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学位の種類	博士（生命科学）
学位記番号	生博第252号
学位授与年月日	平成25年3月27日
学位授与の要件	学位規則第4条第1項該当
研究科，専攻	東北大学大学院生命科学研究科 （博士課程）生態システム生命科学専攻
論文題目	Phylogenetics and population dynamics of tintinnid species in the neritic water of Sendai Bay, Japan（仙台湾沿岸に出現する有鐘繊毛虫類の系統関係と個体群動態）
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Tintinnid ciliates are protozoan zooplankters in aquatic ecosystems including oceans and lakes. These protozoans use bacteria and phytoplankton as foods and are preyed upon by larger zooplankters such as copepods. Because they appear abundantly in neritic and estuary areas, tintinnids are one important component in the zooplankter community in coast ecosystems. However, although the abundance and species composition of tintinnids are known to fluctuate temporally, it is not yet clear factors regulating these temporal changes. Because the environmental condition in neritic water is unstable, physicochemical factors such as salinity and water temperature have been thought to be crucial for regulating tintinnid populations. In addition, there is also circumstantial evidence suggesting the importance of biological factors such as copepod predators on the community composition of tintinnids. However, there is little knowledge about the relative importance of physicochemical factors and the biological factors. In tintinnids, species identification and classification have been made by the morphological characteristic of the vase-shaped shells called loricae. With development of the recent molecular biological techniques, classification and phylogeny of ciliates including tintinnids are reexamined. However, due to low accumulation of DNA sequence data from various specimens, it is not yet clear how morphological characteristics of the loricae reflect species identities and the classifications of tintinnids. To examine the population dynamics of tintinnids at species level, it is necessary to clarify species identification based on phylogenetic and classification by the loricae morphologies. In this study, therefore, we first examined relationships between the loricae morphology and phylogeny of tintinnid ciliates appeared at Sendai Bay using a molecular technique. Then, we examined relative importance of physicochemical factors and biological factors in regulating tintinnid populations in coastal the areas of Sendai Bay by temporally high frequent samplings and in-situ experiments.

In the first chapter, I collected 23 tintinnid morphospecies (11 genera) at various sites of Sendai Bay and examined sequences of nuclear small subunit rRNA (SSrRNA) gene. Then, to examine if the morphological characteristics of loricae reflect the phylogenetic relationships, I reconstructed phylogenetic trees based on 84 partial sequences of SSrRNA fragments of 44 morphospecies from a total of eight families (55 sequences from this study, and 29 sequences from the literature). I found that tintinnid ciliates could be classified into six clades consisting of five major clades. While one clade consisted of one family, each of the other five clades consisted of more than two families. Although two families were found only in one clade, the remaining six families were found in more

than two clades. In addition, each of six morphospecies was found in more than two major clades, indicating that they are polyphyletic. Principal coordinate analysis showed that morphology of the lorica overlapped substantially between the clades. These results imply that most of the morphological and morphometric traits of marine tintinnid species do not reflect SSrRNA genetic distances even at family levels. Thus, it is necessary to check genetic identity when species identification is made based on the loricae morphologies. This study indicates necessity of new characteristics in tintinnid species that reflect the phylogenetic relationships robustly.

In the second chapter, to examine environmental factors regulating the population dynamics of tintinnid species, I performed a temporally high frequent observation of tintinnid ciliates for a month in Hiroura, an estuary, in Sendai Bay. I made sampling every other day during July 5 to August 2, 2010 at three sites of the estuary. At each sampling, I collected seawater using a tube sampler from all layers, and tintinnids are collected using a 20- μm mesh. Water temperature and salinity in each site were measured with a CTD profiler, and tidal level change was recorded with a water level logger installed at the estuary. I also determined chl *a* concentration in different size classes (<2, 2–20 and >20 μm) fluorometrically. The abundances of bacteria and HNF were measured with a fluorescent microscope. Crustacean zooplanktons were enumerated after concentrating them with 100- μm mesh net. Species identification of the tintinnids was made based on the loricae morphologies after confirming genetic identities using the molecular method mentioned above. The relationship between the abundance of each tintinnid species and environmental factors was examined with a redundancy analysis (RDA). The effects of environmental factors on population change rates of the tintinnids were examined with a generalized linear mixed model (GLMM).

During the study period, a total of 20 species was appeared and the species richness was positively correlated with salinity. However, the species composition of tintinnids changed drastically in a few days, resulting in different assemblages from the first to the second half of the study period. The analyses showed that relationships between the abundance and environmental factors were species-specific. In addition, effects of environmental factors on population change rates were species-specific. Among factors, water temperature, salinity and tidal trend were revealed to affect the abundances of species in most cases. Some species were also influenced by the abundances of bacteria

and heterotrophic nanoflagellates (HNF) that were potential foods for tintinnid ciliates. However, any effects of predators such as copepods were detected on the neither abundance nor population change rates of tintinnids. These results suggest that physicochemical factors play fundamental roles in short-term temporal changes in tintinnid populations. During the study period, HNF and phytoplankton as well as copepods were abundant. Because copepods prefer HNF and phytoplankton as food source, predation pressures to tintinnid species may have been low during the study period. This possibility examined experimentally in the next chapter.

