Due to ongoing climate change, all the water bodies such as ocean, seas, river and lakes experiences impingement of natural and anthropogenic stressors including fluctuations in temperature, salinity and feeding levels in the next 100 years. Global climate change will finally affect physiological and biochemical performance of clam species subjected to brackish environment.

The present study was carried out at Lake Hinuma, a brackish lake in Ibaraki, Japan (36°16′N, 140°30′E). Sea water flows from the Pacific Ocean during high tides and can affect the lake environment due to fluctuations of salinity. Global warming effects of representative aquatic organism Corbicula japonica in brackish environment is widely unknown, although clams are expected to be affected by ocean warming. The main objectives of this study are ; 1) to investigate oxidative stress responses to natural environmental factors such as feeding activity and salinity level, 2) to assess global warming effects on physiological and biochemical biomarkers and 3) to integrate multiple biomarker approaches for environmental impact assessment.

At first, three laboratory-scale experiments were conducted to assess the oxidative stress responses of C. japonica to feeding activity and salinity level. Biomarkers such as oxygen radical absorbance capacity (ORAC) and adenosine triphosphate (ATP) were measured in those experiments. The experimental results revealed that the availability of SS to C. japonica strongly affects ORAC. Furthermore, the combination of feeding activity and salinity level was proved to be significant in determining ORAC behavior in C. japonica. In addition, good correlation was found between changes in ATP content and ORAC.

Secondly, field investigation of Hinuma Lake/river was conducted to find out the effects of water quality on antioxidant capacity ORAC. For water quality experiments, clams were placed in water of totally five sites. ORAC assay showed that there were significant differences between upstream and downstream of river/lake near ocean. To estimate the water quality parameters that affect ORAC, multiple regression analysis was carried out. The analysis revealed that water quality factors such as temperature, salinity and Chl. a were the most affective to ORAC.

Thirdly, to assess global warming effects, three factors such as temperature, salinity and feeding activity were selected. Clams were exposed in a full factorial design with two levels (control, elevated) of temperature, salinity and SS creating all together eight exposures conditions. The two selected temperatures were chosen to represent the average water temperature in clam C. japonica at the time of collection and (t+5°C) (25°C ) increase predicted for the year 2100 by an IPCC scenario. The individual and interactive effects of these factors were tested after one-week acclimatization. For physiological responses, condition index (CI) and energy reserves (ER) were measured whereas for biochemical responses, antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), antioxidant capacity (ORAC) and oxidative damage or (lipid peroxidation (LPO)) were measured. The results obtained showed that C. japonica presented higher LPO activity (p < 0.01) at warming (elevated temperature (25°C)) when compared to ambient (20°C). In the present work, the inhibition in enzyme activity SOD (p < 0.001), CAT (p < 0.05) and antioxidant capacity (ORAC) (p < 0.001).
contributed to strong increase in LPO activity especially at (25°C) under low feeding (0.5 mg SS/ind/day). In contrast, under high feeding (2.0 mg SS/ind/day), LPO activity were stable (p > 0.05) though there was inhibition of these enzymes such as SOD (p < 0.001), CAT (p < 0.01) and antioxidant capacity (ORAC) (p < 0.01). SOD was induced (p < 0.05) under the combine effect of elevated salinity (20 psu) and elevated SS (2.0 mg SS/ind/day) at (20°C)

...decreasing LPO activity (p < 0.05). Combined effect of elevated temperature (25°C) and elevated salinity (20 psu) at both feeding levels, resulted lipid membrane damages (LPO) (p < 0.01) (0.5 mg SS/ind/day)) and ((p < 0.05) (2.0 mg SS/ind/day)) suggesting that antioxidants mechanisms were not enough to prevent it though enzyme activity (SOD) ((p < 0.01) (2.0 mg SS/ind/day)) and antioxidant capacity (ORAC) ((p < 0.01) (0.5 mg SS/ind/day) and ((p < 0.05) (2.0 mg SS/ind/day)) was induced greatly. Also, result showed that C. japonica ER were seen higher at high feeding (2.0 mg SS/ind/day) at both salinity levels (5 psu) (p < 0.05) and (20 psu) (p < 0.001) under elevated temperature (25°C). Both SOD (p < 0.05) and CAT (p < 0.05) activity were induced at elevated salinity (20 psu) under low feeding (0.5 mg SS/ind/day) at ambient temperature (20°C) with stable LPO activity (p > 0.05). Similarly, LPO activity (p > 0.05) were stable at elevated SS (2.0 mg SS/ind/day) under low salinity (5 psu) at ambient temperature (20°C) with activation of enzyme activity (CAT) (p < 0.05) and antioxidant capacity (ORAC) (p < 0.05). CI activity was significantly affected by temperature levels (p < 0.001). The highest CI in clams were observed in control (8.76%) as compare to all exposure conditions. Furthermore, results of condition index (CI) suggest that elevated temperature may act as stressor influencing the growth and reproduction of C. japonica. In addition, multi-biomarker approaches (PCA) and cluster analysis clearly categorized experimental exposures into three groups such as low impact stress, moderate impact stress and high impact stress.

