Hrbáček, 1969), and *Daphnia* body size decreases as the predation pressure from planktivorous fish increases (Jeppesen et al., 2002). Thus, PCL

increases as predation pressure from planktivorous fish decreases (Amsinck, Jeppesen, & Landkildehus, 2005; Perga, Desmet, Enters, & Reyss, 2010). The PCL of *Daphnia* community was measured for each layer of two sediment core samples (Lim2 and Mac3) as an index of fish predation pressure following Korosi, Jeziorski, and Smol (2011), using photos taken by a digital camera (ARTCAM-130MI) at 200× magnification (n = 1097).

Statistical analysis

Although I used all data from all core samples for the following analyses of the cladoceran community and phytoplankton pigment, I omitted the samples Lim1 and Lim2, in which a sampling error occurred, for the measurement of TP concentration in the following analysis of TP. To determine the long-term dynamics of the cladoceran community, TP, and phytoplankton pigment concentration, I smoothed the flux of cladoceran subfossils, TP concentration, and Chl.a + Pheo.a concentration by LOESS smoothing with the `qplot` and `stat_smooth` functions of the R package “ggplot2” (Wickham, 2016). I then examined the main changes or shifts in cladoceran community assemblages and trophic conditions using the Constrained Incremental Sum of Squares cluster analysis (CONISS, Grimm, 1987), with the broken stick model to assess the significance of CONISS-delineated zones (Bennett, 1996). I conducted CONISS on all cladoceran subfossil data. These analyses were performed with the R packages `vegan` (Oksanen et al., 2017) and `rioja` (Juggins 2017).

I used Multivariate Auto-Regressive models (MARs) (Ives, Dennis, Cottingham, & Carpenter, 2003) to evaluate whether changes in the cladoceran community could be caused by biotic interactions and eutrophication associated with changes in TP and phytoplankton pigment concentration. This analysis was performed using the R package `MAR1` (Scheef, 2015). I used the fluxes of seven cladoceran subfossils as variables in the MAR model and added the TP and Chl.a + Pheo.a fluxes as exogenous co-variables to assess the effect of eutrophication. In addition, to evaluate the effect of shifts in predators, I added the *Chaoborus* subfossil flux as a variable as I had done with the cladoceran subfossil fluxes.

First, I prepared the dataset using the “prepare data” function. I replaced the data of 0 flux as 1 and then log-transformed all data. I standardized all data to have equal means and deviations for comparing between taxa. I fitted

these data to the MAR model (Ives et al., 2003) by generating all possible models. MAR model follows to below equation (2-1).

$$X_t = A + BX_{t-1} + CU_{t-1} + w_t \quad (2-1)$$

where X_t is the $p \times 1$ vector of flux if each of the p species at time t , A is the $p \times 1$ vector of intrinsic growth rate of each p species, B is a $p \times p$ matrix of b_{ij} meaning the effect of the flux of species j on the per capita growth rate of species i , U_{t-1} is the $q \times 1$ vector of covariate values at time $t-1$, C is the $p \times q$ matrix of c_{ij} which describe the effect of covariate j on species i , w_t is the process errors during the change from time $t-1$ to time t described as the $p \times 1$ vector of elements randomly sampled from a multivariate normal distribution with a mean of 0 and covariate matrix S .

And then selecting the best-fit model as the one with the lowest Akaike's Information Criteria (AIC). Then, I used bootstrapping ($n = 500$) on the best-fit model to obtain 95% Confidence Interval (CI) for the coefficients, which describe the degree and direction of the effects of each species and covariables on each species, in the model. Finally, I calculated the conditional R^2 for each taxon to evaluate the model's ability to predict the temporal changes in abundance. Model selection and estimation were performed using the "run.mar" function.

In addition, I examined the differences in *Daphnia* PCL between the periods using ANOVA and the post-hoc comparison with the Tukey-Kramer test. All statistical analyses were performed using R version 3.5.2 (R Core Team 2017), and significance was considered at $P < 0.05$.

Results

Constrained clustering analysis CONISS (Grimm, 1987) was carried out on all cladoceran data. Seven periods were significantly identified for the cladoceran community dynamics (Figure 2-1, Figure S2-1): 1662 to early 1880 (period I), 1880 to early 1950 (period II), early 1950 to early 1980 (period III), early 1980 to early 2000 (period IV), early 2000 to mid-2000 (period V), mid-2000 to around 2014 (period VI), and around 2014 to 2016 (period

VII).

Briefly, the long-term dynamics of the cladoceran community suggested by the subfossils were as follows.

First, the cladoceran community consisted only of benthic species in period I when both TP and Chl.a + Pheo.a were low. Then, small pelagic species of *Bosmina* appeared, and the ratio of pelagic taxa to benthic taxa increased in period II when the first peak of TP and Chl.a + Pheo.a. occurred. Then, the large cladocerans *Daphnia* appeared continuously from period V and increased in period VI when *Chaoborus*, which I used as a proxy of planktivorous fish, increased. Most recently, small *Bosmina* increased again in period VII when *Chaoborus* decreased.

In period I, the flux of cladoceran subfossils was entirely low. Benthic cladocerans, *Chydorus* and *Alona*, appeared and were continuously detected up to the surface of the sediments (i.e., 2016). In addition, before the cladoceran subfossils appeared, benthic chironomid larvae were observed. Until the later part of period I, TP flux and Chl.a + Pheo.a flux were low. From the end of period I to period II, small pelagic *Bosmina* appeared and increased. At this stage the ratio of pelagic species to the total number of cladocerans began to increase (Figure 2-1, Figure S2-2). By the end of period II, *D. ambigua* was periodically but not continuously detected. Total phosphorus and Chl.a + Pheo.a fluxes were high at the beginning of period II but decreased by the end

From period III to period IV, *Bosmina* further increased. Even though benthic *Chydorus* and *Alona* also increased, the proportion of pelagic species was still much higher than that of benthic species. Large cladocerans, such as the *D. pulex* and *D. ambigua*, occurred only in some layers in period III and not continuously. At the beginning of period III, the flux of TP and Chl.a + Pheo.a increased again. From period IV to period V, the cladoceran community became diverse, and *D. ambigua*, *D. pulex* and *Ceriodaphnia* were continuously detected. Large cladocerans, *D. ambigua* and *D. pulex*, increased whereas small *Bosmina* decreased during this time. The flux of TP and Chl.a + Pheo.a increased and peaked during this time. After that, the flux of TP and Chl.a + Pheo.a remained at a high level up to the surface of the sediments (i.e., 2016).

In period VI, the two *Daphnia* peaked, although they tended to decrease at the end of the period. Conversely, small cladocerans, *Bosmina* and *Ceriodaphnia*, increased towards the end of period VI and period VII. In period VII, the large *Daphnia* continuously decreased (Figure 2-1, Figure S2-2).

Chaoborus larvae, an indicator of fish abundance, increased from the latter part of period IV to period V and decreased from period VI to period VII (Figure 2-1). This result indicated that planktivorous fish decreased from period IV to V and increased from period VI to period VII.

The ratio of the defended type (i.e., pellucida-type) antennules of *B. longirostris*, a proxy of copepod community change, also showed long-term changes (Figure 2-2). Cornuta-type, which is the non-defended type, was more abundant from period II to period IV. Conversely, the layers in which the ratio of defended type exceeded 0.5 began to be detected after period V, although they were also observed in period I when the abundance of this species was very low (probably due to sampling error associated with low abundance).

Daphnia PCL was significantly different between CONISS periods based on ANOVA results ($P < 0.001$, Figure 2-3). The post-hoc multiple comparison with the Tukey-Kramer test indicated that there were significant differences between periods VI and VII and periods IV and V ($P < 0.001$). In periods VI and VII, the PCL of *Daphnia* was shorter than that in periods IV and V.

The results of MAR indicated the following regarding the factors affecting the cladoceran community (Figure 2-4, Table 2-2). Chl.a + Pheo.a flux had a significant positive effect on *Bosmina* flux, and TP flux had a significant positive effect on both *Bosmina* flux and *Chydorus* flux. *Chaoborus* larvae positively affected the *D. ambigua* flux and *D. pulex* flux, whereas the negative effect of this predator on *Bosmina* flux was not significant, indicating that planktivorous fish negatively affected large *Daphnia* and but not small *Bosmina*.

Discussion

In this study, I revealed the long-term dynamics of the cladoceran community from lake formation onward and the change in both bottom-up and top-down effects on the cladoceran community. These results supported the hypothesis based on the previous studies about assembly and dynamics of the cladoceran community and temporal changes in controlling mechanisms as nutrient levels increase, including the replacement of the benthic with the pelagic community due to eutrophication (Taylor, 2006; Davidson et al., 2011; Jeppesen et al., 2011; Bennion et al., 2015) and the increased importance of top-down effects with eutrophication (McQueen, Post, & Mills, 1986;

Jeppesen et al., 1997; Davidson & Jeppesen, 2013). The cladoceran community consisted of benthic species under oligotrophic conditions in the lake formation period, which were replaced by pelagic species due to rapid eutrophication. These dynamics must be mainly controlled by bottom-up effects. Under conditions in which nutrient levels became sufficiently high after rapid eutrophication, large *Daphnia* became established in the later part of period IV. In addition, high-order consumers also became established at that time. As a result, the cladoceran community was mainly controlled by top-down effects, initially affecting the establishment of large *Daphnia* and latter reducing cladocerans' body size.

Bottom-up effects on cladocerans during the early periods

In the early stage of lake formation, TP and Chl.a + Pheo.a were low (Figure 2-1–period I), indicating that the lake was oligotrophic. In this period, only benthic cladocerans, *Chydorus* and *Alona*, were present, and the ratio of pelagic to benthic taxa was continuously low (Figure 2-1). In addition, benthic chironomids had already appeared (Figure 2-1). These results suggest that the benthic cladoceran community dominated under oligotrophic conditions in which the main primary producers were not phytoplankton (Taylor, 2006; Davidson et al., 2011; Jeppesen et al., 2011; Bennion et al., 2015). This finding supports the hypothesis that the benthic community first assembled during the early stages of the cladoceran community.

The present study also showed the cladoceran community changing from a benthic to a pelagic community in response to the phytoplankton becoming the dominant primary producer with increasing nutrient level (Taylor, 2006; Davidson et al., 2011; Jeppesen et al., 2011; Bennion et al., 2015). In Lake Fukami-ike, rapid eutrophication has occurred twice (Figure 2-1). Fluxes of TP and Chl.a + Pheo.a increased around 1850 and 1950–1960, indicating eutrophication of the lake and an increase in phytoplankton. When eutrophication occurred around 1850, small pelagic *Bosmina* appeared and increased, eventually becoming dominant in the cladoceran community (Figure 2-1). This led to the replacement of the benthic cladoceran community with the pelagic community in the transition from period I to period II, which was revealed by the CONISS analysis (Figure 2-1). The first rapid eutrophication event could have caused these changes in the cladoceran community. The results of the MAR model

were consistent with this; both the TP and Chl.a + Pheo.a fluxes positively affected *Bosmina* (Figure 2-4). This result agrees with those of previous studies reporting that *B. longirostris* increased as eutrophication proceeded (Gašiorowski & Szeroczyńska, 2004; Ohtsuki et al., 2015). Furthermore, pelagic *Bosmina* did not have a negative effect on the benthic *Chydorus* and *Alona* according to the results of the MAR model analysis (Figure 2-4). Therefore, the replacement of the benthic community by the pelagic community seen in my study was possibly not due to competitive interactions with benthic species but rather to changes in primary producers, which both the pelagic and benthic taxa use.

Previous studies suggesting that the replacement by the pelagic community was due to eutrophication analyzed the change in the community before and after industrial eutrophication around 1950–1970 (Taylor, 2006; Davidson et al., 2011; Jeppesen et al., 2011; Bennion et al., 2015). In my study suggested that such replacement by the pelagic community occurred during the first rapid eutrophication event around 1850 before the second rapid eutrophication event, which was similar to those occurred in lakes worldwide (Schindler, 2006; Sakamoto, 1973; Saijo & Mitamura, 2016) (Figure 2-1). The first eutrophication was possibly caused by extensive agricultural activity combined with a natural disaster.

The land around Lake Fukami-ike was most likely used as mulberry and rice fields in 1850 as shown in the earliest map of the area, published in 1908 (Topographic Map 50000 Geospatial Information Authority of Japan, 1908). In Anan town, chemical fertilizers have been widely used since the 1970s (Record of Anan Compilation Committee, 1987). Before the 1970s, plant ash was used as a fertilizer, and thus many grass fields were maintained to grow material for fertilizer, and wild fields were burned to generate ash (Record of Anan Compilation Committee, 1987). These fertilizers may have been used to treat the mulberry and rice fields surrounding the lake. In 1850, a severe flood, called the “Dai-mansui” flood, occurred around Lake Fukami-ike (Matsushima, 2000). The layers representing this event were formed by the inflow of sediment from the surrounding area due to heavy rain (Figure 1-3, Kawasaki et al., 2004). Thus, eutrophication could have occurred through the fertilized soil flowing into the lake. Furthermore, nutrients accumulated in the lake sediments could be used by phytoplankton through biological decomposition (Schindler, 2006; Keatley, Bennett, MacDonald, Taranu, & Gregory-Eaves, 2011); thus, a

single notable influx of nutrients due to the flood could have supported phytoplankton growth for some years after the flood. Therefore, my study suggests that rapid eutrophication, even if not the recent industrial eutrophication event due to the inflow of chemical fertilizer and sewage water, can cause the same replacement of the benthic cladoceran community by the pelagic community.

When the second rapid eutrophication event occurred around 1950, pre-established cladocerans: *Bosmina*, *Chydorus*, and *Alona* increased from period III to period IV (Figure 2-1). Like the replacement process, this increase in pre-established cladocerans could have occurred due to eutrophication. The MAR model analysis showed that TP positively affected *Chydorus* as well as *Bosmina* (Figure 2-4). The results agree with those of previous studies that showed more abundant *Chydorus spaericus* and *B. longirostris* with increasing nutrient concentration and biological production (Luoto, Nevalainen, & Sarmaja-Korjonen, 2008; Nevalainen & Luoto, 2013). Increasing nutrient concentration can promote zooplankton reproduction by increasing phytoplankton biomass (Vanni, 1987). The MAR model supported the effect of increased phytoplankton: Chl.a + Pheo.a positively affected *Bosmina* (Figure 2-4). In contrast, Chl.a + Pheo.a did not positively affect *Chydorus* (Figure 2-4). *Chydorus spaericus* can live in pelagic habitats as well as in littoral or benthic habitats (Freyer, 1968), as this species can associate with algal filaments (Freyer, 1968) and also feed on small phytoplankton (de Eyto & Irvine, 2001). Thus, *Chydorus* may have been influenced by increased TP with more production in benthic and pelagic habitats but not by Chl.a + Pheo.a, which represented only the pelagic food resources.

Top-down effects on cladocerans during the later periods

After increases in nutrient levels from two eutrophication events, the relative importance of the top-down effects on the cladoceran community increased in Lake Fukami-ike. After eutrophication occurred, the cladoceran community diversified from around 2000 and became dominated by small species in more recent periods. *Daphnia* and *Ceriodaphnia* were continuously detected from later in period IV and became established since period V. Large *Daphnia* decreased, and small *Bosmina* and *Ceriodaphnia* increased during the most recent periods VI and VII (Figure 2-1). Since period V, TP, and Chl.a + Pheo.a fluxes did not change much (Figure 2-1), and the MAR model

suggested that the cladoceran species, which became established in period V were not significantly affected by either TP or Chl.a + Pheo.a (Figure 2-4). In contrast, *Chaoborus* larvae, a proxy of planktivorous fish abundance (Sweetman et al., 2006; Palm et al., 2011), had a significant positive effect on the larger *D. pulex* and *D. ambigua*, which became established in period V (Figure 2-4). This result suggests that the planktivorous fish might have negatively affected *Daphnia* and that the change in the cladoceran community after period V might be relatively influenced by top-down effects rather than bottom-up effects.

The change in fish abundance, based on subfossils of *Chaoborus* larvae and the PCL of *Daphnia*, indicators of planktivorous fish abundance, supported this argument. The abundance of *Chaoborus* larvae and the PCL of *Daphnia* changed since the 2000s. When *Daphnia* increased from the end of period IV and period V, *Chaoborus* larvae also increased (Figure 2-1), and the PCL was longer than in periods VI and VII (Figure 2-3), suggesting that planktivorous fish were less abundant. In contrast, when small cladocerans were dominant from period VI to period VII, *Chaoborus* larvae decreased, and the PCL was shorter, suggesting that planktivorous fish were more abundant. These dynamics and the MAR model analysis indicated that the reduction in planktivorous fish had a positive effect on *Daphnia* (Figure 2-4), facilitating the sustainability of *Daphnia* populations and causing the diversification of the cladoceran community in periods IV and V. In contrast, the body size of the cladoceran community decreased because the increase in planktivorous fish reduced the *Daphnia* population and the small *Bosmina* increased from late in period VI to period VII. Increasing numbers of planktivorous fish might have reduced *Chaoborus* and released small cladocerans from predation risk, leading to the dominance of *Bosmina*. Positive effects of planktivorous fish on small *Bosmina* were also seen in the MAR model; *Chaoborus* larva negatively affected *Bosmina*, although the effect was not significant (Figure 2-4). While *Chaoborus* larvae are important predators of juvenile *Daphnia* (Havel & Dodson, 1984), my statistical analysis showed the effect of *Chaoborus* on *Daphnia* was positive (Figure 2-4). This suggests that the relative effect of predation from planktivorous fish on *Daphnia* could be greater than that from *Chaoborus*. These patterns in cladoceran and fish communities observed in Lake Fukami-ike agree with the size-efficiency hypothesis that planktivorous fish selectively prey on large cladocerans like *Daphnia*, and invertebrate predators like *Chaoborus* and they less selectively prey on small cladocerans like

Bosmina (Brooks & Dodson, 1965; Miner et al., 2012). Invertebrate predators selectively prey on small cladocerans over larger ones (Brooks & Dodson, 1965; Leavitt et al., 1994). These results agree with those of previous studies that reported that both *Chaoborus* larvae and *Daphnia* tended to appear and increase simultaneously (Kerfoot, 1981; Palm et al., 2011), and *B. longirostris* tended to increase when *Chaoborus* larvae disappeared (Luoto et al., 2008; Arcifa et al., 2015). In addition, the results of an enclosure experiment that introduced *Chaoborus* larvae and planktivorous fish were consistent with these changes (Hanazato & Yasuno, 1989).

The fish community dynamics suggested by the *Chaoborus* larvae and the PCL of *Daphnia* agree with the results of previous studies indicating that increases in TP finally lead to a decrease in the relative abundance of planktivorous fish to piscivorous fish (Jeppesen et al., 1997). Historical records of fish invasions and introductions from external sources (Table 2-1) have significant implications for the observed changes in the cladoceran community. *Hypomesus nipponensis*, a planktivorous fish that selectively preys on large zooplankton (Makino et al., 2001; Chang, Hanazato, Ueshima, & Tahara, 2005), was artificially introduced in 1960 (Tanaka, 1992). The presence of the piscivorous fish *Micropterus salmoides* was first recorded in 1974, and then *H. nipponensis* disappeared due to predation by *M. salmoides* (Shimoina Board of Education, 2009). In 2005, the omnivorous *Lepomis macrochirus*, which preys on zooplankton (Sakano & Yodo, 2004) was found in the lake (Kawanobe & Hosoe, 2007). After that, *L. macrochirus* increased greatly in abundance between 2005 and 2007 (Kawanobe & Hosoe, 2007), and has recently dominated the fish community of the lake (Takei, 2010).

In period III, when *H. nipponensis* was artificially introduced, *D. pulex* was first detected but immediately disappeared, indicating that it had failed to establish a sustainable population (Figure 2-1). Similarly, *D. ambigua* was detected in only one layer but not continuously during period III (Figure 2-1). In addition, *Chaoborus* larvae were found in only a few layers from that period (Figure 2-1). These results indicate high predation pressure from planktivorous fish on large zooplankton during period III. Then, after the invasion of piscivorous *M. salmoides*, *Chaoborus* larvae increased from the 1980s, most likely due to the release from *H. nipponensis* predation. *Daphnia* were also able to establish a sustainable population probably due to the decrease in *H. nipponensis* following *M. salmoides* predation. The changes in the cladoceran community recorded in my study agree with

those observed in previous studies that reported *Daphnia* becoming dominant after the introduction of piscivorous fish (Leavitt et al., 1994) and that the dominant taxon switched from *Daphnia* to *Bosmina* after the increase in planktivorous fish (Perga et al., 2010). Overall, my research demonstrated that changes in predator composition caused by human activity could significantly affect cladoceran community dynamics.

In the present study, *Daphnia* became established under conditions in which sufficient nutrients already existed in the lake after the second eutrophication (Figure 2-1). This result suggests that eutrophication relieves *Daphnia* from food limitations and that it could enhance the relative importance of top-down effects on *Daphnia* and the cladoceran community. Increased numbers of *Daphnia* under high-nutrient conditions were observed in some prior studies, including observation and mesocosm experiments (e.g., George, 2012; Declerck, Vanderstukken, Pals, & DeMeester, 2007). For example, Straile and Geller (1998) showed that *Daphnia* biomass increased under a change from oligotrophic to mesotrophic status and mesotrophic to eutrophic status by comparing *Daphnia* biomass during three periods in Lake Constance: oligotrophic (1920s), mesotrophic (1950s–1960s), and eutrophic (1980s–1990s).

In addition, I tried to examine the dynamics of cyclopoid copepods, predators of small zooplankton, based on the changes in *Bosmina* antennule type (Figure 2-2). During periods VI and VII, when small species dominated the cladoceran community, the ratio of defended (i.e., pellucida-type) *Bosmina* increased (Figure 2-2). This result suggests that cyclopoid copepod might have increased since period VI, supporting the argument that high nutrient levels can maintain the presence of high-level consumers.

Conclusion and Future Studies

I observed the long-term community dynamics of cladocerans from the time the lake was formed. Our results suggest that, under the early oligotrophic conditions, the cladoceran community consisted of a benthic community, which was replaced by a pelagic community due to eutrophication. Later, further eutrophication allowed high-order consumers to establish, and the cladoceran community might have become controlled by top-down effects. In the present study, I focused on cladocerans and evaluated phytoplankton change based on fossil pigments only. Thus, if

I could analyze the subfossils of other taxa, including phytoplankton, I would be able to reveal the mechanisms underlying the bottom-up and top-down effects in more detail. Ishihara et al. (2004) analyzed planktonic diatoms in both light-colored laminae and dark-colored laminae to determine whether each laminae couplet represented an annual varve in Lake Fukami-ike. However, since this previous study focused only on planktonic diatoms, I could not compare the eutrophication process indicated by diatoms and my TP and fossil pigment analyses or analyze the relationship between changes in the cladoceran and diatom communities in detail. However, Ishihara et al. (2004) found that *Aulacoseira* spp., reported by several studies as replacing and dominating benthic species under eutrophic conditions (e.g., Doig, Schiffer, & Liber, 2015), were abundant around 1890 (Ishihara, unpublished), which agrees with my finding that the first rapid eutrophication was occurring at that time. Analyzing the relationship between cladoceran and other taxa like phytoplankton and microbes is a topic for future study. Also, accumulating more paleolimnological data sets like those obtained by the present study should provide a more general understanding of the community succession process and its underlying mechanisms, which would be of much interest to researchers in Limnological ecology.

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Tables

Table 2-1. Events preserved in the sediment core as key layers and historical records of fish introduction to and invasion of Lake Fukami-ike

Year	Event	Fish introduction	Reference
1662	Lake formation		Ueno (1952), Kawakami <i>et al.</i> (2004)
1804	“Ne-no-mansui” flood		Matsushima (2000), Kawakami <i>et al.</i> (2004)
1850	“Dai-mansui” flood		Matsushima (2000), Kawakami <i>et al.</i> (2004)
1891	Noubi earthquake		Matsushima (2000), Kawakami <i>et al.</i> (2004)
1945	Nankai earthquake		Matsushima (2000), Kawakami <i>et al.</i> (2004)
1960		<i>Hypomesus nipponensis</i> , <i>Carassius sp.</i> and <i>Cyprinus carpio</i> Ire introduced	Tanaka (1992)
~1974		<i>Micropterus salmoides</i> invaded	Sakurai and Watanabe (1974)
~2005		<i>Lepomis macrochirus</i> invaded	Kawanobe and Hosoe (2007)

Table 2-2. R² of the best-fit multivariate autoregressive model for cladoceran community change in Lake Fukami-ike. R² calculated with the bootstrapped model indicates how well the model predicts changes in density of each species from one step to the next.

