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論文題目　Eco-physiological study on reproduction of the range-extended sea urchin Heliocidaris crassispina (分布拡大したムラサキウニの再生産に関する生理生態学的研究)

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論文内容要旨

Eco-physiological study on reproduction of the range-extended sea urchin *Heliocidaris crassispina*

分布拡大したムラサキウニの再生産に関する生理生態学的研究

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Chapter I. Introduction

Climate change mainly presented by global warming has caused increase in seawater temperature. It potentially propels extension of marine organisms to high latitude areas. Sea urchin is one of the ecologically important species. A few studies revealed high water temperature extended the range of some sea urchin species poleward. *Heliocidaris crassispina* is originally distributed in intertidal and shallow subtidal rocky reefs in southern Japan from Ibaraki in the Pacific Ocean and Akita Prefecture in the Sea of Japan. In August 2014, the dense habitation of *H. crassispina* was found in Toga Bay along the Oga Peninsula in the Sea of Japan. In contrast, *Mesocentrotus nudus*, which was predominant there until a couple of years ago, disappeared. This study aims to figure out (1) the driver of the range extension (2) temperature on replacement of sea urchin species and (3) reproductive adaptation of new extender. It is the first time to study on a new sea urchin population extended during the last decade.

Chapter II. Gonad development and population structure

Spawning of *H. crassispina* is in July to August in southern Sea of Japan. To figure out the driver of the range extension, and to explore the differences in the gonad development in spawning season and population structure of *H. crassispina* between extended and central ranges, the gonad development by sex, sex ratio and age composition of ~100 *H. crassispina,* which collected in a *Sargassum siliquastrum* bed at the depths of 3–5 m in Toga Bay (extended range) in August 2014, and on a barren at a depth of 3 m off Shitsumi, Fukui (central range) in August 2018, were investigated. Age compositions showed a persistent juvenile recruitment in both the localities. The sex ratio was skewed to female in Toga Bay, in contrast, balanced between sexes off Shitsumi. Ovaries were in the partly spawned and the spent stages in both localities. Testes were in the growing and premature stages in Toga Bay, in contrast, in the premature and mature stages off Shitsumi (Fig. 1). Thus, testicular development in both localities were delayed compared to ovarian development. But, testicular development in Toga Bay was more delayed than that off Shitsumi. Male *H. crassispina* may be more vulnerable to low water temperature in the extended range and to low food availability in the central range than females. Long-term water temperature data suggested that the range extension of *H. crassispina* from historic habitat was due to an increase in the temperature, particularly, during the summer spawning season.

Chapter III. Reproductive cycle and nutrient accumulation

To verify the delayed spermatogenesis of *H. crassispina* in Toga Bay, and identify its specific cause, seasonal changes in the gonad development and body (gonad, gut and gut content) indexes in relation to temperature and the carbon and nitrogen contents (C and N) and C/N ratio of the gonad, gut tissues, and gut contents were investigated. Thereby, the nutritional accumulation of the range-extended sea urchins was evaluated by sex. A defined gametogenic cycle with a single spawning per sex was found. The ovaries were in the premature–mature stages during June–July. The spent stage was mainly in August and September. In contrast, the testes were in the growing–mature stages from June to July and in the partly spawned stage from July to August, indicating that spermatogenesis was one month delayed compared to oogenesis (Fig. 2). Thus, spawning was partially synchronized between sexes. Meiotic cell divisions are the bases of spermatid and spermatozoa production. Low temperature inhibit cell proliferation, and alter spermatogenic process. A long-term warming trend in Toga Bay was observed in annual average and May–August, indicating that *H. crassispina* is subjected to the low temperature, particularly during winter in new habitat (Table 1). Past studies reported that higher protein content in testes than that in ovaries from the recovery to growing stages. However, there was no sexual difference in N content of gonad in *H. crassispina* (Fig. 3), indicating that the protein content accumulated in testes was insufficient at low water temperatures in winter. The suppressed meiotic cell divisions for spermatid and spermatozoa production and insufficient protein content in the testes for spermatogenesis might be the causal factors of delayed spermatogenesis.