In the last chapter, I examined if the predation pressure of copepods on tintinnid populations change depending on the abundances of phytoplankton and other food source. For this object, I performed in situ experiments with nutrient and mesozooplankton manipulations. The experiments were carried out from 19 to 23 July, 22 to 26 August and 10 to 14 September in 2011 at Shiogama, a cast of Sendai Bay. In each experiment, in-situ plankton community passed through a 200- μm mesh net was enclosed into 1.25 L transparent polycarbonate bottles. Then, one of the following nutriment mixtures was added: [N-40 μM · P-2.5 μM], [N-40 μM · P-2.5 μM · Si-40 μM], [N-40 μM · P-2.5 μM · Glucose-50 μM] and [no nutrients]. To a half of each nutrient treatment, meso-zooplankton that were collected by a plankton net with a 200- μm mesh and mainly composed of copepods were added. The remaining bottles were used as a control. In each experiment, the bottles were incubated for 2 or 4 days at the surface (1-1.5 m). After the incubation, an aliquot of water in the bottle was collected for the enumeration of bacteria, HNF and phytoplankton. For phytoplankton, enumeration was made according to the taxonomic group and the cell size. Tintinnid ciliates, naked ciliates and crustacean planktons in the bottle were collected by concentrating the remaining aliquots of the water with a 20- μm mesh net. The population change rates of tintinnids were calculated assuming an exponential growth. The effects of incubation days, nutrient addition and copepods addition on population change rates of tintinnids were examined by generalized linear mixed model (GLMM). Similarly, these effects on the abundances of bacteria, HNF, phytoplankton, naked ciliates and nauplii of crustaceans were analyzed by the GLMM to detect indirect effects of the copepod addition.

Increase in the population change rates of tintinnids by nutrient enrichments was detected only in July. Effects of copepods addition on tintinnids were also found but different among three

experiments. Population change rates were higher in treatments with copepod addition than in the control in July and September. It was, however, comparative between these treatments in August. Among these months, abundance of phytoplankton, especially large diatoms, was low only in August. The experiments also showed that abundance of naked ciliates was affected negatively by copepods in July and September, but not in August when the low initial abundance was low. The abundance of HNF and bacteria were increased by copepods addition in August and September, respectively. In July and September, the addition of copepods increased the abundance of tintinnids. In these months, large diatom and naked ciliates were abundant. Because these organisms were preferential food for copepods, the predation pressure to tintinnids would be low. Rather, copepods may have favored tintinnid populations through reducing competitors or increasing the abundance of food such as bacteria through releasing dissolved organic matters. In August, predation pressure by copepods on tintinnids seems to play a role because large diatom and naked ciliates were not abundant. However, these positive indirect effects of copepods on tintinnids via the increase of HNF may have offset by negative effects via the direct predation

To summarize, copepods can positively affect on tintinnid populations by indirect interactions such as reduction of competitor and improvements of the resource conditions. However, strength of these indirect effects was context-dependent and changed depending on abundance of more preferential food for the copepods. To understand population dynamics of tintinnids in various ecosystems, it is necessary to take into account diverse roles of a predator and condition-dependency of the predation pressure.

論文審査結果の要旨

有鐘織毛虫は内湾や汽水域で量的に多く出現する原生動物プランクトンで、その種組成や密度は季節的に大きく変化することが知られている。しかし、有鐘織毛虫の種同定にもちいられてきた殻形態と系統との関係や個体群動態に関与する環境要因はよく分かっていない。そこで本研究ではまず仙台湾沿岸に出現する有鐘織毛虫を対象に、遺伝距離との関係から各種の殻形態特徴と系統関係について再検討を行った。次いで、有鐘織毛虫の個体群動態に及ぼす環境要因や生物相互作用の影響を明らかにするため、仙台湾沿岸域で現場観察と野外実験を行った。

有鐘織毛虫の殻形態と分子系統を比較した結果、従来の殻形質による分類は遺伝距離を正しく反映しておらず、有鐘織毛虫の系統分類を整理するためには殻でなく細胞形質など遺伝距離と整合性のある同定方法が必要であることが明らかとなった。また河口干潟を含む汽水域に出現する有鐘織毛虫類を高い頻度で約1ヶ月にわたり観察した結果、種組成および密度は数日の間に大きく変化することが分かった。この短期間の個体群変動について特に優占種を対象に詳細な解析を行ったところ、その個体群動態には潮汐や餌環境が強く関与していたが、従来から捕食者として重要視されてきたカイアシ類の影響はいずれの種に対しても見られなかった。そこで、有鐘織毛虫に対するカイアシ類の影響をより詳しく検討するために、カイアシ類の密度と栄養塩濃度を操作した野外操作実験を行った。その結果、カイアシ類の有鐘織毛虫群集に対する捕食圧は、カイアシ類の餌となる珪藻や無殻の織毛虫の現存量によって変化すること、カイアシ類は排泄や食いこぼしなどによる細菌や鞭毛虫類などへの影響を介して有鐘織毛虫に間接的な正の効果も及ぼすことを明らかにした。

これら一連の研究は、有鐘織毛虫の形態形質について分類学的な再検討を促すものであるとともに、これまで知見の乏しかった有鐘織毛虫の個体群動態に関与する要因を具体的に示したものとして評価出来る。特に、有鐘織毛虫の個体群動態に理化学的要因のみならず生物間相互作用が関与しており、捕食者は直接的な捕食による負の効果だけでなく、間接的に成長を促進する正の効果も及ぼすことを示した点は説得力があり新規性がある。これら成果は、自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって、風間健宏提出の論文は博士（生命科学）の博士論文として合格と認める。