	<i>Response</i>							
	<i>Bosmina</i>	<i>Daphnia ambigua</i>	<i>Daphnia pulex</i>	<i>Ceriodaphnia</i>	<i>Chydorus</i>	<i>Alona</i>	<i>Camptocercus</i>	<i>Chaoborus</i>
R ²	0.64	0.91	0.91	0.85	0.90	0.49	0.58	0.58

Figures

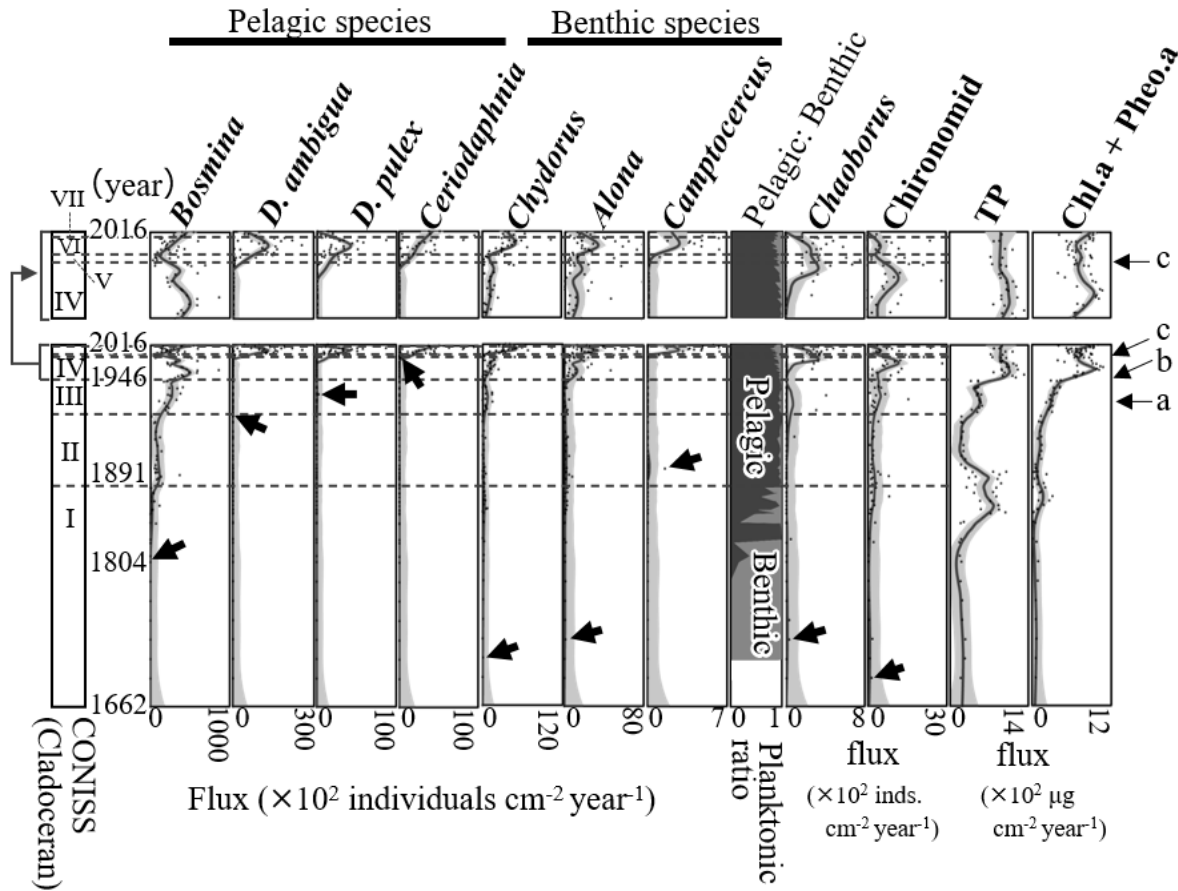


Figure 2-1. Dynamics of the cladoceran community and eutrophication process from the lake formation to the present. The lines represent the loess smoothed change in each species flux (span = 0.2) with 95% confidence intervals indicated by gray bands. Data points are the flux data of each species in each layer. Arrows indicate the year when each species first appeared in the sample. The far-left column shows the periods of cladoceran community dynamics determined by CONISS. Arrows labeled with a–c indicate the timing of fish introduction: artificial introduction of *Hypomesus nipponensis* in 1960 (a); introduction of *Micropterus salmonids* in c. 1974 (b); introduction of *Lepomis macrochirus* in c. 2005 (c).

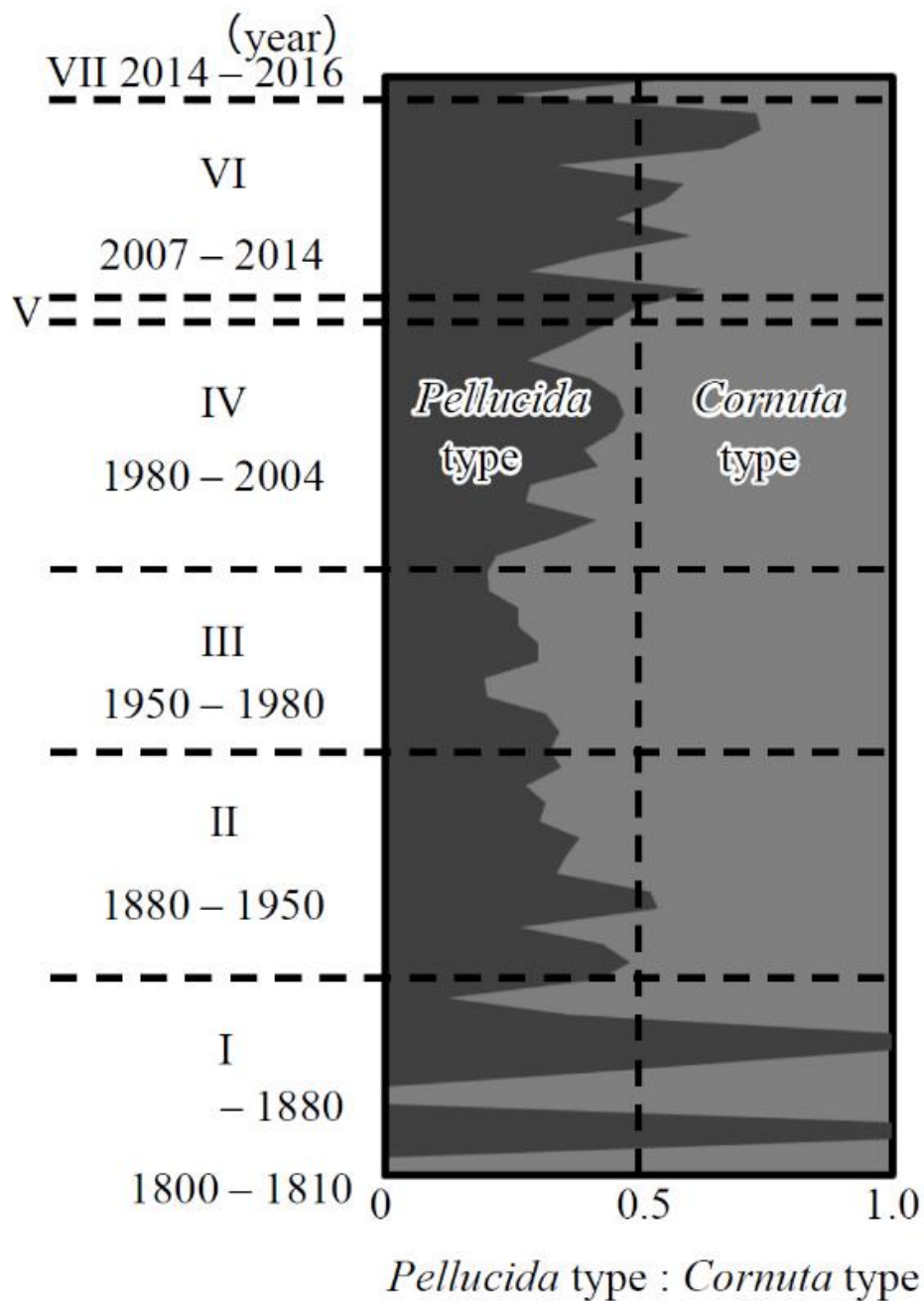


Figure 2-2. Changes in *Bosmina* antennule type shown as the ratio of pellucida-type individuals to cornuta-type individuals. The pellucida-type antennule (dark gray) is a defense trait against cyclopoid copepods, whereas the other cornuta-type (light gray) is a hooked antennule that cannot reduce predation risk from cyclopoid copepods.

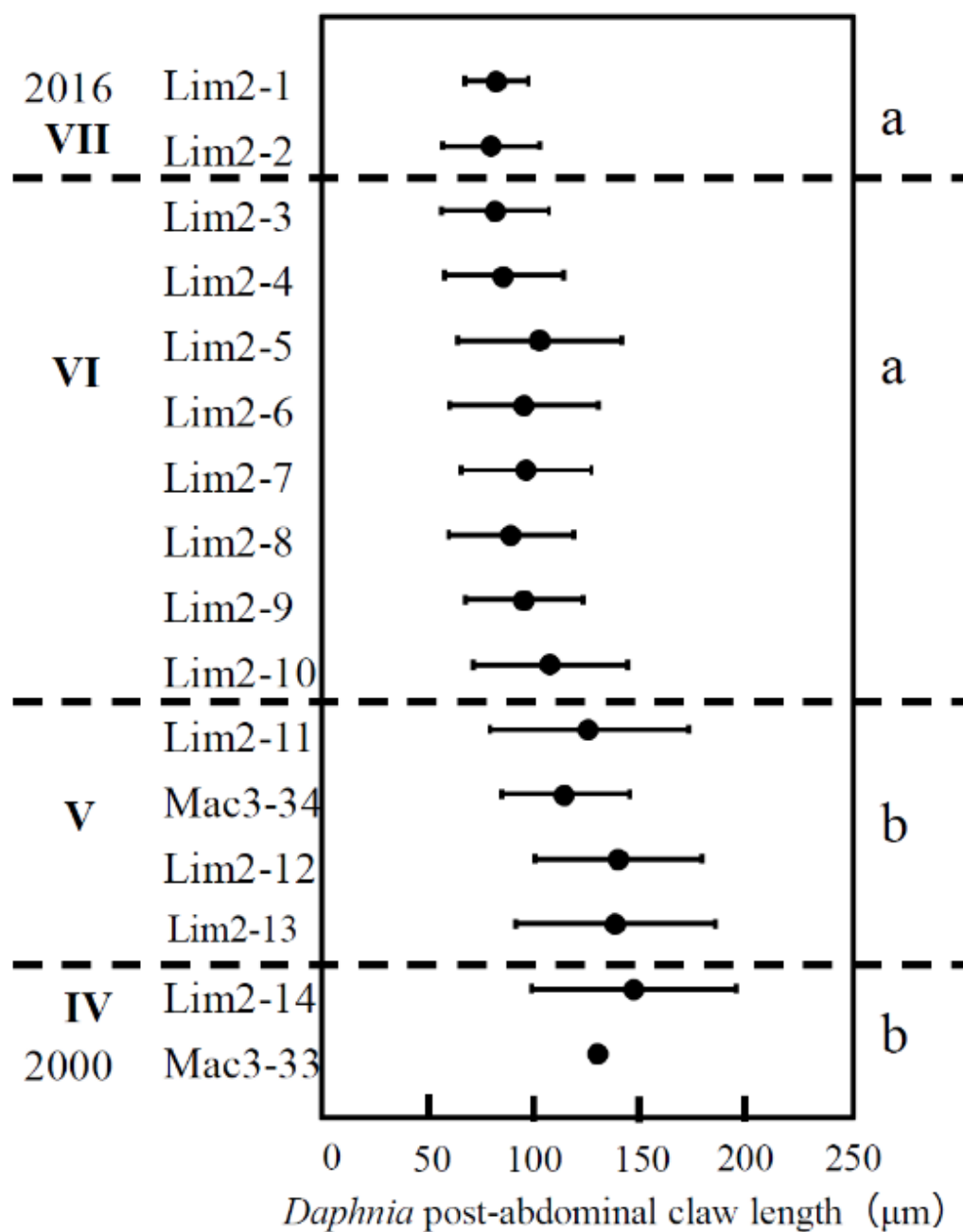


Figure 2-3. Changes in *Daphnia* post-abdominal-claw length ($n = 1097$). Each plot shows the average length of the post-abdominal-claw from all *Daphnia* species in each layer. The bars show SE. IV to VII indicate the period of cladoceran community dynamics determined by CONISS. “a” and “b” are the results of the post-hoc multiple comparison with the Tukey-Kramer test among CONISS periods (a-b: $P < 0.001$).

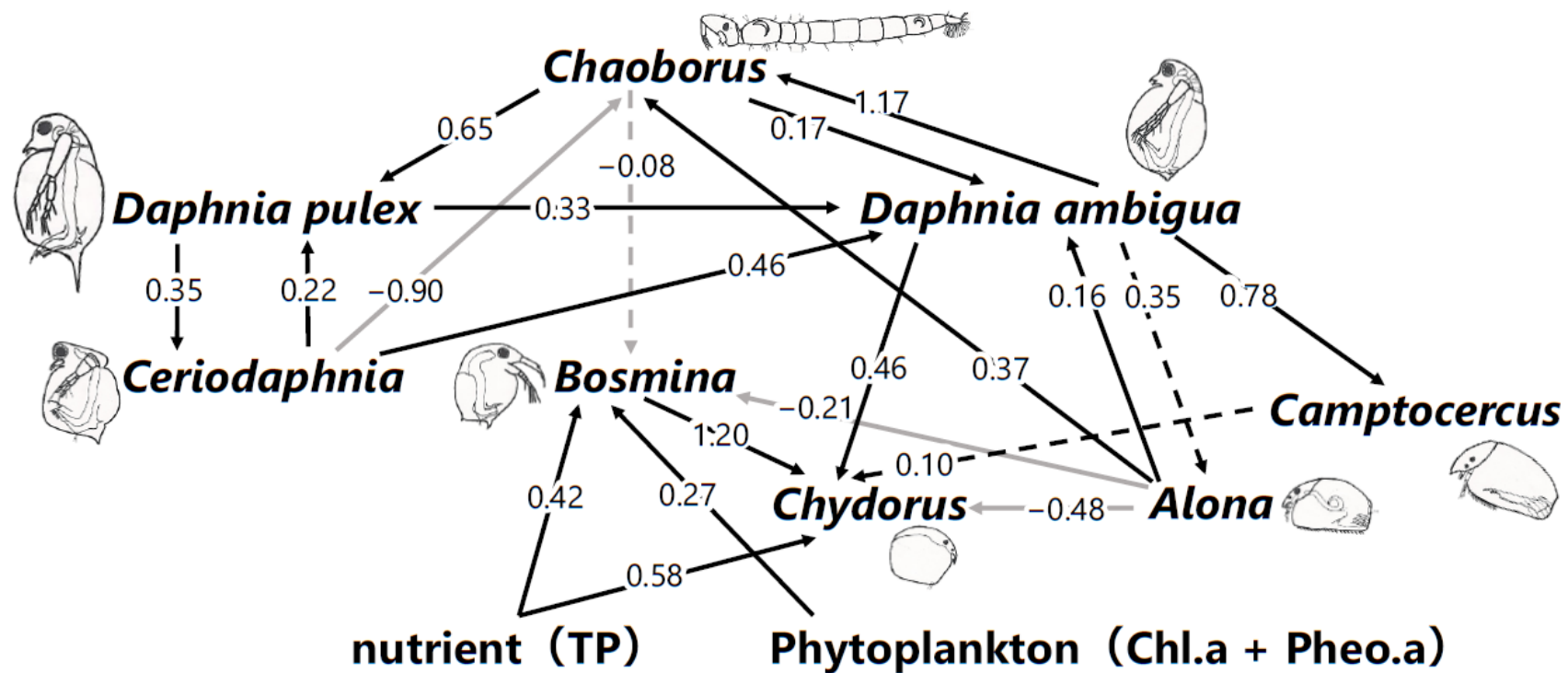


Figure 2-4. Bottom-up effects, top-down effects, and effects of inter-species interactions on the cladoceran community based on MAR model analysis. The coefficients are tested for significance by 500-step bootstrap analysis (significant in bold). Black arrows indicate positive coefficients, and gray arrows represent negative coefficients. Solid arrows indicate significant coefficients, and broken arrows indicate non-significant coefficients based on a bootstrap analysis.

Supporting Information

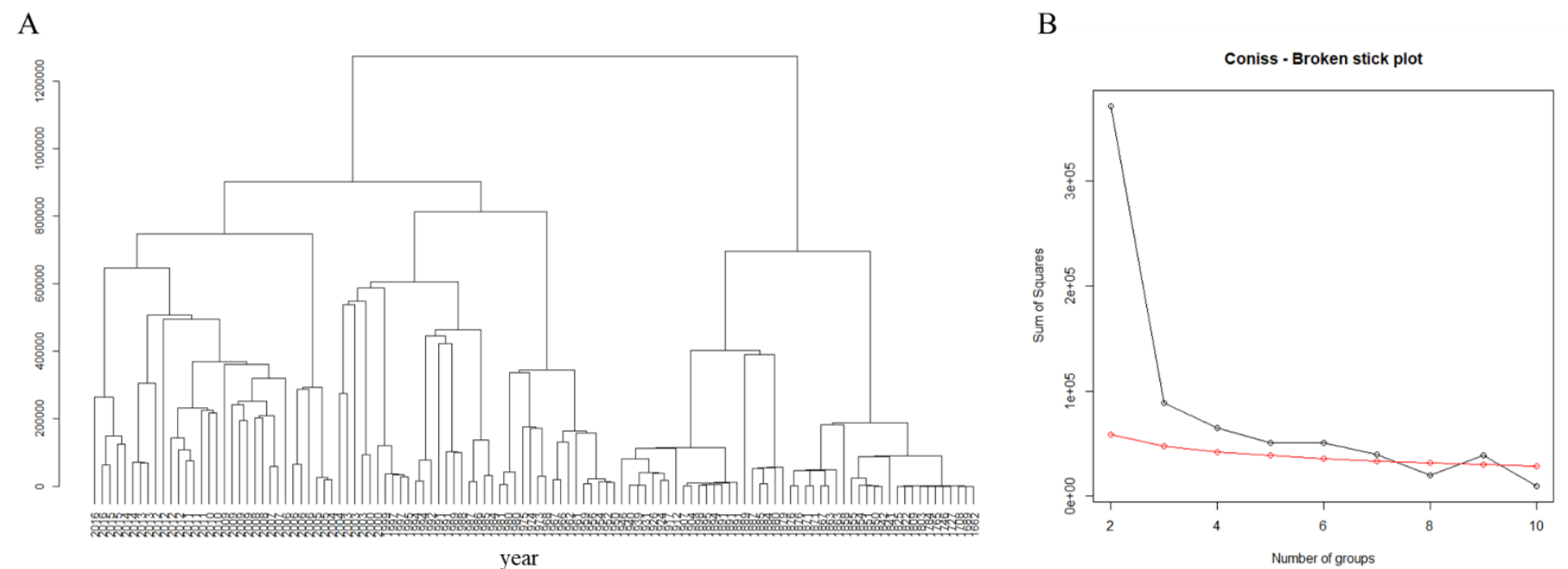


Figure S2-1. A. Cluster plot from the CONISS analysis for cladoceran community. B. Results of the broken stick model to determine the number of significant groups in CONISS analysis (Red line represents the sum of square with broken stick model. Black line represents the sum of square with CONISS analysis).

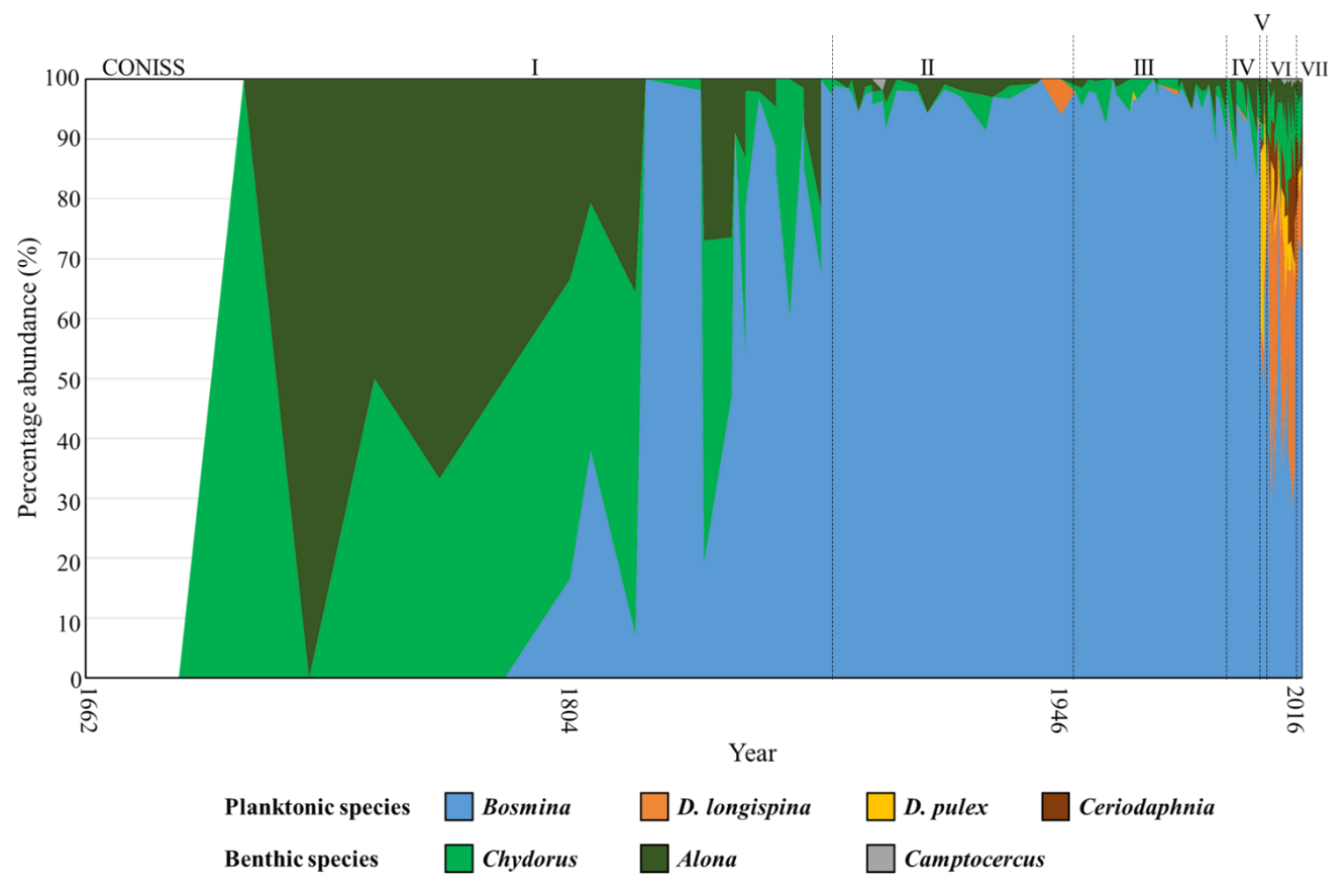


Figure S2-2. Percentage abundances of subfossil cladocerans among total fossil content. CONISS results are the same as in Figure 2-3.

Chapter 3 Genetic dynamics of obligate parthenogenetic *Daphnia pulex* during colonization process from early colonization in Lake Fukami-ike, Japan

Abstract

How genetic diversity and genetic structure change during the colonization process in novel habitats has been of interest in ecology, evolution, and invasion biology. How genetic change affects establishment possibility is controversial. While genetic dynamics at the initial introduction stage are considered to affect the colonization process, temporal observations from the initial stage are rare. The difficulty to conduct long-term observations with prior forecast of introduction occurrence is one of the major factors resulting in incomplete knowledge about genetic dynamics of the colonization process and the lack of knowledge about genetic dynamics from the initial stage. I revealed the long-term, about 13 years, genetic dynamics of the obligate parthenogenetic *Daphnia pulex* population during the entire colonization process from the initial introduction using diapausing eggs preserved in lake sediment. I collected diapausing eggs from each layer and genetically analyzed them with mitochondrial DNA markers. The abundance of ephippia suggested that *D. pulex* established a firm population in the early 2000s. Genetic analysis suggested that the *D. pulex* population was colonized and sustained with only one dominant haplotype and other rare haplotypes without any turnover. Haplotype richness and diversity indexes were low during the colonization process, suggesting that the genetic diversity was consistently low and there was no recovery of genetic diversity. Contrary to limited genetic diversity, morphological analysis in Chapter 4 suggested that adaptive phenotypic change occurred in response to changes in predator community. From these results, the biological population can be maintained long-term with limited genetic diversity. Obligate parthenogenetic taxa may be particularly succeeded to establish with limited genetic diversity.

Introduction

The mechanisms by which genetic diversity and structure change during the colonization process in novel habitats has been of interest in ecology, evolution, and invasive biology. Introduction to novel habitats has also been researched through natural experiments that allow us to test the mechanisms by which biological populations adapt and respond to environmental changes (Moran & Alexander, 2014). While many studies have approached the

colonization process of biological populations, our knowledge about the contribution of genetic diversity to the establishment and long-term maintenance of populations, and the change in genetic diversity through the colonization process have not yet been unified, especially in natural systems. For instance, while some research suggested that genetic diversity recovers during the colonization process (Most et al., 2015) and some suggest that genetic diversity of introduced population do not decrease (Roman & Darling, 2007), others suggested that a decrease in genetic diversity commonly occurs in a new population and that the diversity remains reduced for long-term (Dlugosch and Parker, 2008). Furthermore, some research suggests that reduced genetic diversity might not prevent immigrating populations from surviving in and adapting to a new environment because it does not decrease phenotypic variation (Dlugosch and Parker, 2008; Colautti and Lau, 2015). However, other research suggests that reduced genetic diversity has a negative effect on the ability of populations to establish and adapt (Kinziger, Nakamoto, Anderson, & Harvey, 2011; Signorile et al., 2014).

Apart from such controversial results, our knowledge is limited about the changes in genetic diversity and composition in the early stage after colonization and the high-resolution time series genetic dynamics during the colonization process in natural systems. Genetic dynamics and changes in genetic diversity in the early stage of colonization can continuously affect the colonization process. For instance, the order of arrival of genotypes can affect genetic structure, which can affect the genetic priority effect (De Meester et al., 2002; De Meester et al., 2016). Some experimental research suggest that the priority effect continues to affect genetic structure for some time and reported that the initial genetic diversity of the colonizer affects the strength of the priority effect and future genetic diversity (Holmes, et al., 2016). Furthermore, various events can affect genetic dynamics and the diversity of the introduced population at each stage of the colonization process. For instance, niche filtering selects the genotype at the arrival and survival stages, and multiple introductions due to high propagule pressure can promote gene flow, facilitating sustenance of the population (Rius & Darling, 2014). Several laboratory experiments have suggested that there is a strong effect of the genetic condition of the population in the initial introduction stage on establishment (Szucs, Melbourne, Tuff, Weiss-Lehman, & Hufbauer, 2017; Szucs, Vahsen, et al., 2017). Hence, accumulation of the observation knowledge about genetic dynamics through the colonization process from the initial introduction

stage is important to improve our understanding of the changes in genetic diversity, and thus the introduction and establishment of biological populations to novel habitats and adaptation to those new environments.

One of the factors that have limited our ability to study this process is the difficulty in time-series observations of genetic dynamics from the initial stage of introduction. The large cost (Kuebbing et al., 2018) and difficulty associated with prior forecasting of the introduction of a new population restricts long-term temporal monitoring of the entire colonization process. Most prior studies that have tested the process of altering genetic diversity during the colonization process and its effects on establishment were conducted by the traditional “space for time” method, which compares current population living in introduced sites and native sites. In this traditional method, temporal changes in genetic diversity and structure cannot be observed and may lead to underestimation of the effect of a specific event that occurred during the colonization process. Furthermore, a bias in the population that succeeds in establishing might occur (Wellband et al., 2017). Even in long-term ecological research sites, temporal assessments of genetic and phenotypic diversity are rarely introduced (Brodersen and Seehausen, 2014).