Finally, to determine the stress levels of C. japonica, integrated biomarker response index (IBR) was evaluated based on five different approaches. IBR approach I was selected based on most sensitive biomarkers of three aspects: 1) physiological aspects :2) biochemical aspects in terms of oxidative stress and finally :3) biochemical aspects in terms of Oxidative damage. Results from different IBR approaches were based on rank and highest rank was obtained in anthropogenic stress as compared to natural stress (control). IBR approach I, was capable to detect vulnerability towards thermal sensitivity, salinity and feeding levels understanding overall health condition in clam species. According to this index, the rank of the most affected exposures can be ordered as: Warming > Warming + SS > Warming + Salinity > Warming + Salinity + SS > SS > Salinity > Salinity + SS > Control. Also, the ranking changed between approach I and approach V. Based on physiological and biochemical score, the most responsive biomarkers for most affected zone for approach I was LPO > CI > SOD in warming, CI > LPO > SOD in Warming + SS, LPO > CI > SOD in Warming + Salinity and CI > LPO > SOD in Warming + Salinity + SS.. Except energy reserves, all biomarkers were affected by all warming exposures. Overall, the most responsive biomarkers in decreasing order were CI > SOD ≥ ORAC ≥ LPO > CAT > ER. Altogether, this study provides evidence that global sea water temperature of 25°C may affect more in C. japonica because warming (elevated temperature exposures) shows deleterious effects in fitness.

In this study, two types of multi-biomarker approaches were used (PCA and IBR). Many researchers have used PCA as multivariate analysis method. Recently, to understand the stress level due to anthropogenic effects, IBR was used. Present study gave a clear attention towards selection of most sensitive biomarkers for different IBR approaches. Overall, the IBR index provided an integrated view of C. japonica stress levels allowing the discrimination of the more stressful exposures. Also, IBR approaches were much clearer in terms of stress levels in aquatic organisms than PCA analysis.
論文審査の結果の要旨

学位申請者Preeti Pokhrelから提出された博士学位論文「Integrated assessment of physiological and biochemical responses of Corbicula japonica in brackish environment（水環境におけるヤマトシジミの生化学的応答の総合的評価）」について、茨城大学大学院理工学研究科における博士学位論文評価基準に基づいて審査会において審査を行った。

本学位論文では、水生生物保全の観点から水環境の在り方を検討する手法の構築を目的として、水生性二枚貝ヤマトシジミ（Corbicula japonica）に着目し、自然的要因（塩分、水温、浮遊物質）に対する酸化ストレスマーカー（SOD, CAT, ORAC, LPO）等の生化学的指標、肥満度、タンパク・グリコーデン・脂質保有量等の生理学的指標を室内実験、フィールド調査から明らかにした。自然的要因が時空間的に変動する水環境に生息する二枚貝を対象とする研究は極めてチャレンジングであり、これまでに研究例はほとんどない。このようななかで、既報では考慮されてこなかった二枚貝の酸化ストレスマーカーの応答に対する摂餌・代謝の影響をはじめて明らかにした。また、既存のIntegrated biomarker responses indexの課題を指摘したうえで、本研究で得られた生化学的、生理学的指標を統合した手法を提案した。そして、21世紀末における気候変動を想定し、水生性二枚貝に及ぼす影響を評価した。このように、水生性二枚貝の生化学・生化学的応答や総合的な評価手法に関する極めて重要な知見を方法論を提示している。

本学位論文では、現在の社会的背景を踏まえ、既存の研究成果を幅広く引用したうえで研究目的を設定し、本研究の位置付けが明確に記述されている。研究方法も第三者による再実験が可能なように詳細に記述されている。実験、調査の結果は正確かつ明確に記述されており、新たに明らかにした事項を明確にしながら考察を論理的に展開し記述している。また、水生性二枚貝に対する酸化ストレスマーカーの応答機構や統合評価に関する科学的な知見、工学的な有用性も明確に記述されている。これらの研究成果は、審査付学術論文1編に著されている。

以上を総合して、本学位論文は本学大学院理工学研究科博士後期課程の基準を十分に満たしていると判断し、審査会委員全員一致で合格と判定された。学位申請者Preeti Pokhrelは、博士（工学）の学位授与は可と判定する。