Observation of the genetic dynamics during colonization process can be achieved by the paleolimnological approach and *Daphnia*, which is major key stone zooplankton species in lake ecosystems. Paleolimnological analysis reconstructs long-term changes in the environment and community using lake sediment core samples from past to present (Smol, 2010; Douglas 2013). Most *Daphnia* produce diapausing eggs enclosed in ephippia, which are tolerant to drying and preserved in lake sediment for the long term, from decades (Miner et al., 2012) to hundreds of years (Frisch et al., 2014). I can extract DNA from such preserved diapausing eggs, and reconstruct the genetic dynamics (Burge et al., 2018). While many studies have analyzed the evolutionary processes of *Daphnia* using diapausing eggs (e.g., Decaestecker et al., 2007; Stoks et al., 2016), only two prior studies have observed the long-term genetic dynamics of *Daphnia* species, including the early colonization process using these palaeolimnological methods (Dane et al., 2020; Most et al., 2015). Most et al. (2015) analyzed 40-years’ worth of long-term genetic dynamics in the colonization process of *Daphnia pulicaria* that focused on multiple genotypes using diapausing eggs preserved in lake sediments. The genetic information was mainly constructed with cyclic parthenogenetic lineages and possibly included obligate parthenogenetic lineages. Dane et al. (2020) also analyzed the long-term genetic dynamics of only

obligate parthenogenetic *Daphnia pulicaria* for three centuries, including the colonization process in three lakes in Greenland. Both of these prior studies showed that limited haplotypes succeeded during early colonization. In contrast, the genetic dynamics after colonization were different. While *D. pulicaria* populations, including cyclic parthenogenetic lineages, showed a rapid increase in genetic diversity (Most et al. 2015), obligate parthenogenetic populations were strongly dominated by a single clone that had persisted for 250–300 years on two lakes despite substantial climate change (Dane et al. 2020). The difference in reproduction possibly caused these different genetic dynamics. Dane et al. (2020) reported that the limitations of their study were the small sample sizes (n=12, 23, 57 in each lake) and limited resolution of genetic markers (eight microsatellite loci), which might have caused oversight of cryptic genetic diversity.

In this study, I focused on the Japanese *Daphnia pulex*, which consists of four genetically independent lineages, JPN1 to JPN4, which originated in North America (So et al., 2015). All of these lineages are obligate parthenogenetic (So et al., 2015), and thus the genetic information preserved in diapausing eggs is identical to its parent. Furthermore, while each Japanese lineage has identical microsatellite genotypes (12 microsatellite loci); each lineage has multiple mitochondrial haplotypes. I performed high-resolution population genetic analysis of the long-term genetic dynamics of obligate parthenogenetic *Daphnia* by using a large sample size of diapausing eggs (total 588) and amplified mitochondrial markers, which were used in So et al. (2015) and detected genetic diversity in a lineage with identical microsatellites. Furthermore, in the present study, I successfully collected lake sediment cores that included the lake formation period. Thus, I could analyze the genetic dynamics of the *D. pulex* population from the initial stage of establishment whenever the introduction occurred. Our research will contribute to the accumulation of genetic dynamics during the colonization process and will provide new insights into the genetic dynamics of the *Daphnia* population constructed of only obligate parthenogenetic lineages. I aimed to reveal the long-term genetic dynamics of the *D. pulex* population during the whole colonization process from the initial introduction using diapausing eggs preserved in lake sediment, and to discuss the impact of genetic dynamics on the success of establishment. From these, I attempted to contribute to understanding the change in genetic diversity and structure in the colonization process and its effect on the likelihood of establishment. In particular, I aimed to understand the

translocation dynamics of obligate parthenogenetic populations and the mechanism by which these dynamics differ from those of cyclic parthenogenetic populations. Obligate parthenogenetic *D. pulex* population may show successful colonization and maintain with limited haplotype based on prior studies, and I tested this hypothesis.

Materials and Methods

Sediment core sampling

Our research field, Lake Fukami-ike (35°19N, 137°49E) is located in Anan town, Nagano Prefecture, Japan (Figure 1-2). I collected lake sediment core samples including four short core samples and five long core samples on October 6 and 7, 2016 (Figure 1-2) as described in detail in Chapter 2, and additional five short core samples were collected on November 2, 2017. Three short core samples used in this chapter were collected using a gravity corer (Limnos corer; Kansanen et al., 1991) and one long core sample used in this chapter was collected using a Mackereth corer (Mackereth, 1958) (Figure1-2). Two of the short core samples (17Lim1 and 17Lim4), another short core samples (16Lim3), and long core samples were sliced at 1-cm, 3-cm, and 10-cm intervals, respectively, and then stored at 4 °C in the dark. I dated these sediment core samples (Figure1-3) based on the correlation with the sediment core samples dated by ¹⁴C and counting annual lamination in previous studies (Ishihara et al., 2003; Kawakami et al., 2004) and the sedimentation rate calculated in previous studies (Kawakami et al., 2004; Yagi et al., 2009). Full details of these procedures are described in Chapter 2.

Ephippia preparation, DNA extraction

To estimate the population dynamics of *D. pulex* based on the abundance of ephippia and to observe the genetic dynamics of *D. pulex* using genetic information of diapausing eggs, I collected ephippia of *D. pulex* from each layer of all sediment core samples. Sediments were sieved through 100 µm mesh size nets and the ephippia of *D. pulex* were detected and collected based on morphology. All *D. pulex* ephippia found in each sediment layer were collected in petri dishes and counted. I decapsulated each ephippia and isolated one of the diapausing eggs included in each ephippia to 0.5 ml microtubes under a stereo microscope (Nikon smz1500, Tokyo, Japan). I used 43 eggs per layer in average (maximum 93 and minimum 1). To extract DNA from these diapausing eggs, I added 25 µl Quick Extract

DNA Extraction Solution (Lucigen, Wisconsin, US) per individual and subjected them to a thermal shock consisting of 60 °C for 2 h and 95 °C for 20 min.

PCR and sequencing of mitochondrial genes

To observe the genetic dynamics, each diapausing egg was identified using mitochondrial genetic markers designed in the NADH dehydrogenase 5 (*ND5*) region and the control gene of mitochondrial DNA (So et al., 2015). *ND5* was amplified following the method described in So et al. (2015) using the same primers (ND5 f21 and ND5 r1046). For amplification of the Control region, each 10 µL reaction consisted of 1.0 µL of extracted DNA, 5µL of 2×Type-it™ Multiple PCR Master Mix (QIAGEN), 0.5 µL of 5×Q-solution and 0.2 µmol L⁻¹ of each primer (IAIT fw and IAIT rv1). The thermal cycling conditions were as follows: a five-min initial cycle at 95 °C, followed by 30 cycles of 95 °C for 30 s, 60 °C for 1 min, and 72 °C for 1 min, and finished at 60 °C for 10 min.

All successfully amplified samples were purified using ExoSAP-IT® and sequenced. Sequencing was performed using FASMAC INC. (Atsugi, Japan).

Date analysis

To analyze the genetic composition in each layer, the sequences were aligned with MEGA-X (ver. 10.0.5; Kumar, Stecher, Li, Knyaz, & Tamura 2018) and haplotypes were identified, and thus the frequency of each haplotype in each layer was evaluated. To analyze the genetic relationship of each diapausing egg founded in Lake Fukami-ike and the abundance of each haplotype in each year, a mitochondrial haplotype network was constructed using sequences from the *ND5* and the control region. The haplotype network, including other Japanese *D. pulex*, reported in a previous study (So et al., 2015), was also constructed in addition to the haplotype network only consisting of the current samples alone. Molecular diversity indices, number of haplotypes, haplotype diversity, nucleotide diversity, and k-mean number of pairwise differences were calculated using DnaSP v6.12.03 (Rozas et al., 2017). Visualization of genetic relationships among diapausing eggs was obtained by a median-joining network in Network version 10.0.0.0. (Bandelt et al., 1999). Furthermore, to evaluate the change in genetic diversity, I calculated Simpson's diversity index, 1-λ, and haplotype richness in each period.

In addition, I tried to examine whether each haplotype differently respond to abiotic and biotic factors. I used Multivariate Auto-Regressive models (MARs) (Ives, Dennis, Cottingham, & Carpenter, 2003) to evaluate the interaction between haplotypes and effects of abiotic and biotic factors to each haplotype. This analysis was performed using the R package MAR1 (Scheef, 2015). I used the abundance of each *D. pulex* haplotype as variables in the MAR model. And I added the factors, which controlled cladoceran community in Chapter 2, in MAR model as exogenous co-variables. These exogenous co-variables were Total Phosphorus (TP) and fossil pigments of phytoplankton (Chl.a and Pheo.a), and the flux of *Chaoborus* larva subfossils as indicator of predator community.

First, I prepared the dataset using the “prepare data” function. I replaced the data of 0 flux as 1 and then log-transformed all data. Jpn1A-C1T2, Jpn2C_3, Type8_2 and Type9 were excluded since these haplotypes were detected only one egg in all sediment layers. I standardized all data to have equal means and deviations for comparing between haplotypes. I fitted these data to the MAR model (Ives et al., 2003) by generating all possible models. MAR model follows to below equation (3-1).

$$X_t = A + BX_{t-1} + CU_{t-1} + w_t \quad (3-1)$$

where X_t is the $p \times 1$ vector of flux if each of the p haplotypes at time t , A is the $p \times 1$ vector of intrinsic growth rate of each p haplotypes, B is a $p \times p$ matrix of b_{ij} meaning the effect of the abundance of haplotype j on the per capita growth rate of haplotype i , U_{t-1} is the $q \times 1$ vector of covariate values at time $t-1$, C is the $p \times q$ matrix of c_{ij} which describe the effect of covariate j on haplotype i , w_t is the process errors during the change from time $t-1$ to time t described as the $p \times 1$ vector of elements randomly sampled from a multivariate normal distribution with a mean of 0 and covariate matrix S .

And then selecting the best-fit model as the one with the lowest Akaike's Information Criteria (AIC). Then, I used bootstrapping ($n = 500$) on the best-fit model to obtain 95% Confidence Interval (CI) for the coefficients, which describe the degree and direction of the effects of each species and covariables on each species, in the model. Finally, I calculated the conditional R^2 for each taxon to evaluate the model's ability to predict the

temporal changes in abundance. Model selection and estimation were performed using the “run.mar” function.

Results

Ephippia of *D. pulex* was first detected in 1950, but was not detected after that for some time. Since around 2002–2003, ephippia have consistently appeared and increased (Figure S3-1). After that, ephippia significantly decreased in the recent 2016–2017 period. These dynamics coincided with the dynamics of post-abdominal-claw subfossils of *D. pulex* (Chapter 2; Otake et al., 2020).

A total of twelve haplotypes were detected (Figure 3-1) with 755 bp sequence of the ND5 gene and 817–850 bp sequence including indel of the control region. All twelve haplotypes detected in this study were assumed to belong to either JPN1 or JPN2 (Figure 3-1, S3-2), two of all four genetic distant lineages of *D. pulex* found in Japan (So et al., 2015), based on identical genetic sequence in So et al. (2015). Two haplotypes belonging to JPN1 coincided with haplotypes, Jpn1A-C2T2 and Jpn1A-C1T2, which were previously reported. Two haplotypes belonging to JPN2 also coincide with haplotypes, Jpn2C and Jpn2D, which a prior study reported. Other seven haplotypes belonging to JPN2 lineage were newly detected here (Figure 3-1, S3-2). The most abundant haplotype was Jpn2C, which appeared during the colonization process (Figure 3-1 and 3-2). The other nine haplotypes belonging to JPN2 were genetically close to Jpn2C and were only different in one or two indels or one SNP (Figure 3-1).

The dynamics of each haplotype showed that the dominant haplotype (Jpn2C) appeared from the early establishment stage in 2002–2003 and continuously dominated during the research period (Figure 3-2). Other JPN2 haplotypes repeatedly appeared and disappeared. Since around 2013, two haplotypes belonging to JPN1 appeared but did not dominate (Figure 3-2). Haplotype richness was small during the colonization process, with a maximum of 8 and below 5 for most of the period (Figure 3-2). Simpson’s diversity index was also small during the research period and was smaller than 0.5 in most periods (Figure 3-2).

I examined the response of each haplotype to nutrient TP, fossil pigments as indicator of phytoplankton abundance (Chl.a + Pheo.a), and *Chaoborus* which indicates predator community change. Responses to control factors were different between haplotypes (Table S3-1). The response of Jpn2C and its closely related haplotypes

were similar except for Jpn2D. Jpn2D positively respond to *Chaoborus* and TP, which were opposite direction of responses of other haplotypes belonging to JPN2. Jpn1A-C2T2, which is genetically distant with Jpn2C, also showed positive response to *Chaoborus* and TP.

Discussion

In the present study, I aimed to reveal genetic dynamics from the early establishment of obligate parthenogenetic *D. pulex* using their diapausing eggs preserved in the varved sediment. Population dynamics mirrored by the dynamics of diapausing egg abundance coincided with the dynamics that the post-abdominal-claw subfossils of *D. pulex* showed (Chapter 2; Otake et al., 2020): *D. pulex* first appeared around 1950, but its density remained low and it went extinct until around 2002, when a firm population established (Figure 3-1). Genetic analysis of diapausing eggs suggested that the *D. pulex* population was maintained with limited genetic diversity and one haplotype, Jpn2C, dominated without turnover during the colonization process (Figure 3-2). In addition, Simpson's diversity index and haplotype richness were also low, and these indexes did not increase during the colonization process, suggesting that the *D. pulex* population was maintained with limited genetic diversity without recovery. These results suggest that a biological population is possibly able to maintain itself long-term even if genetic diversity decreases due to the founder effect when the population was first introduced. Furthermore, the results suggest that obligate parthenogenetic taxa in particular tend to experience difficulty in recovering and maintaining genetic diversity from a limited number of haplotypes.

All twelve haplotypes detected in this study were assumed to belong to either JPN1 or JPN2, two of all four genetic lineages of *D. pulex* found in Japan (So et al., 2015). Jpn2D and all of seven haplotypes that newly detected here were belong to JPN2 lineage and genetically very close to Jpn2C (Figure 3-1, S3-2). There were only one or two indels or one SNP between these haplotypes and Jpn2C (Figure 3-1). These eight haplotypes, which were Jpn2C and genetically close to it, possibly originated from Jpn2C in Lake Fukami-ike. Since prior population genetic analysis of *D. pulex* across Japan did not detect eight haplotypes belonging to JPN2 lineage, except for Jpn2C and Jpn2D (So et al., 2015), these haplotypes might not have dispersed from other lakes. Furthermore, the indel mutation

rate is assumed to be higher than the base substitution rate of mitochondrial DNA reported in other *Daphnia* species, *Daphnia magna* (Xu et al., 2012), possibly allowing these newly haplotypes to occur during the 10 years since Jpn2C established in Lake Fukami-ike (So et al., 2015). The present study supported the previous observation that Lake Fukami-ike was found to be a limited lake that had both JPN1 and JPN2 (So et al., 2015). In contrast, three haplotypes, Jpn1A-C2T1, Jpn2E and Jpn2F were not detected in Lake Fukami-ike in the present study, although a previous study found them in Lake Fukami-ike. Since So et al. (2015) collected active *D. pulex* in Lake Fukami-ike from the water column rather than ephippia preserved in lake sediment, these three haplotypes might have appeared only in a limited period and their ephippia were possibly in a relatively low abundance in the lake sediment.

In Lake Fukami-ike, the introduction of *D. pulex* is considered to have occurred at least twice by Jpn2C and Jpn1A-C2T2. These two haplotypes are genetically distant and belong to different independent lineages, JPN1 and JPN2, so that Jpn1A-C2T2 is thought to have been introduced from other lakes rather than occurrence with a mutation from Jpn2C in Lake Fukami-ike. As mentioned above, the other nine haplotypes genetically very close to Jpn2C possibly mutated from Jpn2C in Lake Fukami-ike. Jpn1A-C1T2 is also genetically very close to Jpn1A-C2T2 with only one indel (Figure 3-1), and thus the haplotype possibly occurred from Jpn1A-C2T2, which was introduced to Lake Fukami-ike. Jpn2C was also detected in Arigatani-ike, Shizuoka, Japan (34°69'N, 138°82'E; So et al., 2015). Thus, Arigatani-ike is possibly the source population of the Jpn2C individuals that established in Lake Fukami-ike, but that cannot be tested by the present study alone. Since Jpn1A-C2T2 was detected in multiple lakes, I cannot assume the source population in the present study. To estimate the source population, a higher resolution analysis with SNP markers in multiple population across geographic region is needed as it will be more effective.

In the early stage of firm population establishment, *D. pulex* populations might colonize with only Jpn2C and some JPN2 haplotypes, which are closely related to Jpn2C (Figure 3-2). This colonization by limited haplotypes agrees with the reduction of genetic diversity by the founder effect when a part of the population is introduced to a novel habitat (Leberg, 1992; Frankham, 1997). Since ephippia were relatively abundant in around 2002-2003 (Figure S3-1), Jpn2C and some related haplotypes might have colonized the abundant population and/or left the diapausing egg bank that matured from an early stage of establishment, suggesting that the founder population adequately

reproduced. Rapid reproduction soon after the introduction is consistent with the advantage of clonal reproduction. *Daphnia*, a major taxon that has a diapause stage enclosed with ephippia and can grow clonally, is considered to be able to rapidly colonize and expand habitat by dispersion with ephippia and rapid population growth through the clonal reproduction of one female (reviewed in De Meester et al., 2002). Colonization by one or a few genotypes has been observed in several studies: palaeolimnological observation of *Daphnia pulicria* (Most et al., 2015; Dane et al. 2020), and monitoring of *D. magna* in new ponds (Ortells et al., 2013).

After colonization, there was no increase in genetic diversity in the study population. Haplotype richness was low throughout the research period, which was below 5 during in most years (Figure 3-2), suggesting that genetic diversity was low during the early establishment stage. Similarly, Simpson's diversity index was also continuously low, below 0.5, during most of the year (Figure 3-2). This suggests that a few haplotypes appeared in high ratio rather than the population was composed by multiple types in equal ratio, supporting the continuous dominance of only Jpn2C. Furthermore, there was no replacement of the dominant haplotype (Figure 3-2). These results suggest that the recovery of genetic diversity by multiple introductions, which can buffer the negative effect of genetic diversity reduction by the bottleneck effect at introduction (Rius and Darling, 2014), did not occur in Lake Fukami-ike, and that the *D. pulex* population was maintained without genetic diversity recovery. After present observation period, an increase in genetic diversity by multiple introductions possibly occur, as prior studies have shown (Most et al., 2015; Haileselasie et al., 2016), but in any case, reduction of genetic diversity in the initial introduction stage by the founder effect did not interfere with population establishment and persistence in the present *D. pulex* population.

Although genetic diversity was limited and the dominant haplotype did not change during the colonization process, the *D. pulex* population in Lake Fukami-ike changed their traits, including body size of matured females and induced defensive traits, and it was possibly an adaptive response to the change in the predator community (Chapter 4). The results of Chapter 4 and the present study suggest that the introduced population could adapt to novel environments and environmental changes in novel habitats even if genetic diversity reduced at immigration. Phenotypic plasticity possibly contributes to changes in these traits and succeed to establish. Indeed, *Daphnia* species, including *D. pulex*, have been reported to have a highly plastic response in various traits, including morphological,

behavioral, and life history traits to environmental change, which includes predators, temperature, phytoplankton, and more, in both the experimental and natural populations (Herrmann et al., 2017; Loose, 1993; Havel & Dodson, 1984). Phenotypic plasticity is considered to aid colonization of the novel environment and permits persistence despite environmental change (Richards et al., 2006; Hulme, 2007). Phenotypic plasticity is a possible mechanism that buffers the negative effects of genetic diversity reduction and promotes establishment in novel habitats, as invasive plant species or populations have relatively higher plasticity than native species or populations (Bock et al., 2018; Davidson et al., 2011). The contribution of phenotypic plasticity to successful colonization and the relative contribution of phenotypic plasticity to genetic adaptation deserve further study. For instance, research that focused on whether functional genes changed through the colonization process and/or whether genetic selection occurred will be effective.

Two prior studies have revealed the long-term genetic dynamics of *Daphnia* populations with diapausing eggs in lake sediment core samples (Most et al., 2015; Dane et al., 2020). Colonization by limited haplotypes in the present study was consistent with these prior studies. In the present study, the *D. pulex* population was dominated by only one haplotype, Jpn2C, which was already dominant in early stages of colonization. Such genetic dynamics after colonization consisted of an obligate parthenogenetic *D. pulicaria* population reported by Dane et al. (2020). While both my study and Dane et al. (2020) reported that biotic and abiotic fluctuations occurred (predator community change showed in Chapter 2 and climate change, respectively), an obligated parthenogenetic *Daphnia* population sustained with limited haplotypes and dominated with only one haplotype. In addition, the appearance of haplotypes that are genetically very close to the dominant type is also consistent between the present study and Dane et al. (2020). These results suggest that while my study was conducted with a larger sample size and higher genetic resolution than Dane et al. (2020), obligate parthenogenetic *Daphnia* showed similar genetic dynamics from early colonization to the long-term. These results suggest that biological populations can colonize with limited diversity, and this trend may be especially strong for asexual reproductive populations. This concept is consistent with the notion that *Daphnia* can increase rapidly by asexual reproduction in newly introduced areas (e.g., monopolization hypothesis (De Meester et al., 2002)). Furthermore, no increase in genetic diversity was observed in both the present study and Dane et al.

(2020) using obligate parthenogenetic populations, which is contrast to the cyclic parthenogenetic populations reported by Most et al. (2015). This suggests that the obligate parthenogenetic *Daphnia* populations may be less likely to recover genetic diversity after colonization than cyclic parthenogenetic *Daphnia* populations. In the cyclic parthenogenetic *D. pulicaria* population of Most et al. (2020), recombination, which is unlikely to occur in obligate parthenogenetic populations, and multiple introductions could promote a rapid increase in genetic diversity. This advantage of cyclic parthenogenesis against obligate parthenogenesis in creating genetic diversity, which as a resource of evolution, is consistent with the classical concept of maintaining sexual reproduction (Agrawal, 2009). The mechanism by which obligate parthenogenetic organisms are able to sustain populations with low genetic diversity despite biotic and abiotic fluctuations requires further study.

I examined the response of each haplotype to control factors, predator community and bottom-up factors like nutrient and phytoplankton. Consequently, the direction and degree of responses possibly differed depending on haplotypes (Table S3-1). Such ecological difference of haplotypes might affect to dynamics and success of colonization of each haplotype. Detailed analysis of intraspecific difference in ecological character is one of the future research perspectives.

In this study, I observed the genetic dynamics of the *D. pulex* population in only one lake, and *D. pulex* was recently established: within approximately 15 years. Limited study sites and limited genetic markers did not allow us to estimate the source population of each haplotype living in Lake Fukami-ike. This time period was small to estimate the demographic history of the colonization process. To accurately estimate the number of introduction events is one of future perspectives. In addition, to evaluate the genetic diversity within a haplotype that was detected based on the mitochondrial markers, and to discuss the adaptive mechanism in the colonization process are also future studies as mentioned above. One of the future questions is whether Jpn1A-C2T2 can be introduced to Lake Fukami-ike later and can coexist with Jpn2C. In Chapter 5, I tried to compare the traits of two haplotypes to estimate the mechanism that allows two lineages to coexist in Lake Fukami-ike.

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Figures

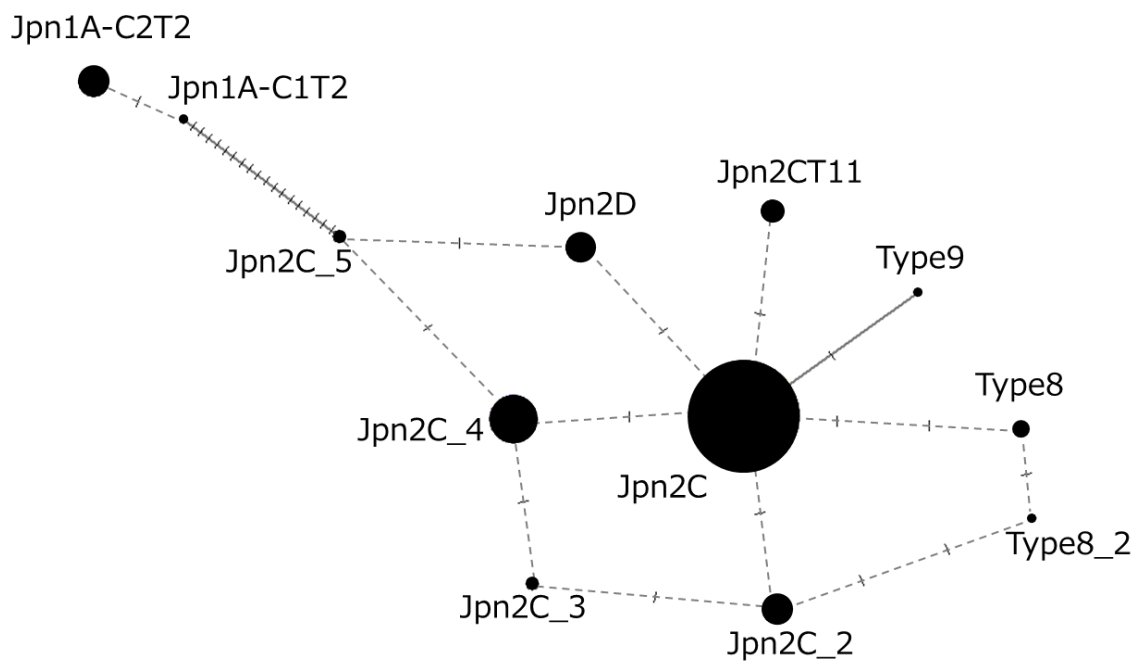


Figure 3-1. Haplotype network showing the relation of all *D. pulex* haplotype detected in lake sediment core samples of Lake Fukami-ike. The circle size indicates the number of individuals of each haplotype through research period. The number of horizontal lines indicates the number of different sites between haplotypes of ends. Dash lines indicate that the haplotypes of ends differ only by indel.

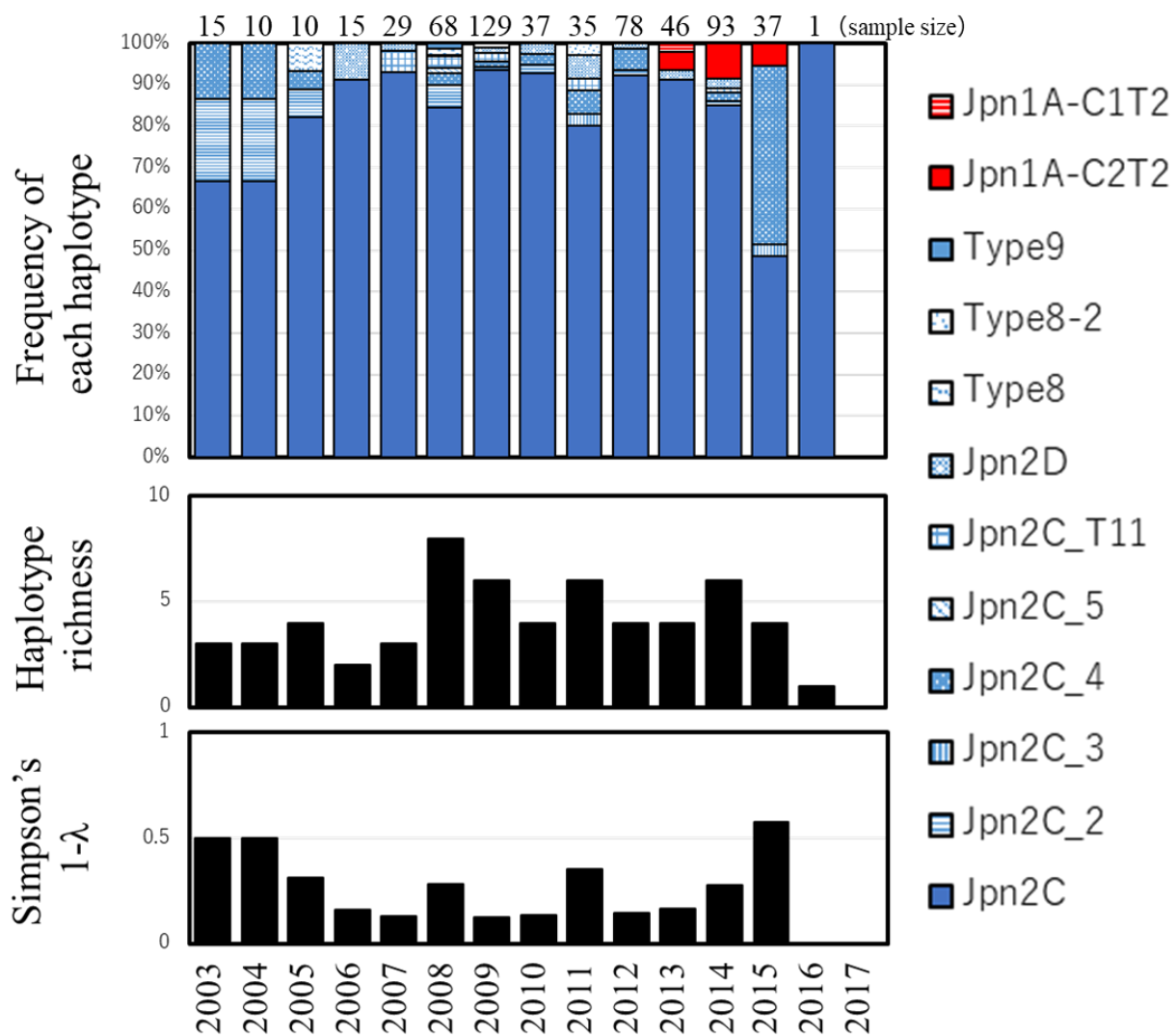


Figure 3-2. The temporal change of frequency of each haplotype (upper row), haplotype richness (middle row) and Simpson's diversity index (lower row) in *D. pulex* population in Lake Fukami-ike since the early stage of colonization. The numbers above bar-graph indicate the number of diapausing egg samples used for population genetic analysis in each layer.

Supporting Information

Table S3-1. Interaction matrix from the best-fit multivariate autoregressive model for genetic dynamics of *D. pulex* population in Lake Fukami-ike. The coefficients were tested for significance by 500 step bootstrap (all coefficients were significant). In this table, positive coefficients were represented by blue and negative coefficients were represented by red. Brank cells mean that coefficients were not selected in best-fit model. R² calculated with the bootstrapped model indicates how well the model predicts changes in density of each species from one step to the next.

Response	Predictor											R ²
	Jpn1A-C2T2	Jpn2C	Jpn2C_2	Jpn2C_4	Jpn2C_5	Jpn2C_T11	Jpn2D	Type8	<i>Chaoborus</i>	TP	Chl.a + Pheo.a	
Jpn1A-C2T2		1.74	0.7	-0.57	-0.74	-0.85	0.26	0.23	0.80	0.02	0.98	1
Jpn2C	-2.44	2.19	-0.01	-0.65	0.40	-0.71	0.98	0.40	0.08	-0.15	1.47	1
Jpn2C_2	-1.42	0.38	-1.65	0.04	1.33	-0.16	0.17	1.02	-2.19	-1.26	0.50	1
Jpn2C_4	-2.60	2.48	0.23	-1.21	0.39	-1.72	1.96	1.05	-0.71	-0.58	0.80	1
Jpn2C_5	-0.87	1.54	-0.56	-0.61	0.37	-0.15	0.31	0.16	-0.92	-0.83	1.15	1
Jpn2C_T11	-2.00	0.84	-1.03	-0.25	2.02	0.33	-0.04	-0.07	-1.84	-1.13	0.33	1
Jpn2D	-1.34	2.55	0.90	-1.32	0.06	-1.89	0.61	0.29	1.06	0.72	0.92	1
Type8	-0.67	-1.39	-0.96	0.12	1.64	1.34	-0.74	-1.12	-0.18	-0.33		1

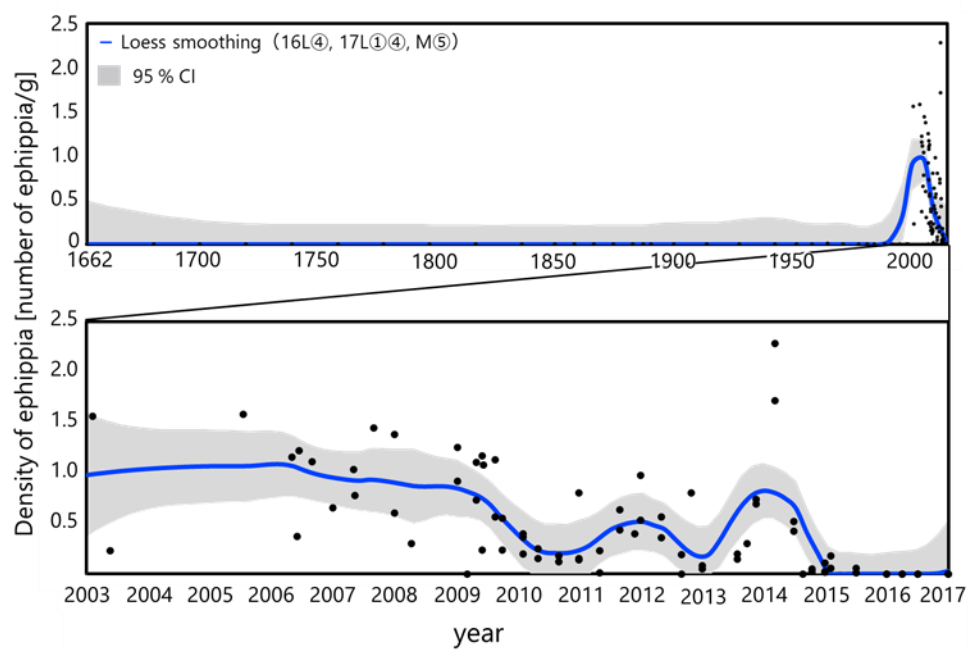


Figure S3-1. *Daphnia pulex* population dynamics estimated based on ephippia density from lake formation.

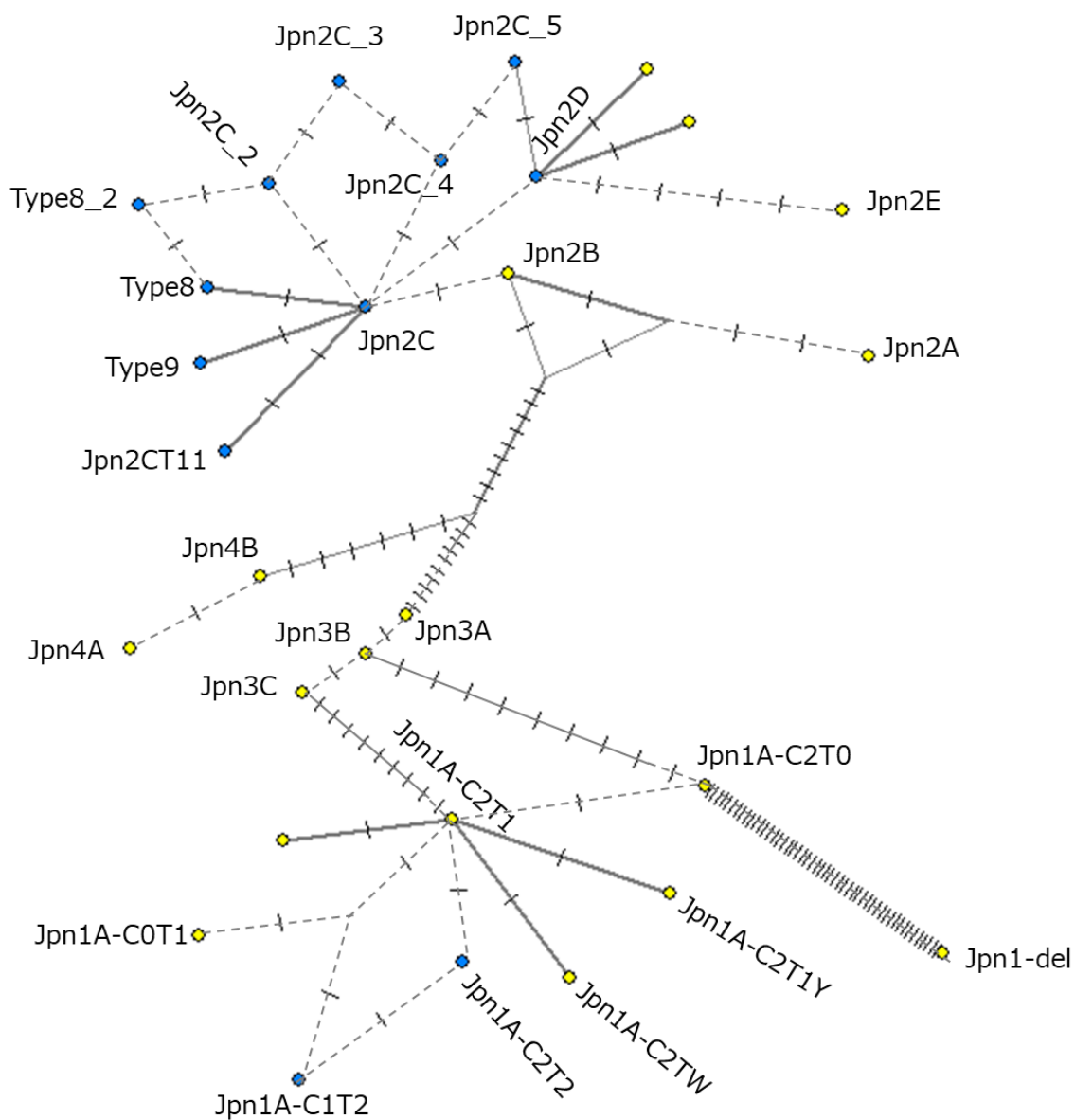


Figure S3-2. The haplotype network including other Japanese *D. pulex* lineages (yellow points) in addition to lineages detected in present research in Lake Fukami-ike (blue points). The circle size indicates the number of individuals of each haplotype through research period. The number of horizontal lines indicates the number of different sites between haplotypes of ends. Dash lines indicate that the haplotypes of ends differ only by an indel.

Chapter 4 Long-term changes in morphological traits of *Daphnia pulex* in Lake Fukami-ike, Japan

Abstract

How a population adaptively respond to environmental changes is one of the central topics in ecology, but long-term changes in phenotype of an organism have been rarely studied. In this study, I examined the morphological changes of *Daphnia pulex* from the initial establishment stage in early 2000s by paleolimnological analysis in Lake Fukami-ike (Nagano Prefecture, Japan). I measured morphological traits of ephippia and subfossils of *D. pulex* and compared the results with the population dynamics assessed in Chapter 2. Length of ephippia significantly increased in the mid-2000s and then decreased, whereas length of post-abdominal claw as a proxy of body size did not show significant changes. These suggests that their adult body size was larger, and time to mature delayed or shift to diapause phase got later in the mid-2000s, although the mean body size did not change. Subfossils of *Chaoborus* and the historical records suggest that planktivorous fish was less abundant and *Chaoborus* larvae were more abundant in the mid-2000s compared to more recent periods. These results suggest that *D. pulex* adaptively changed phenotypic traits in response to the change of predator community in the studied lake.

Introduction

Rapidly adaptive response of a biological population to environmental changes via phenotypic plasticity and/or evolution can commonly occur in nature and it would affect ecological processes in turn (Agrawal, 2001, Hairston et al., 2005). Laboratory experiments and field observation studies revealed that freshwater zooplankton can adaptively change their life cycle, behavior, and morphology when they are exposed to predators (Reviewed in Havell and Tollrian, 1999; Riessen & Gilbert, 2018). However, only a few studies have examined the long-term phenotypic changes of zooplankton and their associated environmental changes longer than one growing season (e.g., Hairston & Dillon, 1990), although such studies will contribute to our understanding of rapid adaptation occurring in natural populations.

In this study, I examined long-term changes in morphological traits of a *Daphnia* species in response to

predation from the initial stage of population establishment by paleolimnological analysis. Some phytoplankton and zooplankton leave a part of body of them and their diapause stage as subfossils in lake sediments for long-term, which can be used as indicators of their abundance and community composition (Douglas, 2013). Also, these subfossils provide morphological information of plankton (e.g., Korosi et al., 2011), allowing to reconstruct the long-term changes of morphological traits and population dynamics together. My research field was Lake Fukami-ike located in Nagano Prefecture, Japan (Figure1-2). This small lake was formed by the earthquake in 1662 and became eutrophicated by now (Yagi et al., 2009). Varved lake sediments comprising annual laminae were preserved in the lake, allowing us to examine high-resolution temporal changes (Lamoureux, 2001).

I focused on a population of *Daphnia pulex* inhabiting Lake Fukami-ike. *Daphnia* is a keystone taxon in lake ecosystems and sensitive to biotic and abiotic environmental changes (Carpenter & Kitchell, 1993; Jeppesen et al., 2001, 2011). *Daphnia* species can adaptively respond to predation by phenotypic plasticity and rapid evolution (e.g., Hairston et al., 1999; Cousyn et al., 2001; Stoks et al. 2016; Riessen & Gilbert, 2018). *D. pulex* also changes their body size and tail-spine length as inducible defence against their predators, *Chaoborus* larvae and planktivorous fish (Dodson, 1989; Riessen & Gilbert, 2018; Nagano & Yoshida, 2020) as well as their resource allocation to reproduction or growth depending on the predator (reviewed in Tollrian & Dodson, 1999; Riessen & Gilbert, 2018).

Daphnia species remain the post-abdominal claw composed by chitin and diapausing eggs wrapped by ephippia as subfossils preserved in lake sediments for long-term (Korhola & Rautio, 2001; Jeppesen et al., 2001). Thus, I can examine the change of body size by measuring the size of these subfossils (Korosi et al., 2011; Jeppesen et al., 2001) as well as the length of tail-spine remaining on ephippia. A prior study examined the morphological changes of *D. pulex* in the studied lake when exposed to the kairomone of *Chaoborus* larvae and planktivorous fish (Nagano & Yoshida, 2020). The study showed that *D. pulex* in this lake tended to become larger in body size and elongate tail-spine when exposed to *Chaoborus* larvae kairomone, whereas they tended to get smaller in body size and have shorter tail-spine when exposed to planktivorous fish kairomone. In this study, I examined whether *D. pulex* showed morphological changes as found in the prior study in response to the long-term changes in predators in Lake Fukami-ike. I argued the results in combination with our previous findings of population dynamics and the associated

factors (Chapter 2).

Materials and Methods

Sediment core sampling

Lake Fukami-ike (35°19'N, 137°49'E) is located in Anan town, Nagano Prefecture, Japan (Figure 1-2). I collected lake sediment core samples including four short core samples and five long core samples on October 6 and 7, 2016 as described in Chapter 2, and additional five short core samples were collected on November 2, 2017. I used four short core samples (16Lim1, 16Lim2, 16Lim3 and 17Lim4) and three long core samples (Mac1, Mac2 and Mac3) for this study (Figure 1-3). One of the short core samples (17Lim4), other short core samples, and long core samples were sliced at 1-cm, 3-cm, and 10-cm intervals, respectively, and then stored at 4 °C in the dark. I dated these sediment core samples (Figure 1-3) based on the correlation with the sediment core samples dated by ^{14}C and counting annual lamination in previous studies (Ishihara et al., 2003; Kawakami et al., 2004) and the sedimentation rate calculated in previous studies (Kawakami et al., 2004; Yagi et al., 2009). Full details of these procedures are described in Chapter 2.

Examination of subfossils and ephippia

I examined the temporal changes of three traits, body size of entire population or matured females and induced defensive tail-spine, of *D. pulex* based on post-abdominal claw and ephippia that remained as subfossils in lake sediments. Subfossils were analyzed according to the methods of Korhola & Ratio (2001). Briefly, 1 g wet weight of sediments from each layer of core samples was extracted and dissolved in 50 ml distilled water. Inorganic particles did not prevent observation, so I did not remove them. Then, 1 ml of the 50-ml sample was put into a 1-ml Sedwick-Rafter chamber, and subfossils were examined at a 200× magnification using a microscope. I searched post abdominal claw of *D. pulex* and measured post abdominal claw length (PCL) as a proxy of body size (Hrbáček, 1969) according to Korosi et al. (2011) using the samples of 16Lim2 and Mac3. I took photographs of post abdominal claw using a microscope and then measured PCL later using the photos. I also measured the length of ephippia as a proxy of body size of adult female bearing eggs, collected from the samples of 16Lim4, 17Lim4 and Mac3, as previous studies

revealed that length of ephippia linearly related to body size of the egg-bearing females (Verschuren & Marnell, 1997; Jeppesen et al., 2002). In addition, I examined the defensive trait, tail-spine, of matured *D. pulex* using the ephippia. I took photographs of ephippia and then measured the length of ephippia and the attached tail spine.

I examined whether each trait of *D. pulex* changed among the separate periods of population dynamics (described below) by ANOVA and the post-hoc comparison with the Tukey-Kramer test. I used PCL, length of ephippia and the ratio of tail-spine length to ephippia length as a response variable.

In addition, the length of tail-spine is considered to be elongated in correlation with the body size. Hence, to evaluate the tail-spine length independently of body size, we estimated allometric equation between the tail-spine length and the ephippia length with liner regression and calculated residuals with its model. We estimated the allometric equation by liner regression of the log-transferred tail-spine length on the log-transferred ephippia length. First, we tested whether allometric relations between the tail-spine length independently of body size differed among separate periods of population dynamics by ANCOVA. And then, we examined whether residuals indicating the tail-spine length independent of the body size changed among the separate periods of population dynamics (described below) by ANOVA. All statistical analyses were performed using R version 3.5.2 (R Core Team 2017), and significance level was set at $\alpha = 0.05$.

The long-term changes of morphological traits were compared to the population dynamics reported in Chapter 2. Here, I briefly explain the methods and more details are described in Chapter 2. The population dynamics of *D. pulex* were examined by counting their post abdominal claw. I also counted subfossils of *Chaoborus* larvae to examine the top-down effect on *D. pulex* population. *Chaoborus* larvae abundance can be used as a proxy of planktivorous fish abundance as they were selectively preyed upon by planktivorous fish (Sweetman & Smol, 2006; Palm et al., 2011; Tolonen et al., 2012). I calculated the flux of subfossils per year (number cm⁻² year⁻¹) as an index of the abundance of *D. pulex* and *Chaoborus*. Then, I analyzed the population dynamics of *D. pulex* by examining major changes in their abundance that can be detected by the Constrained Incremental Sum of Squares cluster analysis (CONISS: Grimm, 1987) with the broken stick model for assessing the significance of CONISS-delineated zones (Bennett, 1996). The analysis was performed with the R packages *vegan* (Oksanen et al., 2017) and *rioja* (Juggins,

2017).

Results

In Lake Fukami-ike, the post-abdominal claw of *D. pulex* was firstly found around 1930–1950 but was not found continuously after that period. Then, it appeared again in early 2000s and continuously found until around 2016 (Chapter 2; Figure 4-1). The flux of their post-abdominal claw increased from 2000 to 2014, and then decreased after 2014. CONISS analysis suggested that the population dynamics of *D. pulex* can be divided into five periods (Figure 4-1). From the later part of period I to period V when *D. pulex* established and maintained the firm population, *Chaoborus* was also relatively abundant. After that, during the period V, both *D. pulex* and *Chaoborus* gradually decreased.

Post abdominal claw length (PCL) as an indicator of body size of entire active population showed no significant changes during these periods ($P = 0.452$; Figure 4-2 A). In contrast, the length of ephippia as an indicator of body size of matured females was significantly larger in mid-2000 (period II to period IV) than the early establishment (period I) and the most recent periods (period V) ($P < 0.001$; Figure 4-2 B). The ratio of tail-spine length to ephippia length was significantly larger in the early establishment period (period I) than later periods ($P < 0.001$; Figure 4-2 C), whereas the ratio tended to become larger again in the most recent period (period V) though not statistically significant.

We evaluated the tail-spine length independent of the body size using residuals from allometric regression. First, as a result of evaluation the allometric relation of the log-transferred tail-spine length and the log-transferred ephippia size, interaction between the CONISS periods and the log-transferred ephippia length showed no significant effect to the log-transferred tail-spine length (Table S4-1A, B). Based on this result, we examined the regression model with only the log-transferred ephippia length as explanatory variables. In this regression model, the log-transferred ephippia length had a significant effect on the log-transferred tail-spine length (Table S4-1A, C). ANCOVA showed no significant difference between model 2 (explanatory variables are log-transferred ephippia length and CONISS periods) and model 3 (explanatory variables are log-transferred ephippia length, CONISS

periods and these interactions), between model 1 (explanatory variable is log-transformed ephippia length) and model 3 (Table S4-1 B, C). According to these results, we evaluated the residuals which were calculated from a regression model using all the samples across all periods. In this regression model, the log-transformed ephippia length showed a significant negative effect on the log-transformed tail-spine length (Fig. S4-2 A). The residuals indicating the log-transformed tail-spine length independent of the log-transformed ephippia length showed no significant difference among the periods (Fig. S4-2 B; Df = 4, Sum of square = 0.638, Mean square = 0.160, F value = 1.905, P = 0.11).

Discussion

The present study showed that *D. pulex* changed their traits during colonization process from early 2000s (Figure 4-2 B, C). Body size of adult female, age to mature, age to shift from asexual phase to diapause phase, and defensive trait against planktivorous fish, were changed. The change of body size agrees with prior experiment study that examined the response of *D. pulex* from Lake Fukami-ike to fish (Nagano & Yoshida, 2020). Thus, these changes were possibly adaptive response to the change of predator community. Contrary, change of defensive traits of *D. pulex* was different from prior examination (Nagano & Yoshida, 2000), suggesting that the change of defensive trait in our study was not possibly response to the change of predator community.

While length of ephippia was larger in mid-2000s (period II-period VI) and was decreased in the most recent period (period V), PCL which indicates the body size showed no significant change since early establishment to recent periods (Figure 4-4 A, B). It suggests that not the body size of entire *D. pulex* population but the body size of adult females became larger in the period when a firm *D. pulex* population was maintained in mid-2000s. *Chaoborus* larvae abundance can be used as a proxy of planktivorous fish abundance (Sweetman & Smol, 2006; Palm et al., 2011; Tolonen et al., 2012), and the flux of *Chaoborus* subfossils increased in mid-2000s and decreased in the most recent period (period V) (Figure 4-1). This suggests that planktivorous fish was less abundant in mid-2000s and became more abundant in the most recent period. Historical records agree with this change in planktivorous fish. *Hypomesus nipponensis*, a planktivorous fish that selectively preys on large zooplankton (Makino et al., 2001; Chang, Hanazato, Ueshima, & Tahara, 2005), was artificially introduced in 1960 (Tanaka, 1992). The

presence of the piscivorous fish *Micropterus salmoides* was first recorded in 1974 and then *H. nipponensis* disappeared due to the predation of *M. salmoides* (Shimoina Board of Education, 2009). In 2005, the omnivorous *Lepomis macrochils* that preys on zooplankton (Sakano & Yodo, 2004) was found in the lake (Kawanobe & Hosoe, 2007). After that, *L. macrochils* increased their abundance greatly between 2005 and 2007 (Kawanobe & Hosoe, 2007) and have dominated the fish community of the lake recently (Takei, 2010).

Ephippia length of *D. pulex* became larger when *Chaoborus* was abundant and planktivorous fish was less abundant (Figure 4-2 B). This suggests that *D. pulex* would have changed the size of matured female in response to the change in predators. Larger body size of adult female suggests the possibility that the time to mature and to switch to diapause phase became later in less planktivorous fish condition. Prior laboratory examination that tested the response of *D. pulex* from Lake Fukami-ike to planktivorous fish kairomone suggested that body size of adult *D. pulex* can become smaller when exposed to fish kairomone (Nagano & Yoshida., unpublished data). Our result agrees with this prior laboratory experiment. Furthermore, it has been known that *Daphnia* can change their life history and resource allocation depending on their predators (reviewed in Tollrian & Dodson, 1999; Riessen & Gilbert, 2018). When planktivorous fish that prefers larger prey is abundant, *Daphnia* tends to start reproduction earlier and mature at smaller size (Riessen, 1999; Tollrian & Dodson, 1999; Stoks et al., 2016; Carter et al., 2013). In contrast, when *Chaoborus* that prefers smaller prey is abundant, *Daphnia* tends to delay reproduction and mature at larger size (Riessen, 1999; Tollrian & Dodson, 1999). Our results that *D. pulex* matured at larger size and therefore delayed reproduction when *Chaoborus* was abundant and planktivorous fish was less abundant (Figure 4-2 B) coincide with these previous studies. Cladoceran, especially *Daphnia*, have been showed the trend that larger the body size individuals show the higher the filtration rate and the better the resource competition based on measuring threshold food concentration (Gliwicz, 1990; Kreutzer & Lampert, 1999). Hence, under the condition without planktivorous fish that prefer larger prey, large body size increase fitness. Indeed, predation can cause the replacement of competitive ability of different body size *Daphnia* (Hülsmann, Rinke, & Mooij, 2010).

The ratio of tail-spine length to ephippia length indicating the developmental degree of inducible defense

against predators did also significantly change (Figure 4-2 C). The ratio was bigger in the initial establishment stage (period I) than in the later periods when *D. pulex* maintained a firm population in mid-2000s. The ratio again showed an increasing trend in the most recent period, although not statistically significant (Figure 4-2 C). The results suggested that *D. pulex* tended to develop defensive long tail-spine in the period when planktivorous fish was more abundant and *Chaoborus* larva was less abundant. However, this is incompatible with the prior laboratory examination of *D. pulex* from Lake Fukami-ike that found the shorter tail-spine in adult stage when exposed to planktivorous fish kairomone and longer tail-spine when exposed to *Chaoborus* kairomone (Nagano & Yoshida., unpublished data). Thus, the observed change of tail-spine length may not be the response to predators in my studied lake, although some previous studies reported that *Daphnia* can develop longer tail-spine when exposed to planktivorous fish kairomone (Dodson, 1989, Spaak & Boersma, 1997). Instead, trophic and food condition may have affected the length of tail-spine as Spaak & Boersma (1997) found that *Daphnia* elongated their tail-spine only under high food condition, although it remains unclear whether this is the case.

On the contrary, residuals from allometric regression of the log-transferred tail-spine length on the log-transferred ephippia length, as indicator of the defensive tail-spine length independent of body size, showed no significant difference among the CONISS periods (Fig. S4-2 B, $P = 0.110$). Furthermore, interaction between the CONISS periods and the log-transferred ephippia length showed no significant effect to the log-transferred tail-spine length in ANCOVA (Table S4-1 A, B), suggesting that allometric relation between tail-spine and adult body size could not change during the colonization process. From these, the temporal variation in defensive morphological development, as indicated by the ratio of the tail-spine length to the ephippia length (Fig. 4-4 C), might be largely caused by variation in body size. However, the relationship between the log-transferred tail-spine length and the log-transferred ephippia length was significantly negative (Fig. S4-2A). Therefore, it is suggested that smaller individuals tended to extend longer spines, rather than larger individuals having larger spines as predicted by the intuitive growth relationship. The large adult body size and short spines were found in middle 2000s when planktivorous fish might decreased, and the small adult body size and long spines were found in early establishment stage and recent periods when planktivorous fish might be abundant, suggesting that these traits are considered to be adaptive to each predator

condition as discussed above. Therefore, *D. pulex* population in Lake Fukami-ike might responded to predator fluctuations in both adult body size and defensive tail-spine, with a particularly large response in adult body size.

Chapter 4 showed that *D. pulex* in Lake Fukami-ike changed their traits in an adaptive way in response to changes in predators. Whether the change was due to phenotypic plasticity or genetic evolution remains to be studied and population genetic analysis would contribute to the understanding, which I am conducting using diapausing eggs collected from the bottom sediments in Chapter 3.

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Figures

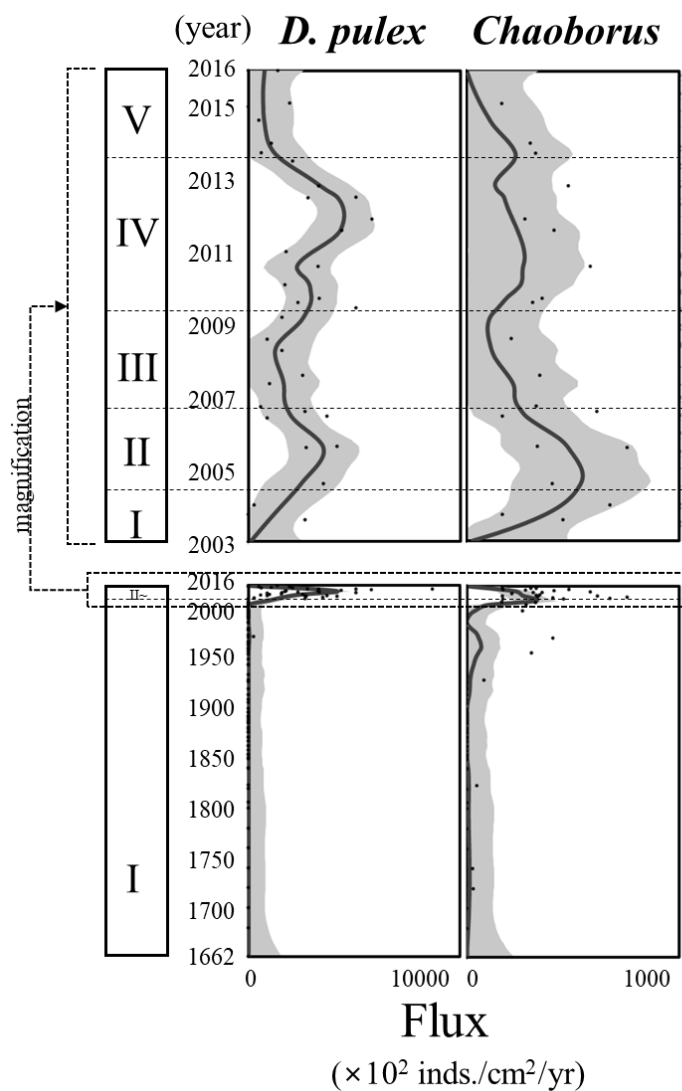


Figure 4-1. Dynamics of the *D. pulex* population and *Chaoborus* population from the lake formation to the present. The lines represent the loess smoothed change in each species flux (span = 0.2) with the 95% confidence intervals indicated by gray bands. Data points are the flux data of each species in each layer. Arrows indicate the year when each species first appeared in the sample. The far-left column shows the periods of *D. pulex* population dynamics determined by CONISS. These pictures are parts of Chapter 2 and Otake et al, 2020.

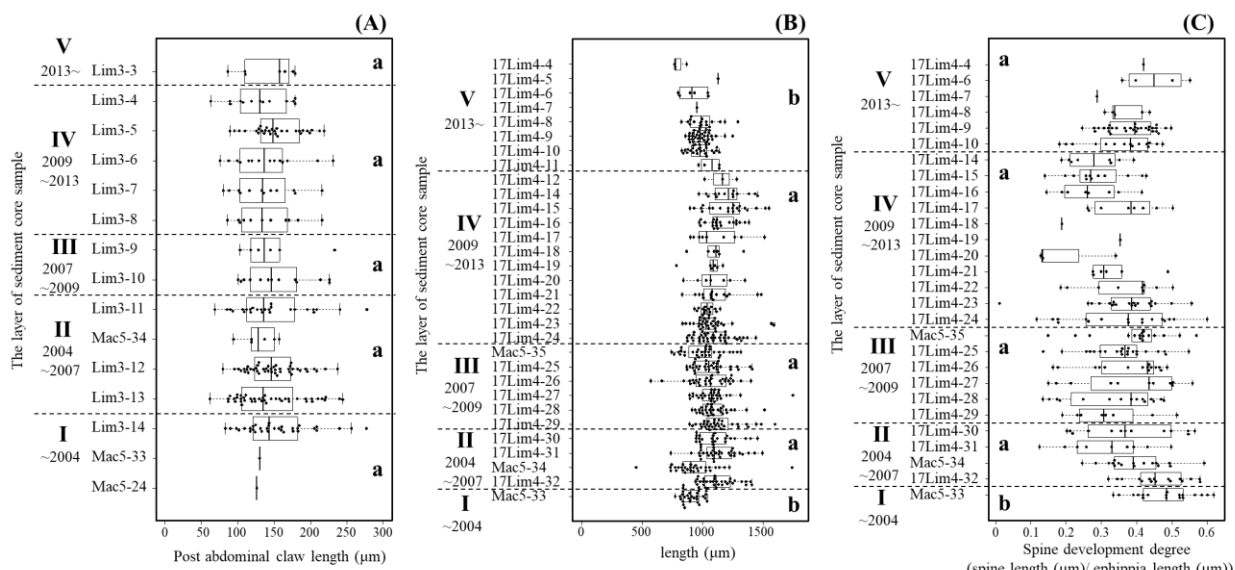


Figure 4-2. The change morphological traits of *D. pulex*. The change of post abdominal claw length as indicator of body size (A). The change of ehippia length as indicator of adult female (B). And the change of the ratio of spine length to ehippia length as the indicator of development degree of defensive morphology (C).

Supporting Information

Table S4-1. Results of ANCOVA and each liner regression models used to it. (A) showed the results of each regression models. For all regression models, a response valuable was log-transferred the tail-spine length. Explanatory variables were log-transferred the length of ehippia in Model 1, log-transferred the length of ehippia and CONISS delineated zones in Model 2, and log-transferred the length of ehippia, CONISS delineated zones and their interaction in Model 3. (B) showed the result of the test whether the allometric relations between the length of ehippia and the tail-spine length are differed depending on CONISS delineated zones. (C) showed the result of the test how the length of ehippia affects tail-spined length.

A

	Model 1					Model 2					Model 3				
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Df	Sum Sq	Mean Sq	F value	Pr(>F)
log_length	1	0.739	0.739	8.692	0.00344**	1	0.739	0.739	8.798	0.00325***	1	0.739	0.739	8.736	0.00337**
CONISS						4	0.653	0.163	1.943	0.103	4	0.653	0.163	1.930	0.105
log_length : CONISS											4	0.157	0.0393	0.464	0.762
residuals	307	26.110	0.085			303	25.457	0.084			299	25.300	0.0846		

B

	Res. Df	residuals	Df	Sum of Sq	F value	Pr(>F)
Model 2	303	25.457				
Model 3	299	25.300	4	0.157	0.464	0.762

C

	Res. Df	residuals	Df	Sum of Sq	F value	Pr(>F)
Model 1	307	26.110				
Model 2	303	25.457	4	0.653	1.943	0.103

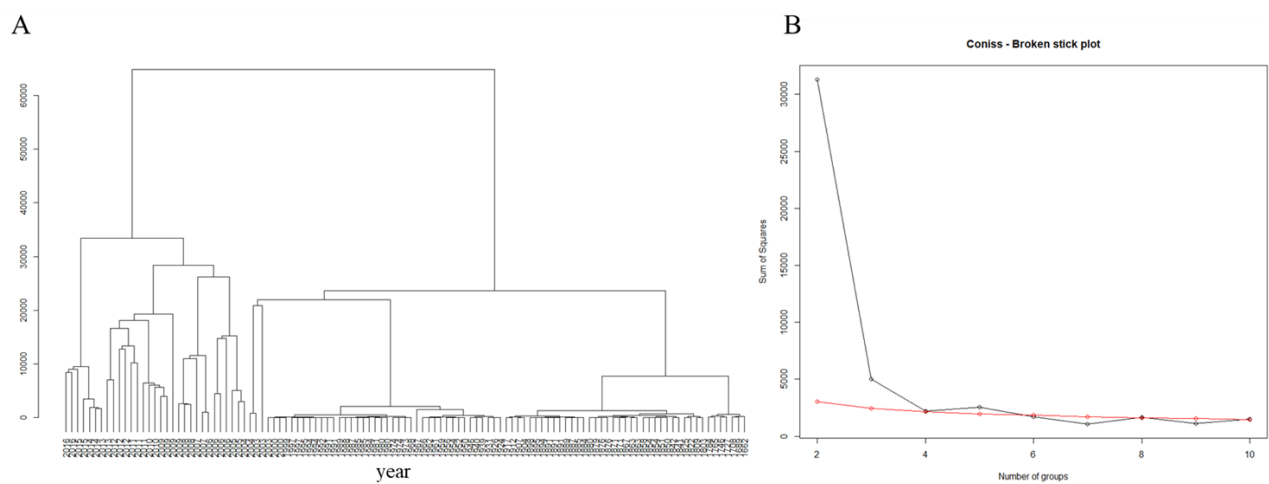


Figure S4-1. A. Cluster plot from the CONISS analysis for the cladoceran community. B. Results of the broken stick model to determine the number of significant groups in CONISS analysis (Red line represents the sum of squares with broken stick model. Black line represents the sum of squares with CONISS analysis).

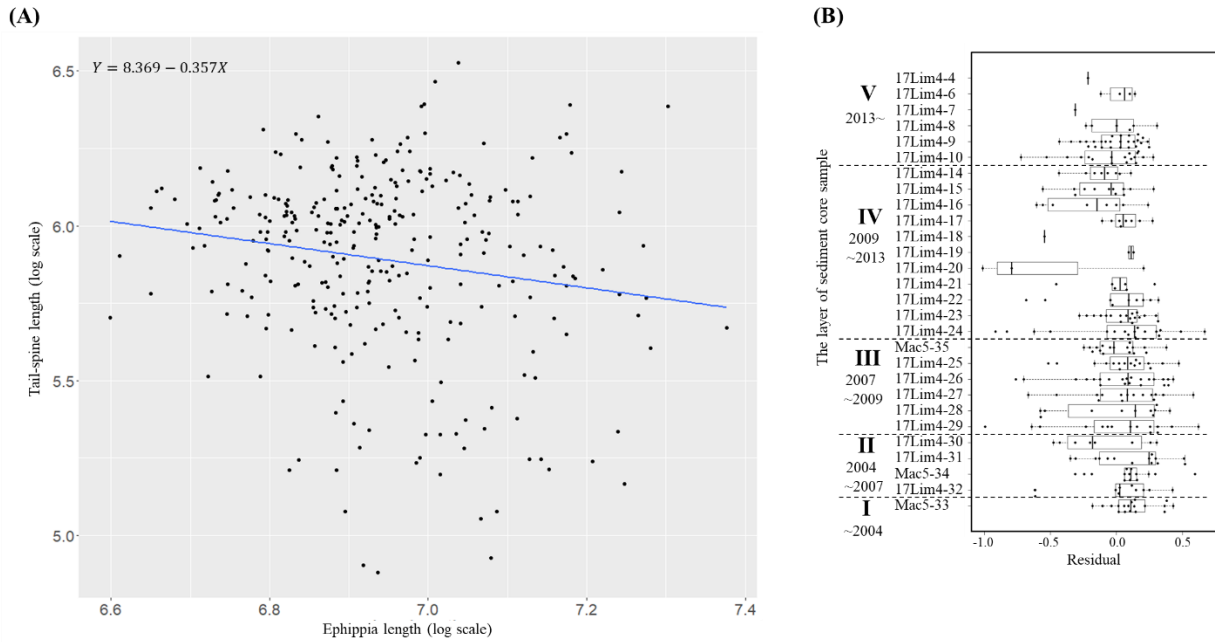


Figure S4-2. Residuals of tail-spine length from the allometric regression model against the ehippia length. The allometric relation between the tail-spine length and the ehippia length (A). Solid blue line is regression one of tail-spine length on the ehippia length (estimate = -0.357, SE = 0.121, t-value = -2.948, $p = 0.00344$). Each point is an individual ehippia. The change of the residuals as indicators of the defensive tail-spine length during the *D. pulex* population colonization process (B). The ANOVA analysis showed that residuals indicating the tail-spine length independent of the body size (response variable) did not significantly differed among CONISS period (explanatory variable) (Df = 4, Sum of square = 0.638, Mean square = 0.160, F value = 1.905, $P = 0.110$).

Chapter 5 Different photoperiodic responses in diapause induction can promote the maintenance of genetic diversity through the storage effect in *Daphnia pulex*.

Abstract

Understanding mechanisms that promote the maintenance of biodiversity (including genetic and species diversity) have been a central topic in ecology and evolutionary biology. Researchers have revealed that the storage effect is an important coexistence mechanism in temporally fluctuating environments owing to density-dependent covariance between the environment and competition as well as buffered population growth via, for example, dormant stages. While differences in diapause timing are important for the storage effect, few studies have examined the effects of diapause timing on the storage effect and the maintenance of genetic variation. I tested whether different photoperiod responses in diapause induction can promote the coexistence of two parthenogenetic (asexual) lineages of *Daphnia pulex*. Laboratory experiments showed that a competitively inferior genotype tended to produce diapausing eggs earlier than the other genotype. Theoretical model analyses suggested that such different photoperiod responses and interannual fluctuations in growth conditions can promote the coexistence of the two genotypes via the storage effect, as the competitively inferior genotype avoids competition by diapause induction. Although germination timing has been investigated as an important factor for the storage effect in plants, I show the potential importance of diapause timing induced by photoperiodic changes to promote the maintenance of genetic diversity.

Introduction

How is biological diversity (including genetic and species diversity) maintained despite ecological niche overlap? Researchers have investigated this question in ecology and evolutionary biology for decades (Hutchinson, 1959). One important factor that can promote the maintenance of genetic variation and species coexistence is the induction of dormancy. Dormant stages such as diapausing eggs, cysts, spores, and seeds are widespread in bacteria, fungi, algae, rotifers, inland-water crustaceans, and plants (reviewed in Weider, Jeyasingh, & Frisch, 2018). Previous theoretical and empirical studies demonstrated that dormant stages can prevent extinction and promote coexistence (Cáceres, 1997; Ellner, & Hairston, 1994; Turelli, Schemske, & Bierzychudek, 2001; Cáceres & Tessier, 2004;

Montero-Pau & Serra, 2011). The timing of dormancy induction may prevent the extinction of competitively inferior lineages (i.e., species or genotype) by the following two mechanisms: (i) the competitively inferior lineage becomes dormant earlier than the competitively superior lineage and (ii) the competitively inferior lineage becomes dormant later than the competitively superior lineage.

The first mechanism works as it is sometimes advantageous to become dormant. For example, when winter comes earlier than usual in temperate lakes, the competitively superior lineage may not be able to produce many diapausing eggs owing to the low temperatures. In such a case, coexistence becomes possible via the storage effect (Cáceres, 1997; Chesson, 2000); the competitively inferior lineage can take advantage in the years when winter comes earlier because they can produce abundant diapausing eggs due to early diapausing, and this inferior can persist population even in the year when winter comes later, favourable condition for competitive superior, by stored diapausing eggs. Previous studies have revealed the potential importance of temporally fluctuating environments in promoting the maintenance of genetic variation (Dempster, 1955; Haldane & Jayakar, 1963; Ellner, & Hairston, 1994; Turelli et al., 2001) and species coexistence (Hutchinson, 1961; Chesson & Warner, 1981; Chesson, 2000). The temporal storage effect promotes coexistence of competing species when one of them is competitively superior at some point in time (e.g., in dry years), whereas the other species dominates otherwise (e.g., in wet years) and population growth is buffered (Yuan & Chesson, 2015). Without buffered population growth, species with the highest geometric mean growth rate will dominate, and other species will go extinct. Several factors can buffer population growth; however, the most common factor is a dormant stage and the resultant generation overlap (Chesson & Warner, 1981; Ellner & Hairston, 1994). In addition, earlier dormancy of inferior lineages may also promote coexistence by buffering the effect of competition like that inferior competitors avoid intense competition resulting the decrease difference of growth rate between competitors.

The second mechanism works when the competitively superior lineage shows density-dependent dormancy induction (Montero-Pau & Serra, 2011). As dormant populations do not reproduce, the density-dependent production of dormant stages results in a negative density-dependent population growth. This negative density-dependent population growth of the competitively superior lineage can result in negative frequency dependence in community

dynamics, as the competitively inferior lineage can grow without competition after the induction of diapause of the competitively superior lineage (Montero-Pau & Serra, 2011; Aránguiz-Acuña & Ramos-Jiliberto, 2014). Therefore, interannual environmental variation is essential in mechanism (i), whereas coexistence is possible in stable environments with mechanism (ii). Despite the potential importance of maintaining biodiversity through the above two mechanisms, few studies have examined it empirically. Rather, previous studies have tended to focus on germination timing of dormant plant seeds (i.e., termination of dormancy instead of the induction of dormancy) (Pake & Venable, 1996).

In this study, I tested whether differences in diapause timing contribute to the maintenance of intraspecific genetic variation. I used two obligate parthenogenetic (asexual) lineages of *Daphnia pulex* found in Lake Fukami-ike (Nagano Prefecture, Japan). *Daphnia*, the keystone cladoceran group in lake ecosystems, has often been studied to understand mechanisms for the maintenance of genetic variation and species coexistence. Schaffner et al. (2019) reported that dominant genotypes in a temperate lake change seasonally, and diapausing egg banks may contribute to the maintenance of multiple genotypes. The timing of hatching and diapause induction is different between multiple *Daphnia* species that coexist in the same lake (Cáceres, 1997; Cáceres & Tessier, 2004; Hairston, Hansen, & Schaffner, 2000). Production of diapausing eggs is induced by several cues including temperature, food abundance, chemicals released from fish (kairomones), overabundance, and photoperiod (reviewed in Gyllström & Hansson, 2004). Photoperiod is an accurate predictor of seasonal changes and may thus be responsible for different diapause timings. However, the role of photoperiod responses in promoting the coexistence of competing lineages has seldom been discussed.

To test whether different photoperiodic responses in diapause induction contribute to the coexistence of two genotypes, I conducted two experiments using *D. pulex*. First, I measured the competitive ability of the two genotypes and examined the effects of photoperiod on the outcome of the competition. Second, I measured the tendency of producing diapausing eggs under different conditions of food abundance and photoperiod. Finally, I constructed and analysed a mathematical model to understand the conditions for coexistence of the two genotypes.

Materials and Methods

Experimental materials

The Japanese *D. pulex* is an obligate parthenogenetic and comprises four genetically distant lineages (JPN1–JPN4) originating from North American lineages (So et al., 2015). Although any one of the lineages singly appears in most Japanese lakes, multiple lineages coexist in a few lakes. As lineages and genotypes of Japanese *D. pulex* are the same species, they are presumed to need a similar ecological niche and to be in exploitative competition. I studied one of the lakes in which two lineages of *D. pulex*, JPN1 and JPN2, coexist. Prior paleolimnological research showed that JPN2 established first approximately in 2002–2003 and JPN1 migrated around 2013, and since then, the two lineages have coexisted in Lake Fukami-ike (Chapter 3). I used seven *Daphnia* clones of each lineage, JPN1 and JPN2; thus, I used 14 *Daphnia* clones in total. These clones were descendants of single females that were collected by hatching diapausing eggs isolated from ephippia preserved in the sediment core samples from Lake Fukami-ike, Japan. Lake Fukami-ike (35°19N, 137°49E) is located in Anan town, Nagano Prefecture. The lake is naturally formed and is currently eutrophic with a maximum depth of 7.8 m and a surface area of 2.2 ha (Figure1-2). I collected and analysed the varved sediment core samples from Lake Fukami-ike in previous chapters. In Chapter 3, I observed the population and genetic dynamics of the *D. pulex* population. The results suggested that a genotype (Jpn2C) belonging to JPN2 is dominant among *D. pulex* and was first established recently, and genotypes belonging to JPN1 (Jpn1A–C2T2) appeared around 2012. I isolated *D. pulex* ephippia from the layers in which the two lineages, JPN1 and JPN2, coexisted. Next, I decapsulated the ephippia and isolated diapausing eggs and exposed these eggs to hatching stimuli, which are conditions of a long-day photoperiod (16:8 h light (L): dark (D)) and 18 °C (Cambronero & Orsini, 2018). Hatched individuals were kept in monoclonal cultures in aged tap water under laboratory conditions (23 °C, 14:10 h L:D, fed 0.63 mg carbon/L of *Scenedesmus*). These clones were identified genetically based on mitochondrial markers in NADH dehydrogenase 5 (ND5) and the control region of mitochondrial DNA (So et al., 2015). Full details of these genetic identification processes are described in Chapter 3. Seven clones were randomly selected for each dominant type (Jpn2C) and JPN1 lineage (JpnN1–AC2T2) in Lake Fukami-ike.

Laboratory experiment 1: competition

To evaluate the competitive ability of each lineage when they coexist with another lineage in the water column, I prompted experimental competition under two photoperiodic conditions: long-day (14:10 L:D) and short-day (10:14 L:D). Temperature (18 °C) was common in both photoperiodic conditions. The experimental competition was conducted between two *D. pulex* lineages, JPN1 and JPN2, with all possible combinations of clones. Therefore, I had a total of 49 combinations. I had three replicate experiences for each combination under the two photoperiodic conditions. Experimental competition jars were filled with 180 mL aged tap water and four newborn female juveniles of each clone, one clone per lineage, were added to each jar. Newborn females for the experiment were obtained using the following process: I transferred clutched females from subculture lines to each experimental photoperiodic condition and the G0 offspring of each lineage born within a day was transferred to the same experimental competition 200 mL jar, which was filled with aged tap water that was refreshed every 4 d. I fed *Scenedesmus* at high-food concentrations (0.63 mg C/L) every two days until 15 d, with day 0 being the start of the experiment. After that, I continued the experiment with no-food until 30 d. At days 15 and 30, I conducted sampling. Before sampling, the contents of all jars were mixed. At day 15, 40 mL from each jar was poured into a petri dish and all animals present were fixed with 99% EtOH in a 1.5 mL micro tube. At 30 days, all animals that lived in each experimental jar were fixed with 99% EtOH in a 5 mL micro tube. Fixed animals were stored at -30 °C.

The change in the frequency of each clone was analysed based on the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method following Ohtsuki and Urabe (personal communication). Individuals (8–16) were isolated from each sample and fixed with 95 % EtOH, and then, they were dried for a day. To extract DNA from these individuals, I added 25 µm Quick Extract DNA Extraction Solution (Lucigen, Wisconsin, US) per individual and subjected them to thermal shock of 60 °C for 2 h and 95 °C for 20 min. PCR amplification of fragments of NADH dehydrogenase 5 (ND5) of mitochondrial DNA (775 bp) was performed using ExTaq Hot start Version (Takara) and the primer set comprising DpuND5b and DpuND5n. Each 5 µL reaction consisted of 0.5 µL of extracted DNA, 0.02 µL of TaKaRa Ex Taq™ HS, 0.4 µL of dNTP, 0.5 µL of 10 × ExTaq™ buffer and 0.2 µmol L⁻¹ of each primer. The thermal cycling conditions were as follows: 94 °C for 2 min, followed by 30 cycles of 95 °C for

30 s, 48 °C for 30 s, and 72 °C for 1 min and finished at 72 °C for 10 min. I then conducted PCR-RFLP with two restriction enzymes, Hpy 188III and MnlI. The lineage of each individual was identified their lineage based on the band pattern of electrophoresis. Each 10 µL reaction consisted of 5 µL of PCR products, 1.0 µL of CutSmart Buffer (New England Biolabs), and 0.05 µL of each restriction enzyme. The thermal shock consisted of 37 °C for 2 h and 65 °C for 20 min. Electrophoresis was performed at 50 V for 60 min on a 2 % agarose gel. Based on PCR-RFLP, I counted the individual numbers of JPN1 and JPN2.

The results of each experimental competition were evaluated statistically to determine whether JPN1 or JPN2 dominated. I used the *t*-test to check whether the ratio of the two lineages changed significantly at the end of competition (30 days) from that at 0.5 (0 days). I then calculated the dominance rate of JPN2 as the ratio of the number of competitive match combinations in which the frequency of JPN2 significantly exceeded that of JPN1 at day 30 to the total number of competitive matches (49 matches). To evaluate the competitive ability of each lineage in each photoperiodic condition, I compared the dominance rate of JPN2 between the long-day and short-day conditions using “prop.test” function.

Laboratory experiment 2: life history measurement

I measured the diapause rate of 14 clones in each photoperiodic condition (long-day or short-day). I used F3 offspring to reduce potentially confounding maternal and grandmaternal effects (Alekseev & Lampert, 2001). First, clutched adult females were isolated from subculture lines and reared individually in 200 mL jars under long-day or short-day experimental photoperiodic conditions. When the F2 offspring were born, they were reared individually in 200 mL jars under four experimental conditions: long-day (14:10 L:D) and high-food (0.63 mg C/L), long-day and low-food (0.063 mg C/L), short-day (10:14 L:D) and high-food, and short-day and low-food. I reared F3 female newborns individually in 50 mL plastic tubes under the above-mentioned four experimental conditions until the third clutch. I changed the water and fed them chemostat-grown *Scenedesmus* (UTEX1359) every two days. During this experiment, I observed the reared females daily and recorded whether they had asexual eggs or ephippia or released asexual offspring or ephippia. When asexual offspring were born, I detected their sex and counted the number of female and

male offspring under a stereo microscope (Nikon smz1500). I calculated the diapause rate in each clutch of each clone as the ratio of ephippial females to the total number of reared individuals. In addition, I calculated the intrinsic growth rate (r) based on the data until the third clutch of each clone using the Euler equation (Roff, 2001): $r = \int_{t=1}^{\infty} l_x m_x e^{-rx}$. Here, l_x represents the proportion of survivors at day x and m_x represents the number of offspring released on day x . To test the effect of lineage (JPN1 or JPN2), photoperiodic conditions, food conditions, and their interactions on diapause rate and intrinsic growth rate analysis was performed using a three-way ANOVA. I then compared the diapause rate and intrinsic growth rate between experimental conditions by multiple comparisons based on the Tukey's honestly significant difference (HSD) test followed by Bonferroni correction. All statistical analyses were performed using R version 3.5.2 (R core Team, 2018), and $P < 0.05$ was considered significant.

Theoretical model analysis

Using a theoretical model, we tested whether competitive interactions and dormancy rates of each lineage observed in the laboratory experiment promote coexistence. We analysed the diapausing egg dynamics of two lineages, JPN1 and JPN2, based on the lottery model (Ellner & Hairston, 1994; Cáceres, 1997). The number of diapausing eggs of lineage j ($j = 1, 2$) at year t ($N_{j,t}$) changes as follows:

$$N_{j,t+1} = \{[(1 - H_j)]s_j + H_j Y_j\} w_j N_{j,t}, \quad (5-1)$$

where H_j is the hatching rate of diapausing eggs, s_j is the probability of survival of diapausing eggs that did not hatch in spring over summer in the egg bank, Y_j is the per capita fecundity, and w_j is the probability of survival of diapausing eggs in the egg banks during winter. Assuming a saturated growth model (Levin, Cohen, & Hastings, 1984), Y_j can be written as:

$$Y_j = \frac{KR_j(e_t)}{\sum_{k=1}^2 H_k R_k(e_t) N_{k,t}}, \quad (5-2)$$

where K is the total production of diapausing eggs after the growth season and $R_j(e_t)$ is the relative fecundity of lineage j at year t represented as cumulative production of diapausing eggs by the end of growing season, e_t :

$$R_{j,t} = \frac{a_j}{1 + \exp[b_j(c_j - e_t)]}, \quad (5-3)$$

where a_j is the maximum fecundity, meaning the maximum production of diapausing eggs reflecting the abundance of the active population in the water column, b_j is the shape function of diapausing egg production, and c_j is the threshold to reach half of the maximum production of diapausing eggs. We assumed an inter-annual fluctuation in the length of the growth season. In equation 5-3, e_t is the end of the growth season, fluctuating according to the normal distribution of mean μ and variance σ^2 .

When only one lineage exists, the equilibrium abundance of diapausing eggs is $\bar{N}_j = Kw_j / (1 - \gamma_j w_j)$ and the amount of generation overlap is $\gamma_j = (1 - H_j)s_j$. Coexistence of two lineages occurs when each lineage is able to increase when rare, meaning that the geometric mean of the following invasion growth rate is larger than 1:

$$\frac{N_{i,t+1}}{N_{i,t}} = \left[(1 - H_j)s_j + \frac{KH_i R_{i,t}}{H_j R_j \bar{N}_j} \right] w_j. \quad (i,j=1,2) \quad (5-4)$$

and the amount of generation overlap is $\gamma_j = (1 - \gamma_j w_j)$.

Coexistence of two lineages occurs when each lineage is able to increase from low numbers, meaning that the invasion growth rate is larger than 1:

$$\frac{N_{i,t+1}}{N_{i,t}} = \left[(1 - H_j)s_j + \frac{KH_i R_{i,t}}{H_j R_j \bar{N}_j} \right] w_j. \quad (i,j=1,2) \quad (2.4)$$

Based on the laboratory experiments, we assumed that JPN1 starts to produce diapausing eggs earlier than JPN2 ($c_1 < c_2$), JPN2 can produce more diapausing eggs at the end of the growth season than JPN1 ($a_1 < a_2$) because

of their highly competitive ability in the water column. We evaluated the effects of interannual growth season fluctuation, dormancy strategy of the two lineages, and competitive ability in the water column to coexist using the described theoretical models.

Results

Competitive ability of the active population and diapause rate

The ratio of the competitive combinations that JPN2 dominated was significantly higher in the short-day condition (46.94%) than in the long-day condition (Figure 5-1, 6.12%, $P < 0.001$, prop.test). No competitive match was dominated by JPN1. The change in frequency of each lineage and the results of competition for all experimental combinations are shown in Figure S5-1.

The two lineages of *D. pulex* showed different diapause responses to the photoperiodic conditions (Figure 5-2). JPN1 showed a higher diapause rate in the short-day condition than JPN2, regardless of food concentration. Statistical analysis supported the effect of photoperiodic conditions on the diapause rate ($P < 0.001$, Table 5-1). This analysis also supported that the difference in diapause response to photoperiodic conditions depending on lineage showed as the effect of interaction between lineage and photoperiodic conditions ($P = 0.013$, Table 5-1). In addition, the intrinsic growth rate was significantly affected by only food concentration ($P < 0.001$, Fig S2, Table S5-1).

Mathematical analysis

Figure 5-3 shows the assumptions of the theoretical model (Figure 5-3 A, B) and the dynamics when the two lineages can coexist (Fig 5-3 C). Assuming that the competitively inferior JPN1 switches to the diapause phase earlier than the dominant JPN2 based on the laboratory experiments, the two lineages are able to coexist under an interannual fluctuation in growth season length (Figure 5-3). Similarly, Figure 5-4 A shows the effect of interannual fluctuations in growth season length. If there is no interannual fluctuation in growth season length ($\sigma^2 = 0$), the coexistence of the two lineages cannot be maintained. Furthermore, the coexistence area expanded as the fluctuation in growth season length increased (Figure 5-4 A). Figure 5-4 B shows that the coexistence was larger as c_1 , the timing of starting to produce diapausing eggs in JPN1 was lower. Conversely, b_1 , the rate of increase in diapausing egg reproduction in

JPN1 as the season progressed, showed little effect on the coexistence area (Figure S5-3 A). As a_1 , which is the maximum production of diapausing eggs of JPN1 reflecting the active population abundance, became closer to a_2 , the coexistence area became a little smaller (Figure S5-3 B). As w_1 , which is the survival rate of diapausing eggs of JPN1 over winter, became larger, the coexistence area became slightly smaller (Figure S5-3 C).

Discussion

In the present study, I tested whether diapause responses to photoperiodic conditions differ between intraspecific genotypes and whether these variations in photoperiodic responses contribute to the maintenance of coexistence among intraspecific competitors. The present results suggest that conspecific genotypes show various diapause responses to photoperiodic conditions, and this variation can contribute to the maintenance of the coexistence of intra-specific genotypes through the storage effect by combining with interannual fluctuations in growth conditions.

The results of the competition experiment showed that there was no competition match in which JPN2 was dominant and the diapause rate of JPN1 was higher in the short-day condition than in the long-day condition (Figure 5-1, Table 5-1). These results suggest that the competitive ability of the active population is different between the two lineages (JPN1 and JPN2) and that of JPN2 is superior, especially under short-day conditions. As I tested the variation in diapause rate in response to photoperiodic conditions between lineages using a laboratory experiment, JPN1, whose active population showed a lower competitive ability than that of JPN2, showed significantly higher diapause rates in the “short-day and low-food” and “the long-day and low-food” conditions (Figure 5-2). Statistical analysis also supported that the diapause response to photoperiodic conditions was significantly different between lineages (Table 5-1). Furthermore, all interactions between lineage, photoperiodic condition, and food concentration also significantly affected the diapause rate (Table 5-1), suggesting that diapause responses to photoperiodic conditions and food concentration were different between lineages. Given the results of the laboratory experiment, it is possible that JPN1, which is inferior to JPN2 in the water column, avoids competition with JPN2 and invests in a diapausing egg bank through which JPN1 more easily switches to the diapause phase from the clonal reproductive phase in response to short-day stimuli. Such competition buffering through diapause stages is consistent with

concepts which suggest these mechanisms in the maintenance of genetic and species diversity by the storage effect rather than the effect of the diapause stage on the competitive ability of competitively superior organisms (Montero-Pau & Serra, 2011). Based on these results, I hypothesised that intraspecific differences in diapause responses to photoperiodic conditions facilitate the coexistence of intraspecific lineages by temporal niche differentiation through the storage effect.

The above hypothesis was tested and supported through mathematical analysis. According to the mathematical analysis, the two lineages cannot coexist without the standard deviation σ of the end date of the growth season, whereas the coexistence area became larger as σ increased (Figure 5-4 A). Interannual fluctuations can be caused by interannual environmental fluctuations such as temperature. These results suggest that the coexistence of two intraspecific lineages cannot persist without year-to-year fluctuations in living environmental conditions. Furthermore, c_I , which indicates threshold switching to the diapause phase for JPN1, also affected the coexistence area. The lower the c_I , the larger was the coexistence area (Figure 5-4 B). In contrast, b_I , the rate of increase in diapausing egg reproduction in JPN1 as the season progressed, showed little effect on the coexistence area (Figure S5-3 A). Based on these results, the early transition to the diapause phase of JPN1 is more important than diapause sensitivity to season. These results suggest that early switching to the diapause phase in JPN1, which is inferior to JPN2 in terms of active population competition, can enhance the possibility of coexistence of intraspecific lineages because of environmental fluctuations. It is possible that when winter comes earlier than usual in temperate lakes, the competitively superior lineage, JPN2, which transits to diapause phase later, may not be able to produce many diapausing eggs owing to the low temperatures. The positive effect of environmental fluctuation on maintaining the species and genetic diversity is consistent with previous traditional concepts for non-equilibrium coexistence (Hutchinson, 1961) and results of previous empirical studies (Pake & Venable, 1995; Herben, Krahulec, Hadincová, Pecháčková, & Wildová, 2003; Jiang & Morin, 2007; Shurin et al., 2010). Moreover, coexistence mechanisms by the temporal storage effect also assume environmental fluctuation (Chesson, 2000), and several previous studies have reported that environmental fluctuation and dormant stages promote coexistence (Cáceres, 1997; Adler, HilleRisLambers, Kyriakidis, Guan, & Levine, 2006). Furthermore, I tested the effect of several trade-offs to

coexistence. As the difference in a between JPN1 and JPN2 became smaller, the coexistence area became slightly smaller (Figure S5-3 B). Because parameter a represents active population abundance and competitive ability in a water column, the trade-off between the competitive ability of the active population and the early transition to diapause can promote coexistence. A trade-off between active population growth and diapausing egg production was reported for other *Daphnia* (*Daphnia mitsukuri*, Gu et al., 2020). Furthermore, such a trade-off contributes to coexistence in phytoplankton species (Fox, Nelson, & McCauley, 2010). The higher the w_1 , the smaller is the probability of survival of diapausing eggs in the JPN1-led coexistence (Figure S5-3 C). This result suggests that an increase in diapausing egg quality of JPN1, which switches early to a diapause phase, can prevent the coexistence of two lineages, suggesting that a trade-off between the quality of diapausing eggs and early transition to diapause phase probably promotes intraspecific coexistence.

Integration of the laboratory experiments and mathematical analysis suggested the following. First, an early switch to the diapause phase by a competitor genotype, which is inferior in the active community, can facilitate the coexistence of intraspecific genotypes through the storage effect in *D. pulex* population of Lake Fukami-ike. Second, one of the mechanisms that can cause such intraspecific differences in diapause strategies is the intraspecific variation in the photoperiodic response.

Our results support the hypothesis that the diapause stage promotes intraspecific and interspecific coexistence by buffering competition. JPN1, which is inferior in the active population, possibly avoids competition with JPN2 by an early transition to the diapause stage. This tendency toward diapause in which the inferior organism switches to a diapause phase earlier is consistent with the pattern predicted by the coexistence hypothesis through the long-term change of relative competitive ability rather than the coexistence hypothesis through the density dependent diapausing induction in superior. Based on the latter, the coexistence mechanisms based on the effect of the diapause stage on the competitive ability of superior competitor, the superior competitor may induce diapause earlier, and this coexistence mechanism is suggested to promote local and short-term coexistence (Montero-Pau & Serra, 2011). In contrast, the intraspecific difference in diapause response to photoperiod observed in this study suggests the possibility that it contributes to the maintenance of coexistence for long generation time by theoretical simulation.

These results and those from previous studies suggest that the intraspecific and interspecific diversity in the diapause strategy can contribute to the maintenance of coexistence and biological diversity at various temporal and spatial scales through multiple mechanisms. Recently, phenology was suggested to possibly contribute to coexistence (reviewed in Rudolf, 2019). The coexistence through different diapause strategies showed in our results are consistent with this possibility. Photoperiodic conditions may contribute as one of the mechanisms that differentiates phenology like germination timing in coexistence of plant species (Pake & Venable, 1996).

Unfortunately, as the populations of *D. pulex* have been reduced in Lake Fukami-ike in recent years (Chapter 2; Otake et al. 2020) because of the increase in planktivorous *Lepomis macrochirus*, I did not observe the dynamics of both the active population and the egg bank in the natural system in this study. Therefore, I cannot determine whether the two lineages of *D. pulex* transit to the diapause phase at different times, promoting coexistence in the natural system. I compensated for this limitation through theoretical model analysis and showed that the two competitive lineages can coexist for a long generation time owing to environmental fluctuations and different diapause timings. The dynamics of the active population and of egg banks at the genotypic level in the lake where multiple competitive lineages simultaneously coexist will provide us with the data on the difference in timing to hatch in addition to the timing to transit to the diapause phase. Photoperiodic conditions also affect termination (hatch or germination) of diapausing eggs in natural systems (Gyllström, 2004), and the termination response to photoperiod can also affect coexistence, similar to the diapause response to photoperiod, as shown by previous studies in competitive species (plant species (Pake & Venable, 1996); cladoceran species (Cáceres & Tessier, 2004)). Although our theoretical model hypothesised that the hatching from the diapausing bank in the new growth season occurs at the same time point in both lineages, early hatching of the inferior competitor JPN1 could promote coexistence by allowing growth in the season without JPN2. Furthermore, measuring the hatching and survival rates in winter of the diapausing eggs produced in a year will allow us to test whether the trade-off between the quality of diapausing eggs and the transition to the diapause phase promotes coexistence, as suggested by our theoretical model (Figure S5-3 B, C).

I did not analyse the difference in Tilman's R^* (Tilman, 1982) between the two lineages in this study, and

thus, I cannot discuss the coexistence based on relative nonlinearity (Armstrong & McGehee, 1980). The feedback between fluctuation in resource and replacement by dominant competitor possibly contributes to the coexistence of the two *D. pulex* lineages in addition to the storage effect as suggested by a coexistence hypothesis based on relative nonlinearity. For *Daphnia*, threshold food concentration has frequently been measured to indicate competitive ability (Kreutzer & Lampert, 1999) based on R^* , suggesting intraspecific and interspecific differences in R^* (Maruoka & Urabe, 2020) and different responses of R^* to environmental fluctuation (e.g., food element, Iwabuchi & Urabe, 2012). Although I compared competitiveness based on a competition experiment using co-cultures, measurement of threshold food concentration may enable us to empirically test the theoretical suggestion regarding the relative contribution to coexistence of the storage effect and relative nonlinearity (Yuan & Chesson, 2015). Some previous research that evaluated the relative importance of multiple coexistence mechanics, including the storage effect and relative nonlinearity in a grassland community, suggested that the storage effect contributes relatively little to stable coexistence (Armitage, 2019; Zepeda & Martorell, 2019).

In addition, the intraspecific difference in diapause strategy could have affected the establishment of each lineage. In Lake Fukami-ike, paleolimnological analysis and population genetic analysis of diapausing eggs revealed that JPN2 was established earlier than JPN1 (Chapter 3). Further evolutionary and genetic research can contribute to our understanding of evolutionary dynamics with intraspecific interaction and its effect on community structure. In addition, comparison the diapause response between the lake which multiple lineages coexist and the lake which only one lineage appears will be also effective to examine the adaptive response to intraspecific competition.

Conclusion

This study showed that the Japanese *D. pulex* lineages whose active populations have different competitive abilities, have intraspecific variation in diapause responses to photoperiodic conditions, and this variation in diapause strategy can promote the coexistence of these lineages through the storage effect. This perspective supports the importance of the storage effect through the dormant stage in maintaining diversity and can contribute to

understanding how genetic and species diversity are maintained in the natural environment. Life-history strategies and photoperiodic stimuli can contribute much more than previously thought to the coexistence of competitors and the maintenance of diversity.

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Table**Table 5-1.** Difference in diapause rate between lineages, photoperiod, and food concentration estimated by ANOVA (significance was considered at $P < 0.05$)

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
lineage	1	1.553	1.553	40.065	<0.001
photoperiod	1	0.767	0.767	19.797	<0.001
food	1	1.017	1.017	26.238	<0.001
lineage: photoperiod	1	0.256	0.256	6.611	0.013
lineage: food	1	0.757	0.757	19.544	<0.001
photoperiod: food	1	0.026	0.026	0.674	0.416
lineage: photoperiod: food	1	0.017	0.017	0.439	0.511
residuals	48	1.860	0.039		

Figures

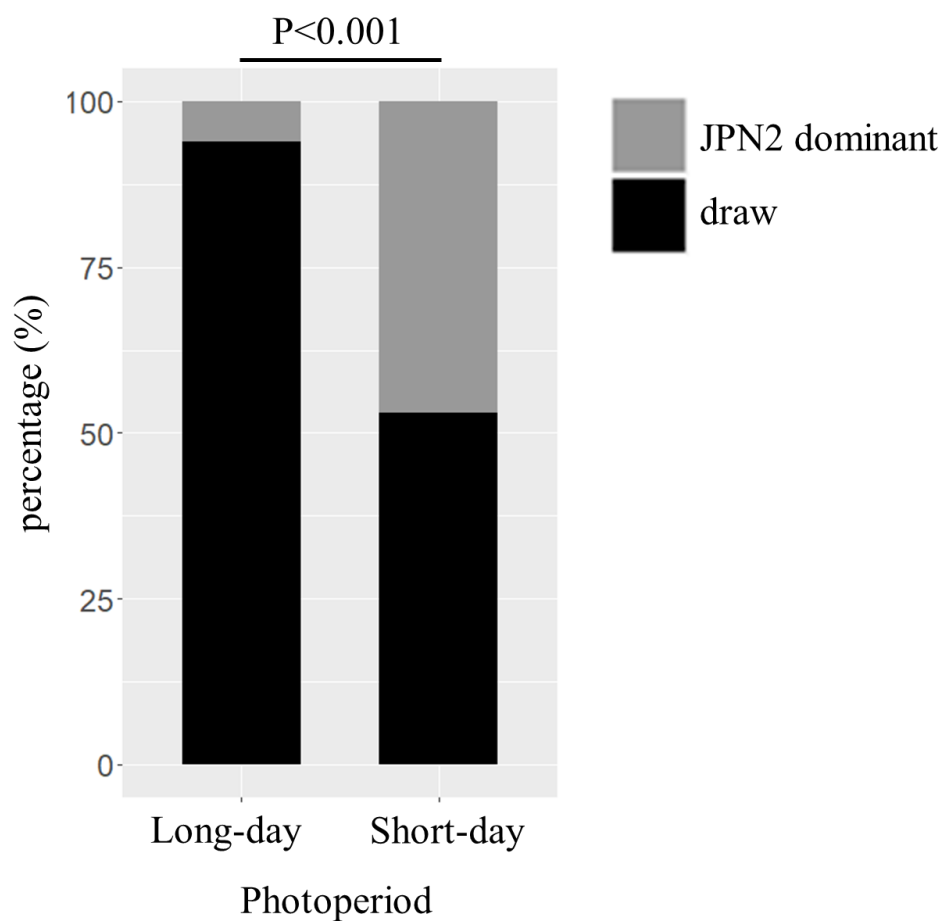


Figure 5-1. Ratio of the number of competitive matches that JPN2 dominated (grey) or drew (black) on day 30 to the total number of matches under long-day condition (14:10 h light:dark) or short-day condition (10:14 h light:dark). No competitive match was dominated by JPN1. Competitive results were detected using the *t*-test. Whether competitive results were different between long-day and short-day was tested using the prop.test (significance was considered at $P < 0.05$).

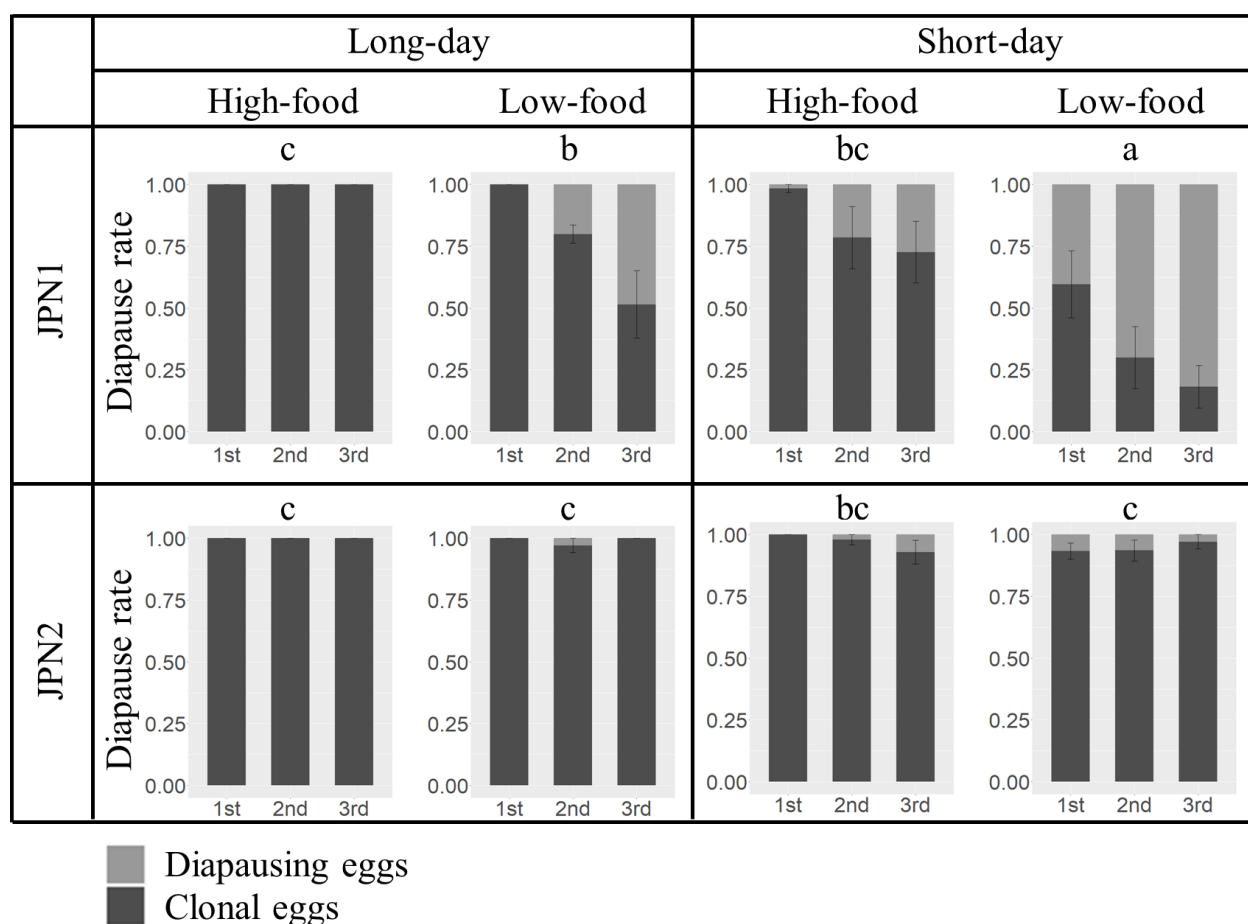


Figure 5-2. Diapause rate at 1st to 3rd clutch of each lineage under each photoperiodic condition, long-day (14:10 h light:dark) or short-day (10:14 h light:dark), and each food condition (high-food (0.63 mg C/L) or low-food (0.063 mg C/L)). The diapause rate of each lineage in each condition was compared using the Tukey HSD test (significance was considered at $P < 0.05$; a-b-c: $P < 0.005$). The error bars indicate the standard error.

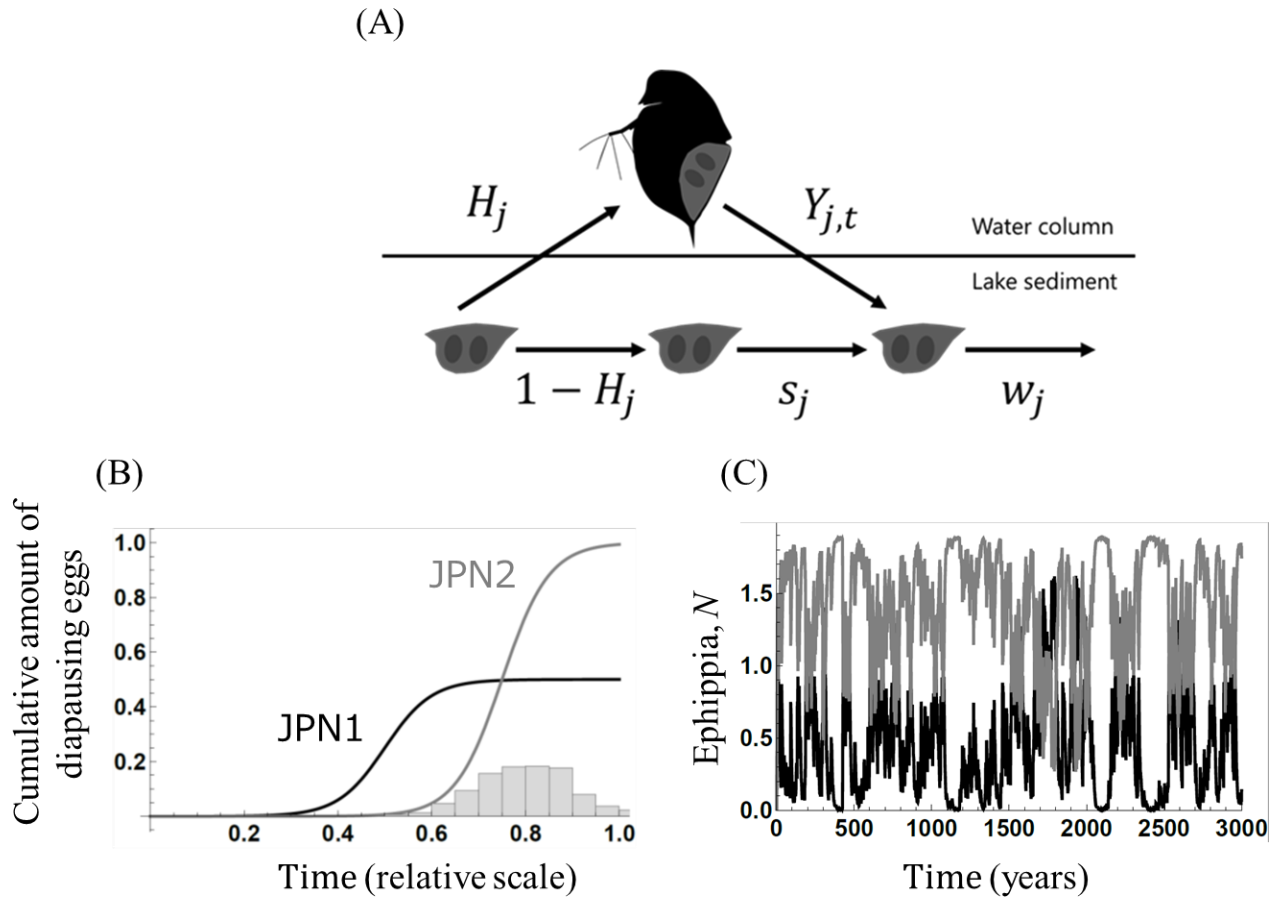


Figure 5-3. Assumptions of the theoretical analysis and one of the conditions that allows the two lineages to coexist. The dynamics of diapausing eggs in equation 5-1(A). In panel B, lines are cumulative curve of diapausing eggs production (black line is JPN1 and grey line is JPN2). Histogram in panel B indicates the inter-annual fluctuation in end date of growth season e_t (in this panel, $\mu=0.8$, $\sigma=0.1$). Based on the laboratory experiments, the theoretical model assumed that although JPN1 started shifting the dormant phase earlier than JPN2, the total production of diapausing eggs is more in JPN2 than in JPN1 owing to a high growth rate in the active population of JPN2 (B). If the end date of growth season fluctuated among the years as shown by the histogram (B), the two lineages can coexist for a long generation time (C).

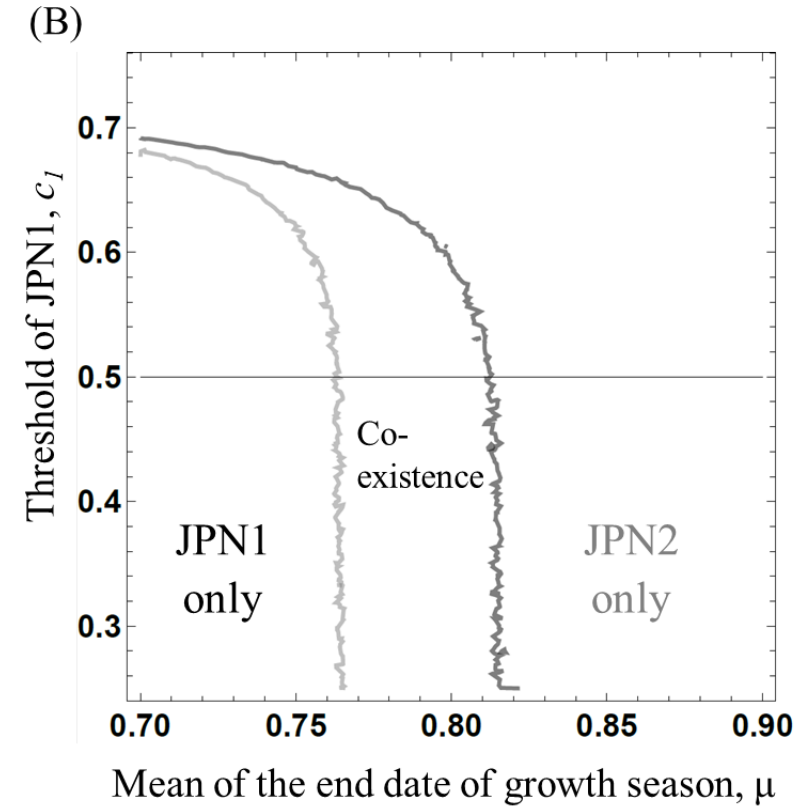
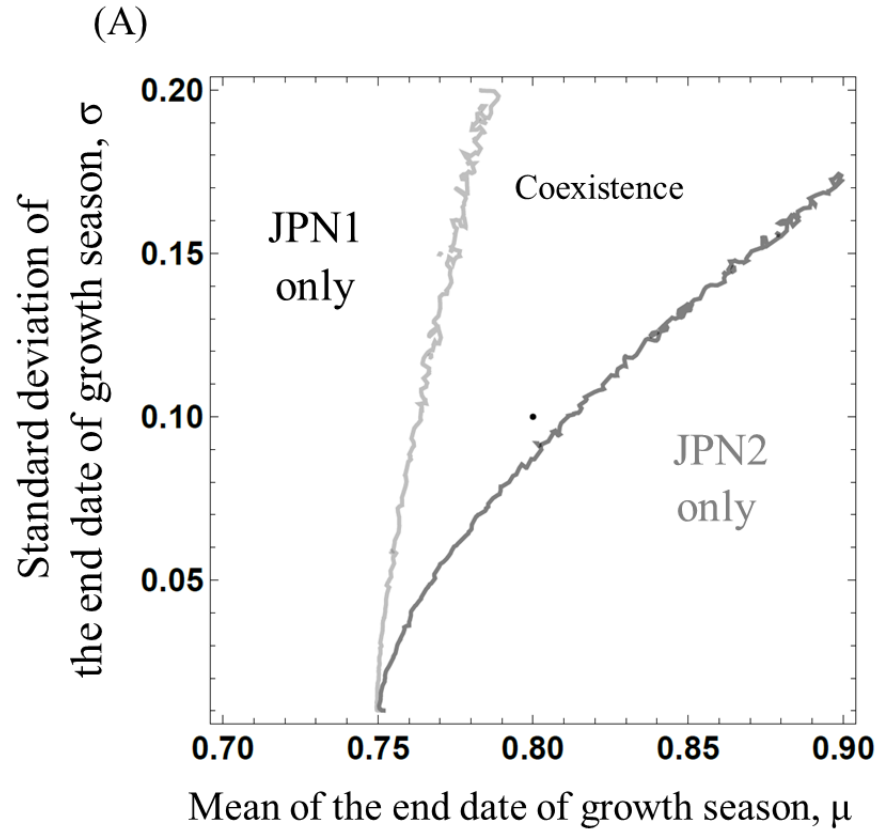


Figure 5-4

Effects of fluctuation in growth season length among years (A) and the diapause timing of the inferior competitor JPN1 to the coexistence of the two lineages suggested by the theoretical model analysis. Lines are invasion growth rate (black line is JPN1 and grey line is JPN2; equation 5-4). Horizontal axes are the mean of normal distribution that the end data of growth season follows. Coexistence occurs in the area where the invasion growth rate of both JPN1 and JPN2 are positive. The invasion growth rate of JPN1 and JPN2 is positive on the left of the black line and right of the grey line, respectively. The black point is one of the coexistence conditions (Figure 5-3C) and the dynamics of diapausing egg abundance at this point is shown in Figure 5-3B.

Supporting Information

Table S5-1. Difference in intrinsic growth rate between lineages, photoperiod, and food concentration estimated by ANOVA (significance was considered at $P < 0.05$)

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
lineage	1	0.00039	0.00039	0.150	0.700
photoperiod	1	0.00364	0.00364	1.399	0.243
food	1	0.0737	0.0737	28.346	<0.001
lineage: photoperiod	1	0.00016	0.00016	0.060	0.807
lineage: food	1	0.00294	0.00294	1.132	0.0293
photoperiod: food	1	0.00027	0.00027	0.105	0.748
lineage: photoperiod: food	1	0.00091	0.00091	0.349	0.557
residuals	48	0.128	0.00260		

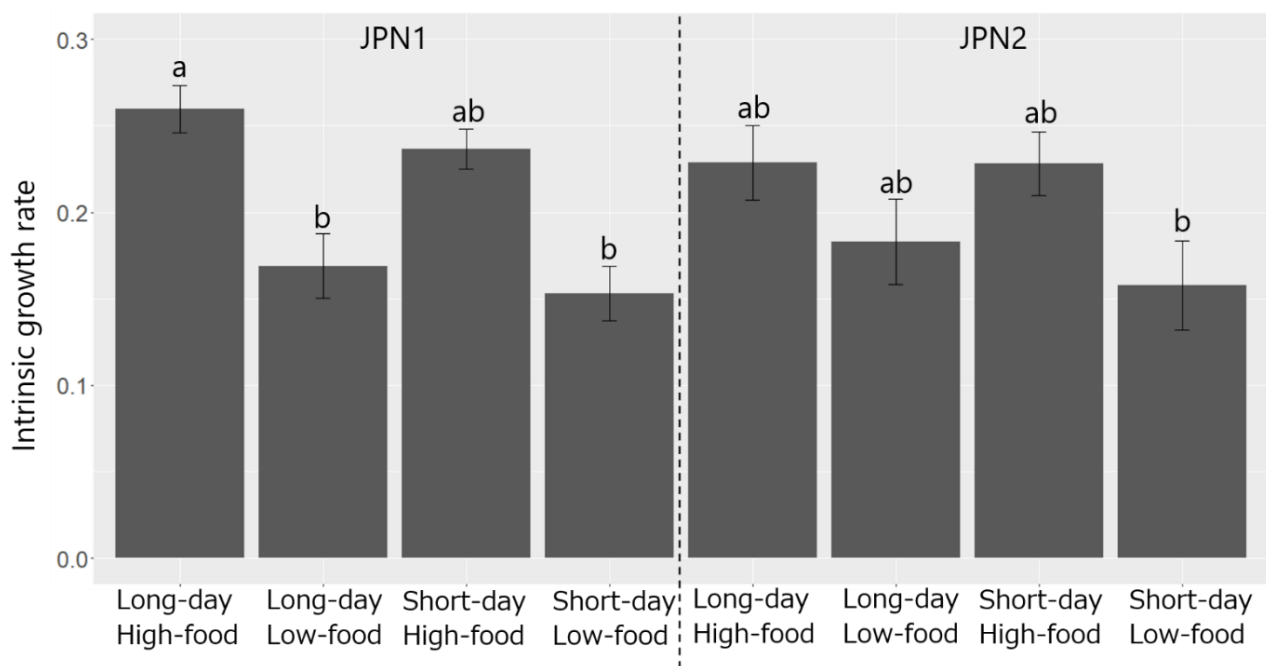


Figure S5-1. Intrinsic growth rate of each lineage under each photoperiodic condition, long-day (14:10 h light:dark) or short-day (10:14 light:dark), and each food condition (high-food (0.63 mg C/L) or low-food (0.063 mg C/L)). The intrinsic growth rate of each lineage in each condition was compared using the Tukey HSD test (significance was considered at $P < 0.05$; a-b-c: $P < 0.005$). The error bar is the standard error.

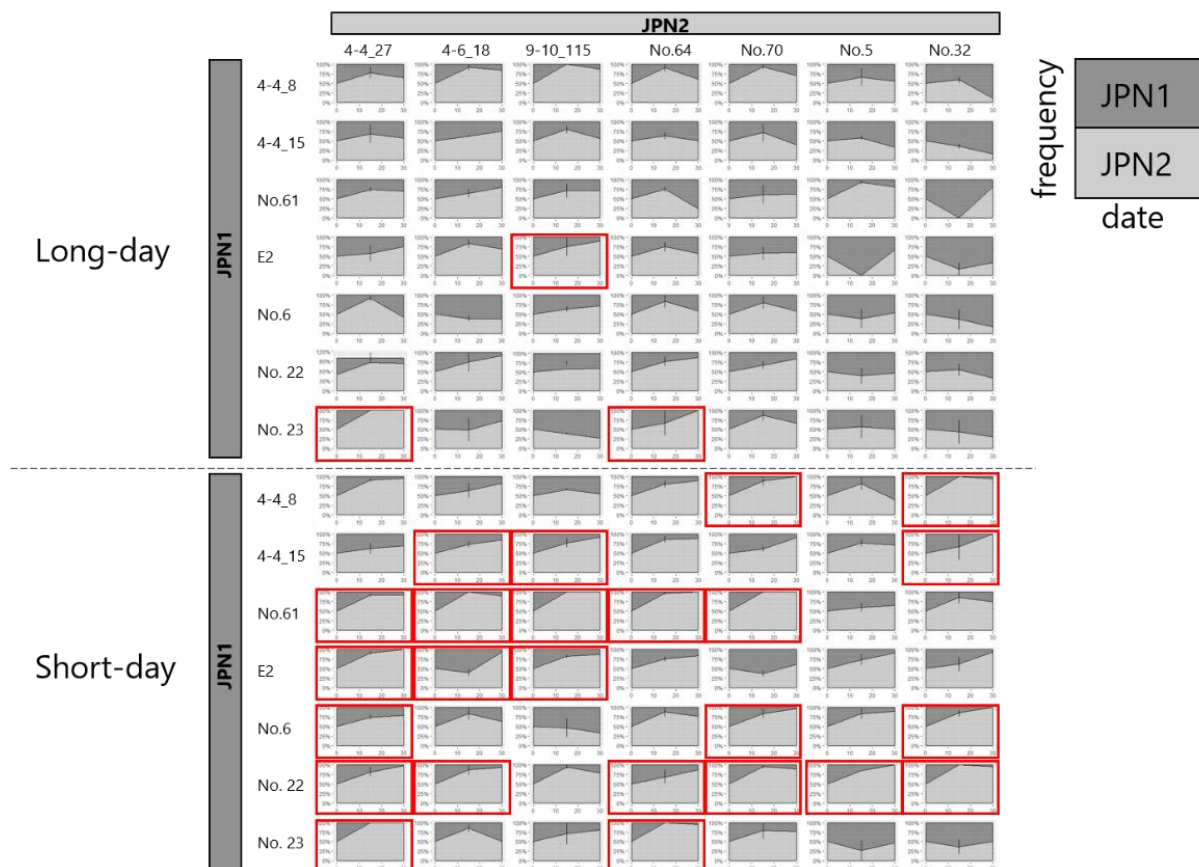


Figure S5-2. Results of each competitive match. The temporal change in frequency in each genotype. Red frames indicate that the ratio of JPN2 to JPN1 at day 30 changed significantly from 1:1 by *t*-test (significance was considered at $P < 0.05$; a-b-c: $P < 0.005$). The error bar is the standard error.

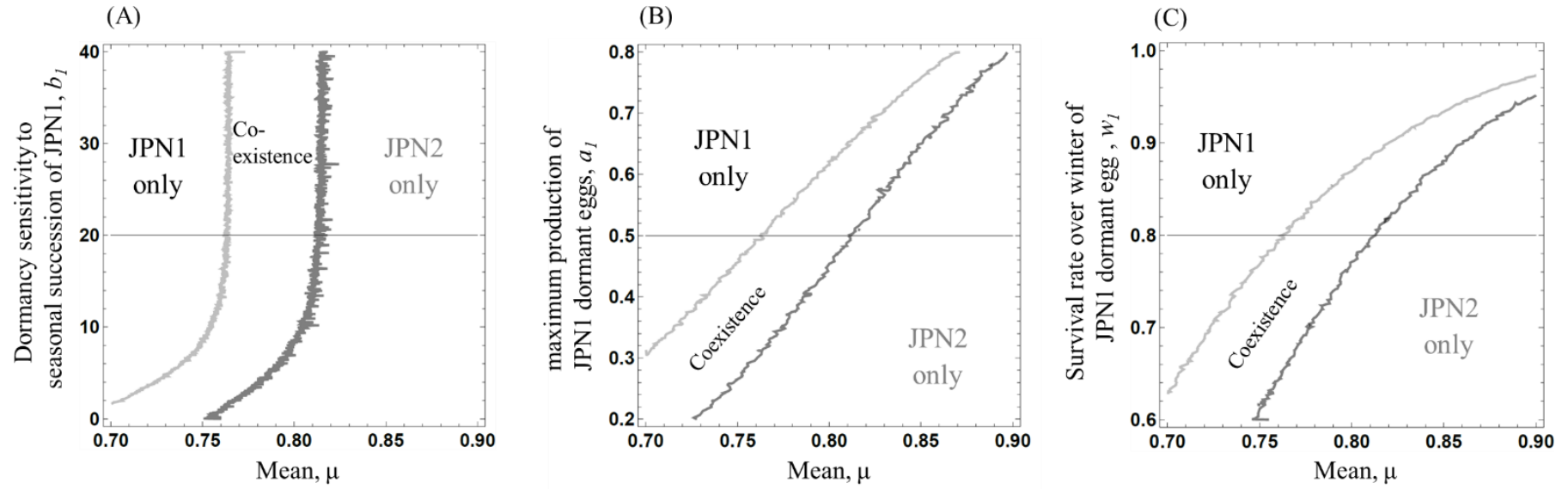


Figure S5-3. Effect of parameter b_I , a_I , and w_I on coexistence. The effect of b_I , increased rate of dormant eggs reproduction as the season progress in JPN1, means the effect of dormancy sensitivity (A). The effect of a_I , the maximum production of dormant eggs of JPN1 reflecting active population abundance, means the effect of trade-off between growth rate and diapause (B). The effect of w_I , survival rate over winter of JPN1 dormant eggs, means the effect of dormant egg quality (C). Lines indicate invasion growth rate (black line is JPN1 and grey line is JPN2; equation 5-4). Horizontal axes are the mean of normal distribution that the end data of growth season follows. Coexistence occurs in the area where invasion growth rate of both JPN1 and JPN2 are positive. The invasion growth rate of JPN1 and JPN2 is positive on the left of the black line and right of the grey line, respectively.

Chapter 6 General Discussion

Summary of the present doctoral thesis

In the present study, I simultaneously observed the multiple levels that compose a natural lake ecosystem, right from the genetic structure of *Daphnia pulex* population to the cladoceran community and the ecosystem including its abiotic environment. I found the multiple interactions between ecological levels, the cascading effects from higher levels to lower levels, in natural lake ecosystem. I also confirmed that these interactions may occur between distant ecological levels, for instance community to phenotype. These results support that interactions between ecological levels, including cross-level interaction, commonly exist in natural systems. I also observed multiple interactions between ecological levels during the initial stage of colonization process, including those that could relate to successful colonization. These observations suggest that colonization process and the cross-ecological level interactions which occur during the process could affect later ecosystem structure and ecological changes. Furthermore, predator communities affected multiple ecological levels, and *D. pulex* population was affected by multiple ecological levels. These results suggest that some biological groups may facilitate cross-level interactions. On the contrary, additional research is needed to observe and understand feed-back responses including evolutionary response, focusing on the biological groups which may have interacted with the cladoceran community and *D. pulex* population. Further detailed genetic analysis or experiments assessing evolutionary responses remains necessary. Through the present study, I showed the effectiveness of paleolimnological analysis to study interactions between different ecological levels in natural systems.

In **Chapter 2**, I reconstructed the community and population dynamics of cladocerans and the changes in biotic as well as abiotic factors that influenced them. In this chapter, I focused on the ecosystem including abiotic environmental factors, cladoceran community, and *D. pulex* population. I revealed the relative importance of controlling factors that affect the cladoceran community as it changes through the eutrophication process. In the early stages of lake formation and community assemblage, when nutrient and primary production were limited, cladocerans were strongly restricted by bottom-up effects like nutrient input and the change in primary production for each microhabitat (Figure 6-1: 2-a). As eutrophication proceeded, cladocerans and higher predators were released from food restriction, and top-down control by the change in the predator community began to strongly affect the

cladoceran community (Figure 6-1: 2-b), which related to the establishment and dynamics of large *Daphnia* (Figure 6-1: 2-c). This transition of control mechanisms and the response of the cladoceran community to these biotic and abiotic effects agree with prior studies (Bennion et al., 2015; Davidson et al., 2011; Jeppesen et al., 2011; Taylor et al., 2006; McQueen, Post, & Mills, 1986).

In **Chapter 3** and **Chapter 4**, I focused on the *D. pulex* population and its genetic and phenotypic characteristics, starting from early colonization. I observed the genetic dynamics and the change of phenotypic characteristics of the *D. pulex* population throughout the whole colonization process. As a result, the *D. pulex* population was colonized with greatly limited haplotypes: one dominant type and several haplotypes closely related to it. Moreover, the *D. pulex* population maintained low genetic diversity without recovery and the dominant haplotype did not change during the colonization process (Chapter 3). While the genetic structure had limited haplotypes, the *D. pulex* population showed phenotypic changes, including body size of adult females and development of defensive traits during the colonization process (Chapter 4). In these processes, I found possible interactions between the predator community and phenotype of *D. pulex* (Figure 6-1: 4-a). In addition, this phenotypic change apparently affected the *D. pulex* population dynamics (Figure 6-1: 4-b); it may be an adaptive response to the change in the predator community, which suggests that the phenotypic change possibly promoted colonization and sustainability of the population. Furthermore, since genetic diversity had been limited during the colonization process, this phenotypic change could be caused by phenotypic plasticity (Figure 6-1: 3-a); the haplotype most able to adapt to various predation risks by phenotypic plasticity might successfully establish itself (Figure 6-1: 3-b). Highly plastic haplotypes, which can adapt to broad environmental conditions, could be established without an increase of genetic diversity. Limited haplotypes establishment was also detected in prior studies (Dane, Anderson, Osburn, Colbourne, & Frisch, 2020; Griebel, Giessler, Yin, & Wolinska, 2016; Most et al., 2015; Ortells, Vanoverbeke, Louette, & De Meester, 2013). The findings of the current study agree with previous study, suggesting that adaptive phenotypic plasticity might aid colonization and persistence in a novel environment (Hulme, 2007; Richards, Bossdorf, Muth, Gurevitch, & Pigliucci, 2006). Another possible genetic mechanism of adaptive phenotypic change is the rapid genetic adaptation of the dominant haplotype in a genetic region outside of present mitochondrial markers.

In **Chapter 5**, I focused on the intraspecific genetic diversity in the *D. pulex* population by examining the coexistence mechanism of intraspecific lineages, as well as the contribution of different diapause responses to photoperiodic conditions to coexist. I found that two genetically distant haplotypes of *D. pulex*, Jpn1A-C2T2 and Jpn2C, could be promoted to coexist through the storage effect that buffers competition by early diapause of the inferior competitor; the different diapause response to photoperiodic conditions possibly contribute to the temporal deviation of diapausing timing. Some cross-level interactions are hypothetically related to coexistence and different life-history strategies of intraspecific haplotypes. First, in the *D. pulex* population, multiple haplotypes are possibly in exploitative competition due to the similar resource niche based on phylogenetic relationships (Figure 6-1: 5-a). Genetic differences in diapause strategy (haplotype to phenotype, Figure 6-1: 5-c) will contribute maintaining genetic diversity (phenotype to haplotype, Figure 6-1: 5-b) despite the intraspecific exploitative competition. This maintenance of multiple genotypes affects intraspecific competitive dynamics of the *D. pulex* population and might contribute to the increase of the adaptive potential of the whole *D. pulex* population due to genetic diversity (Figure 6-1: 5-d).

Interactions between multiple ecological levels observed in the natural lake ecosystem with a focus on cladoceran community: interactions between multiple ecological levels may commonly exist in natural systems.

The present study showed that the interactions between multiple ecological levels constructing the lake ecosystem existed in this natural system, and that those interactions might occur between distant levels (Figure 6-1), for instance, between community and phenotype. I have described the details of the interactions in the Chapters 2–5. In belief, Chapter 2 addressed the effect of predator community and abiotic environmental factors, like eutrophication, on the cladoceran community and *D. pulex* population. Chapter 4 suggested that predator communities affect the phenotypes of *D. pulex* population and this response could facilitate the colonization and maintenance of *D. pulex* population. In Chapter 5, the analysis of different diapause strategies between haplotypes of the *D. pulex* population suggested the effect of genotype on phenotype. Furthermore, such intraspecific difference in diapause strategies possibly facilitate their coexistence, suggesting an effect on genetic composition and population dynamics. While I found no obvious interactions, Chapter 3 describes how the predator communities possibly affected the genetic composition of *D. pulex*,

as successfully established haplotypes could respond to changes in the predator community.

As an example of effect exerted by lower levels on higher levels of ecosystem, the establishment and increase of the *D. pulex* population possibly affected the function of cladoceran community and ecosystem. Since filter-feeding zooplankton species with large body size have high filtering rates (Downing & Peters, 1980; Gliwicz, 1990; Kreutzer & Lampert, 1999), establishment of *D. pulex* possibly increased the efficiency of filter-feeding of the whole cladoceran community and/or zooplankton community. Indeed, the flux of *D. pulex* subfossils, indicator of *D. pulex* abundance, positively affected the transparency of Lake Fukami-ike (Figure 6-2).

Another possible effect of lower ecological levels is the influence of the establishment of *D. pulex* on interspecific competition in cladoceran community. Interspecific differences in competitive ability are well known in the cladoceran community: species with large body size showed low food threshold concentration compared to smaller sized species, indicating that the species with larger body size show more efficient food utilization and thus, have a higher competitive ability, which is consistent with the size-efficiency hypothesis (Gliwicz, 1990; Kreutzer & Lampert, 1999). Hence, the establishment of large *D. pulex*, which have high resource competitive ability, possibly affected interspecific competition and the dynamics of native small cladocerans including *Bosmina*, *Chydorus*, and *Alona* in Lake Fukami-ike. However, the multivariate autoregressive regression (MAR) model, used to evaluate how each factor affected the cladoceran community, suggested no competitive interaction between cladoceran species (Chapter 2). The establishment of *D. pulex* occurred when eutrophication had already progressed, and thus, intraspecific resource competition in the cladoceran community was possibly relatively weak. Furthermore, although I did not observe other zooplankton residues, such as those of rotifers and copepods, as these are less preserved as subfossils in lake sediments; moreover, these other zooplankton communities and the entire lake ecosystem were probably as affected by the genotypic and phenotypic structure and changes in the *D. pulex* population. Some studies have shown that the colonization of *D. pulex* affected native small rotifers and copepods, as the size-efficiency hypothesis suggests, although a high-nutrient condition buffered this effect (Vanni, 1986). In addition, intraspecific competition also possibly affects genetic diversity. For *Daphnia magna*, interspecific competition with other *Daphnia* species significantly affected the clonal composition by effectuating a change of dominant clones depending on

competitor species, while clonal diversity was less affected (Östman, 2011). In the present study, the genetic structure of *D. pulex* might have been influenced by the presence of other cladoceran and zooplankton species in addition to the predator community and intraspecific competition.

While the interactions detected in this study were mainly the effects of higher ecological levels on lower levels, feed-back responses including “evo-to-eco feed-back” could not be fully observed. I have discussed these possible feed-back responses in a later section.

Multiple interactions between ecological levels occurred in the colonization process of *D. pulex* population and could affect structure and later dynamics of the cladoceran community and ecosystem

My results also showed various interactions in the *D. pulex* population colonization process from the early stage. First, the environmental factors which determine the lake ecosystem affected the *D. pulex* population mainly indirectly through their effects on the community. The increase of phytoplankton and primary production in the pelagic zone by eutrophication possibly allowed planktonic *D. pulex* to establish (Figure 6-1: 2-a). In addition, the establishment and increase of planktivorous and piscivore fish due to the rich primary production caused by eutrophication, could affect the *D. pulex* population dynamics and its colonization success. The analysis of subfossils suggests that the population of *D. pulex* increases when planktivorous fish decrease, and vice versa (Chapter 2). A decrease of planktivorous fish around the year 2000, due to high predation pressure by piscivorous *Micropterus salmoide*, possibly allowed the *D. pulex* population to establish and increase. On the contrary, the first introduction of the *D. pulex* population is considered to have went extinct or maintained in very low densities between 1930 and 1950, which was around the period of time when the artificial introduction of *Hypomesus nipponensis* in 1960s occurred (Tanaka, 1992), suggesting that high predation pressure could prevent the establishment of *D. pulex*. The phenotypic changes observed in body size of the matured females and the presence of a defensive tail-spine was adaptive against these predator changes (Chapter 4). This result suggests the possibility that the predator community may affect phenotypic characteristics in the *D. pulex* population (Figure 6-1: 4-a). Moreover, the predator community might also select the genotypes best adapted to predation risk (Figure 6-1: 3-b), as initial large defensive traits possibly promoted the *D. pulex* population to establish in an early colonization stage before *Hypomesus nipponensis*

went extinct. Furthermore, the intraspecific variation could also affect intraspecific competitive dynamics and the arrival order in the establishment of each haplotype. As mentioned above, the interaction between intraspecific exploitative competition (population dynamics) –diapause response (phenotype) –genotypes possibly existed. In addition, the different diapause strategy of Jpn1A-C2T2, which arrived at Lake Fukami-ike after Jpn2C, and the inferior competitive aptitude in the active population to dominant Jpn2C, possibly allowed Jpn1A-C2T2 to establish by avoiding intraspecific competition with Jpn2C.

Further studies are required to evaluate the possible contributions of preadaptation and local adaptation to the establishment of *D. pulex* and its haplotypes and the relative importance of these genetic adaptations and phenotypic plasticity. Three possible mechanisms that might promote the later establishment of Jpn1A-C2T2 in Lake Fukami-ike have been given regarding haplotypes: 1) Jpn1A-C2T2 had a different photoperiodic response on diapause compared with Jpn2C before the immigration, which promoted JPN1 establishment (preadaptation); 2) after immigration, Jpn1A-C2T2 changed its photoperiodic response while coexisting with Jpn2C by local genetic adaptation of phenotypic plasticity; and 3) the role of stochasticity. The possibility that rapid adaptation to a new environment by both genetic evolution and phenotypic plasticity commonly appears in successful invasions and promotes colonization and establishment has been traditionally suggested (Colautti & Lau, 2015). Lake sediment core samples and preserved diapausing eggs of cladocerans may allow us to test the above hypothesis and the role of rapid adaptation for establishment and colonization in more detail. For *D. pulex*, in the present study, the number of diapausing eggs used as samples for population genetic analysis was limited in each layer of sediment of the core samples. Mitochondrial analysis of all diapausing eggs used to test morphological changes may allow me to evaluate the relative contribution of phenotypic plasticity and genetic adaptation in Jpn2C colonization. In addition, using a broader variety of genetic markers and conducting phylogeographic analysis to detect the source populations of each lineage could contribute to the understanding of the detailed establishment mechanisms of each lineage of *D. pulex* in Lake Fukami-ike. Furthermore, the observation of the temporal variation in genetic composition and phenotypes for multiple cladoceran species, suggested that an investigation should be done to test whether the majority of cladoceran species show the same common pattern found with these observations, which in turn, may provide general

knowledge about the role of adaptation in colonization.

These results and suggestions agree with the possibility that the interactions between ecological levels, including eco-evolutionary dynamics, do affect colonization (Hulme, 2007; Richards et al., 2006), the formation of community structure, and ecosystem function (Hendry, 2017; Hendry, 2019). In addition, our results also support that intraspecific diversity will affect community structure and ecosystem functions (Des Roches et al., 2018; Raffard, Santoul, Cucherousset, & Blanchet, 2019; Violle et al., 2012) like the filtration rate, as mentioned above section. These results suggested that interactions across ecological levels could affect early colonization dynamics, including adaptation, thus affecting later dynamics and structure of ecosystem and community. Such cross-level interactions during the colonization process are difficult to detect by traditional space-for-time analysis. Hence, the present study suggests the importance of long-term monitoring including genetic and phenotypic data to understand the interaction across ecological levels and their effects on early dynamics and the later changes found in populations, communities, and ecosystems.

In addition, the establishment of *D. pulex* population possibly affects the interaction between predator community and other zooplankton; however, further studies are required for a more detailed discussion. Since *D. pulex* is a major large zooplankton selectively preyed upon by planktivorous fish (Brooks & Dodson, 1965 ;Miner et al., 2012), predation risk from planktivorous fishes in other cladoceran species and zooplankton is possibly decreased by the establishment of *D. pulex* population. Particularly, copepods, a large zooplankton, increased in recent periods when planktivorous fish increased (Chapter 2). Although copepods have a large body size, they are less selectively preyed upon by planktivorous fish compared to *D. pulex* (Urabe & Maruyama, 1986; Makino et al., 2001) due to their higher swimming speed (Drenner, Strickler, & O'Brien, 1978) and other factors.

Certain biological groups may facilitate cross-level interactions in ecosystem: predator community changes and *Daphnia* responsiveness

In our research system, changes in the predation community simultaneously affected various ecological levels, as well as the cladoceran community dynamics and structure (Figure 6-1: 2-b), *D. pulex* population dynamics (Figure 6-1: 2-c), phenotypes (Figure 6-1: 4-a), and possibly its genetic composition (Figure 6-1: 3-b). These effects

were possibly constructed both directly and indirectly through eco-evo feedback mechanisms. For instance, the effect of the changes in the predator community on the cladoceran community might include the direct change of predation risk and the indirect effect via adaptive response of the *D. pulex* population to predation. As indirect effects, adaptive response of the *D. pulex* population to predation could lead to the establishment of *D. pulex* resulting change of the cladoceran community structure and function like filtration rate. It is difficult to untangle the relative contribution of these direct and indirect effects. Possible indirect effects across levels cannot be detected without long-term observations across ecological levels like in the present study. Hence, this study showed the indirect effects across ecological levels that possibly occur in natural systems through long-term observation.

Many prior studies showed that the predator community played a key role in controlling changes in each of the ecological levels, lake community dynamics (e.g., Carpenter, Kitchell, & Hodgson, 1985; Hulot, Lacroix, & Loreau, 2014), cladoceran community structure and dynamics, population dynamics of cladoceran species (e.g., Perga, Desmet, Enters, & Reyss, 2010; Ohtsuki et al., 2015), and cladoceran phenotype by phenotypic plasticity, genetic response (e.g., Wathne, Enberg, Jensen, & Heino, 2020), or both (e.g., Stoks, Govaert, Pauwels, Jansen, & De Meester, 2016). Based on these prior studies and my results, factors that have a large impact on each of the levels of the ecosystem tend to simultaneously affect multiple levels, which may be caused by indirect effects on other levels.

Similarly, the species that are sensitive to biotic and abiotic changes, like *Daphnia*, may accumulate such impacts from multiple levels of ecosystem and transmit them to the entire ecosystem. Indeed, climate change had been reported the effect on all ecosystem levels that *Daphnia* belongs (reviewed in Scheffers et al., 2016). The outcomes of present study also suggest that *D. pulex* population and its phenotype and genetic structure were affected by multiple ecological levels as showed above. It is possible that the impacts of environmental change on sensitive species could lead to large overall changes in the ecosystem, as these species accumulate the impacts of environmental change and mediate them to in various ecological levels.

Pinpointing which levels are most likely to be impactful on other levels, may provide practical suggestions for the effective management of natural systems. Environmental changes occurring worldwide like climate change

and artificial disturbances, may also affect various ecological levels simultaneously. For instance, climate change can affect species interactions, communities, and biodiversity (Lavergne, Mouquet, Thuiller, & Ronce, 2010). It is important to understand the effect across levels of ecosystems when considering severe impacts regarding environmental change and human activity on ecosystem health and biodiversity. While my research suggests that long-term monitoring is important to understand any effect across levels of ecosystems, as prior studies suggest, I was unable to achieve a detailed understanding of the process. One conceivable effective approach to untangling these connections would be to test a hypothesis based on long-term cross-level monitoring by experimenting in the laboratory or using mesocosms.

Future perspective: evo-to-eco feedback, intraspecific competition, primary producer community, and effectiveness of paleolimnological methods

Further studies are required to understand feed-back interactions including “evo-to-eco” feed-back, as observed in the lake ecosystem studied here. First, for a detailed analysis on the *D. pulex* population and cladoceran community feed-back effects over the whole biological community, a detailed taxonomic analysis of biological groups is needed, in particular of taxa affecting the cladoceran community and *D. pulex* population, such as predator and phytoplankton community.

In the present study, genetic structure and phenotypic change caused by changes in the predator community had possible feed-back effects on upper ecological levels like population, community, and ecosystem. The present study also highlighted the possibility that *D. pulex* population adaptively responded to predator community variations by undergoing phenotypic changes (Chapter 4, Figure 6-1: 4-a). The morphological traits of small body size and inducible defensive morphology, which were observed when planktivorous fish were abundant, were considered effective in decreasing the predation risk. The dynamics and composition of the predator community were possibly affected by such adaptive morphological response of the *D. pulex* population. While feed-back effects of such responses against predation are less reported in natural ecosystem dynamics, laboratory experiments revealed that predator-prey dynamics changed according to phenotypic response related to defensive morphology and adaptive evolution of the prey population (Kasada et al., 2014; Meyar et al., 2006; Yoshida et al., 2003). Any observed change

in fish community composition and the dynamics of each fish species in the sediment core samples will allow to examine the feed-back effect of *D. pulex* population on the predator community.

Another biological groups that could be associated with *D. pulex* is phytoplankton, which is a primary producer, as well as a food resource for *D. pulex*. While the present study evaluated the effects of phytoplankton by estimating their abundance using the residual photosynthetic pigments, an increase of phytoplankton due to eutrophication allowed the establishment and increase in population of pelagic species, including large *D. pulex* (Chapter 2). Such responses of the cladoceran community to phytoplankton community changes possibly exerted feed-back effects on the phytoplankton community. For instance, the PEG model, typical seasonal dynamics of the lake ecosystem, indicates that an increase in zooplankton due to edible phytoplankton bloom decreases of phytoplankton abundance, leading to a clear water phase (Sommer et al., 2012). Moreover, both intraspecific and interspecific variations in body size in the cladoceran community affect the phytoplankton community structure (Miner et al., 2012). Hence, phylogenetic and intrageneric diversity and structure of *D. pulex* population can affect the abundance and productivity of phytoplankton, with feed-back effect on the establishment of *D. pulex*. Such feed-back effect on phytoplankton community composition and abundance also relates to ecosystem function by impacting its primary productivity. Indeed, changes in the cladoceran community composition induces cascade effects on the productivity of phytoplankton (Post, Palkovacs, Schielke, & Dodson, 2008). Although I did not analyze phytoplankton at the species level, a high-resolution analysis of phytoplankton species or of the genetic level may allow the detection of the interaction and feedback mechanisms relating to the bottom-up effect in addition to the top-down effect detected in the present study; moreover, it may help to evaluate the relative importance of the bottom-up and top-down effect on each ecological level. In addition, genetic variation could also exist in traits relating to energetic efficiency in *Daphnia*, which would have evo-to-eco effects on the phytoplankton community in addition to intraspecific and interspecific competition (Jeyasingh, Weider, & Sterner, 2009). Several prior studies showed food quality affecting clonal fitness (Plath & Boersma, 2001; Vanni & Lampert, 1992; Weider, Jeyasingh, & Looper, 2008) and competitive ability (Iwabuchi & Urabe, 2012) of *Daphnia*.

Paleolimnological methods will be also effective for analyses examining the feed-back interactions in the

ecosystem. Paleolimnological analysis allow us to reconstruct long-term changes in other biological community after analyzing a certain community, such as cladoceran community in the present study, using the same sediment core samples. Thus, we can integrate and analyze long-term changes in multiple biological taxa in detailed taxonomic levels or genetic levels using the same sediment core samples. Long-term changes in the phytoplankton community have been examined on lake sediment core samples using taxa specific fossil pigments (Leavitt & Hodgson, 2002), and diatom subfossils (Gasse, Juggins, & Khelifa, 1995). Moreover, DNA metabarcoding using sediment samples is an effective tool. Recently, retrospective analysis of various taxa with poor subfossil preservation, like fish, microorganisms and other zooplankton including rotifers and copepods, was performed by analyzing environmental DNA (Ellegaard et al., 2020). Such DNA analyses allow us to comprehensively confirm the presence or absence of various taxon with little effort. For instance, the present study indicated the possibility that the fish community broadly affected cladocerans, from their genetic structure to species composition. While I estimated the variation of abundance and species composition of the fish community from subfossils of *Chaoborus* and other past records, environmental DNA analysis on sediment may examine and reinforce these suggestions. Furthermore, changes in the primary producer community due to eutrophication could be analyzed at a higher resolution by environmental DNA analysis. Paleolimnological methods, which allow us to obtain data on other taxa from the same sediment samples, are the most effective to understand interactions across multiple ecological levels. If we use DNA analysis methods on lake sediment core samples, we can analyze more long-term dynamics other than those just between cladoceran and predators or phytoplankton. The microorganism community, including viruses and ciliates, may also interact with the cladoceran community (Wolinska, Bittner, Ebert, & Spaak, 2006; Wolinska, Keller, Manca, & Spaak, 2007) and each *Daphnia* population (Decaestecker, Declerck, De Meester, & Ebert, 2005; Haag & Ebert, 2007; Weigl, Korner, Petrusek, Seda, & Wolinska, 2012). These groups are important for a comprehensive assessment of interactions across levels of the whole lake ecosystem, including the microbial loop (Porter, Sherr, Sherr, Pace, & Sanders, 1985). DNA analysis using sediment core samples also allows to examine the interaction between the microbial and cladoceran communities, and the *D. pulex* population.

In addition, resurrection ecology using lake sediment core samples is effective to additionally examine

feed-back interactions between ecological levels or the effects of lower levels, and to experimentally test the related hypotheses by constructing mesocosm experiments and laboratory experiments using organisms hatched from diapausing eggs preserved in lake sediment core samples. I still need to examine whether genetic structure of the *D. pulex* population affect the composition and function of the cladoceran, phytoplankton, predator communities, and the function of ecosystem like filtration rate or primary productivity. One of the future challenges are to observe and evaluate eco-evolutionary dynamics in natural lake ecosystems. Since *Daphnia* is one of the most well-studied groups regarding eco-evolutionary dynamics (Miner, De Meester, Pfrender, Lampert, & Hairston, 2012), the *D. pulex* population observed in the present study can be effectively used to evaluate eco-evolutionary dynamics. Evolutionarily speaking, the response of *Daphnia* population at the genetic and phenotypic level to changes including those in their environment (e.g., Cuenca Cambronero, Zeis, & Orsini, 2018; Frisch et al., 2014) and in other species-like predators (e.g., Cousyn et al., 2001; Stoks et al., 2016), parasites (e.g., Decaestecker et al., 2007), and primary producers (e.g., Hairston et al., 1999) has been well documented. However, it has rarely been tested how the evolutionary response of *Daphnia* affects ecological dynamics, which means how evo-to-eco feedback mechanisms take place during interactions between *Daphnia* and other species. Schaffner et al. (2019) revealed that *D. mendotae* populations can evolve to increase their tolerance to cyanobacteria by responding to the change in the phytoplankton community structure within a year. However, the feed-back effect of the evolutionary response of *D. mendotae* to cyanobacteria population dynamics and to the phytoplankton community remains to be elucidated. The present study focused on long-term ecological dynamics. More detailed genetic analysis of diapausing eggs would be effective to evaluate evolutionary response, such as the analysis of functional genetic regions and/or an association analysis using genome-wide presence of Single Nucleotide Polymorphisms (SNPs).

Future perspective: does the same mechanism maintain both interspecific and intraspecific diversity?

Similar mechanisms can have roles at many ecological levels: similar mechanisms maintaining biological diversity will work in both intraspecific and interspecific diversity. For example, temporally fluctuating environments have been demonstrated to maintain both types of species (Chesson & Warner, 1981; Chesson, 2000; Cáceres, 1997)

and genetic diversity (Dempster, 1955; Ellner & Hairston, 1994; Haldane & Jayakar, 1963; Turelli, Schemske, & Bierzychudek, 2001). The storage effect, which I showed as a possible mechanism to maintain the coexistence of intraspecific haplotypes of *D. pulex*, is also demonstrated as a the mechanism maintaining both species and genetic diversity (Li & Chesson, 2016).

Several studies reported opposite results about interactions between taxonomic diversity and intraspecies diversity. While a study reported that intraspecies diversity inhibited interspecific coexistence (Hart, Schreiber, & Levine, 2016) and another study reported that intraspecific diversity had little effect on interspecific coexistence (Noto, Hughes, & Schwinning, 2020), other research reported that changes in predation pressure contributed to promoting the coexistence of both taxa and genotypes at the same time and same site (Spaak & Boersma, 2006).

Presently, I do not have enough knowledge to say if the same mechanisms can promote both intraspecific genetic and species diversity at the same time and in the same natural system, or whether both types of diversity negatively interact, and if so, how these different diversities are maintained in natural systems. A diapausing egg bank of the *Daphnia* community preserved in lake sediment allows us to reconstruct both community and genetic dynamics of each species, and thus may be useful to examine the interaction between mechanisms maintaining genetic and species diversity in natural systems. Understanding the mechanisms that maintain both intraspecific genetic and species diversity, including the relationships of these mechanisms in natural systems, is an important concept for the conservation of biological diversity.

Conclusion

My research suggests that interactions occur between different ecological levels, including distant ones, and may play important roles in natural systems. More specifically, during the colonization of natural populations, these interactions possibly affect colonization success, changing the community structure, and ecosystem function. In addition, my research outcomes showed that some factors, such as changes in the predator community, can affect multiple biological levels at the same time. More detailed analysis of other biological communities which cladocerans possibly interacted with, like predator and phytoplankton communities, may highlight the feed-back effects present

in the studied system. Paleolimnological analysis will significantly help research in this field in the future and facilitate integration of long-term dynamics of multiple biological communities. Evolutionary responses of the *D. pulex* population can be examined by high resolution genetic analysis of diapausing eggs used in the present study, eventually focusing on genome-wide SNPs and/or functional genes. The present study showed the effectiveness of paleolimnological and resurrection ecological methods to observe the interaction between ecological levels in natural systems and provides new insights for future research studies aiming to explore such interactions, including feedback responses and eco-evolutionary dynamics.

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Figures

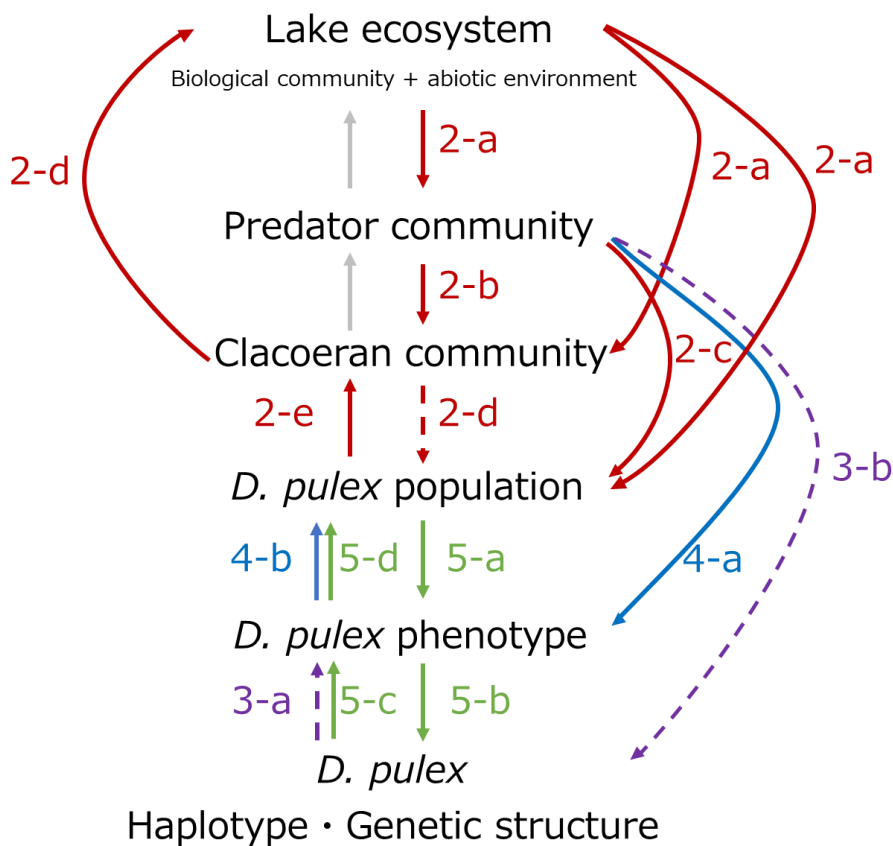


Figure 6-1. Interactions that were detected in the present study among different ecological levels in a lake. Colors indicate the chapters in which each interaction was observed. Solid lines represent the aspects I was able to detect. Dashed lines show the possible interaction that I was unable to detect. 2-a is the effect of change of primary production by eutrophication; 2-b is the top-down control on the cladoceran community by change of the predator community; 2-c is the effect by the predator community on *D. pulex* population dynamics; 2-d suggests the possibility of the effect of intraspecific competition on *D. pulex*; 2-e is the effect on the cladoceran community by establishment of *D. pulex*; 3-a represents the genetic mechanism that caused phenotypic change, which is possibly caused by plasticity; 3-b is the possibility that the predator community determined haplotypes of *D. pulex* that were able to dominate and/or to establish; 4-a suggests the adaptive change of phenotype to predator community changes; 4-b indicates the possibility that adaptive phenotypic change could promote establishment; 5-a suggests that the structure of the *D. pulex* population and the select diapause strategy that can promote coexistence with other haplotypes; 5-b represents the selection of haplotypes that have diapause strategy allowing for coexistence; 5-c is genetic determination of the diapause strategy; 5-d suggests that the diapause strategy determine the *D. pulex* population structure and tolerance against disturbance based on genetic diversity. Grey indicates the interaction that I could not test in this thesis.

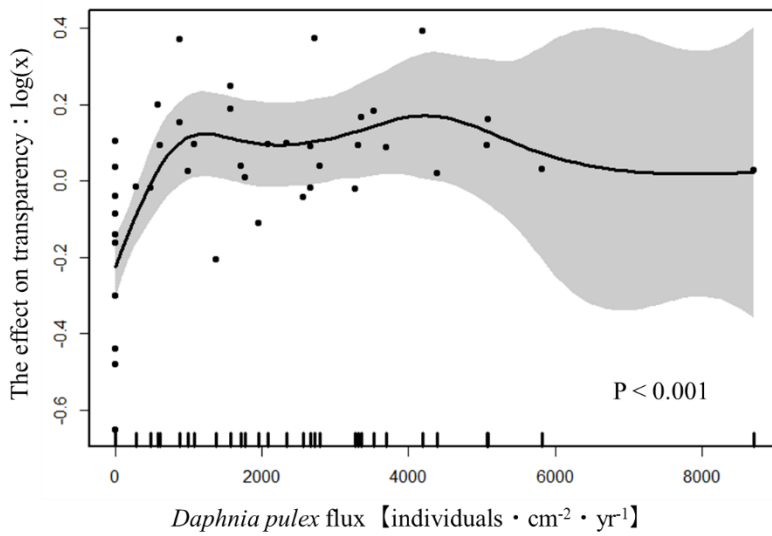


Figure 6-2. The effect of *Daphnia pulex* abundance on transparency in Lake Fuakmi-ike. This effect was analyzed using a generalized additive model. This figure shows the effect that the best model suggested. The solid line indicates the smoothed effect of the *D. pulex* flux on the transparency of the water. The shading shows the 95% confidential interval. The bars at the bottom of the graph indicate the density of the data. The response valuable, transparency, was assumed to follow a gamma distribution. Log function was assumed as the link function. The significance level was set at 5%. Model selection was conducted based on AIC, and the AIC of the best model was 460.9, while the AIC of the null model was 478.9. The transparency data was collected by Akihiko Yagi from 1978 to 2016. The transparency was measured at the center of Fukami Pond using a Secchi transparency board. The annual mean of transparency was calculated for each year, and then averaged for the years included in each core and used as the response variable.

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