Chapter IV. Effect of temperature on early life stage

To explore the effect of temperature (T) on behavior and feeding of juveniles of *H. crassispina* and *M. nudus* collected in Toga Bay and Shizugawa Bay, respectively, in June and December 2018 (Exp. 1), and on the early development from fertilization to larvae from adult *H. crassispina* collected in Toga Bay (Exp. 2) were investigated. In Exp.1, righting response, lantern reflex and feeding rate of 10 urchins of each species were measured every 7 days under T increasing and decreasing rate of 2.5 °C week^{-1}. In Exp. 2, the fertilization, and hatching and development rates at 10 different T (8–35°C) were measured.

The optimal T ranges for behavior and feeding were, respectively, 10.3–31.0°C and 10.3–33.4°C in *H. crassispina*, and 6.1–26.6°C and 11–24°C in *M. nudu*s, suggesting that *H. crassispina* tolerate higher T but do not tolerate lower T than *M. nud*us. The T increasing rate of 2.5 °C week^{-1} is more acute than that of ~3 °C month^{-1} in the wild, suggesting the high T limits of *H. crassipina* (33.3 °C) and *M. nudus* (30.5 °C) are 5–10°C higher than those in the wild. However, no information of defference in the low temperature limits from the T decreasing rates and in the wild is available. Between 26–31°C, the feeding rate significantly increased in *H. crassispina*, but decreased in *M. nudus* (Fig. 4), suggesting that high T in summer drove the replacement of *M. nudus* by *H. crassispina* in Toga Bay. The optimum T for larvae of *H. crassispina* ranged 16.8–29.0°C (Fig. 5), indicating possibility of adaptation in Toga Bay.

Chapter V. Conclusion

In adult *H. crassispina* in Toga Bay, the results of female-skewed sex ratio, partially synchronized spawning, and delayed spermatogenesis would lower the possibility of self-reproduction in the newly extended population. However, the results of a persistent juvenile recruitment, and adjustment of high temperature for juveniles suggest the possible adaptation in Toga Bay. In addition, the optimum temperature range of fertilization to larvae associated with increasing seawater temperature may indicate that the population in Toga Bay is acting as larval sinks or sources in the metapopulation. During the last 100 years, increased temperature in the central Sea of Japan was 1.7°C, which was the highest in all sea areas around Japan. Also, a long-term warming trend during summer in Toga Bay suggests that *H. crassispina* will further extend to the north. Overgrazing of large fucoid beds by the range extender would be a potential threat because of the loss of spawning bed for sandfish stock, which is commercially important species in Akita Prefecture. The assessment of densities of the extended population to clarify whether low population size producing a reduction of fitness by Allee effect is needed. In addition, to demonstrate the mechanism of delayed spermatogenesis, the sexual differences in food consumption, digestive enzyme activity and digestibility at low water temperature are needed to be clarified.

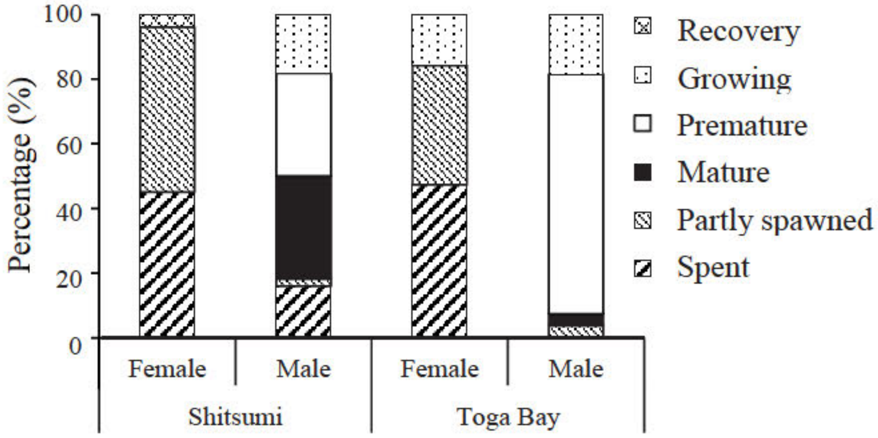

Fig. 1. Percentages of each gonad development stage of *Heliocidaris crassispina* by sex collected off Shitsumi in August 2018 and in Toga Bay in August 2014.

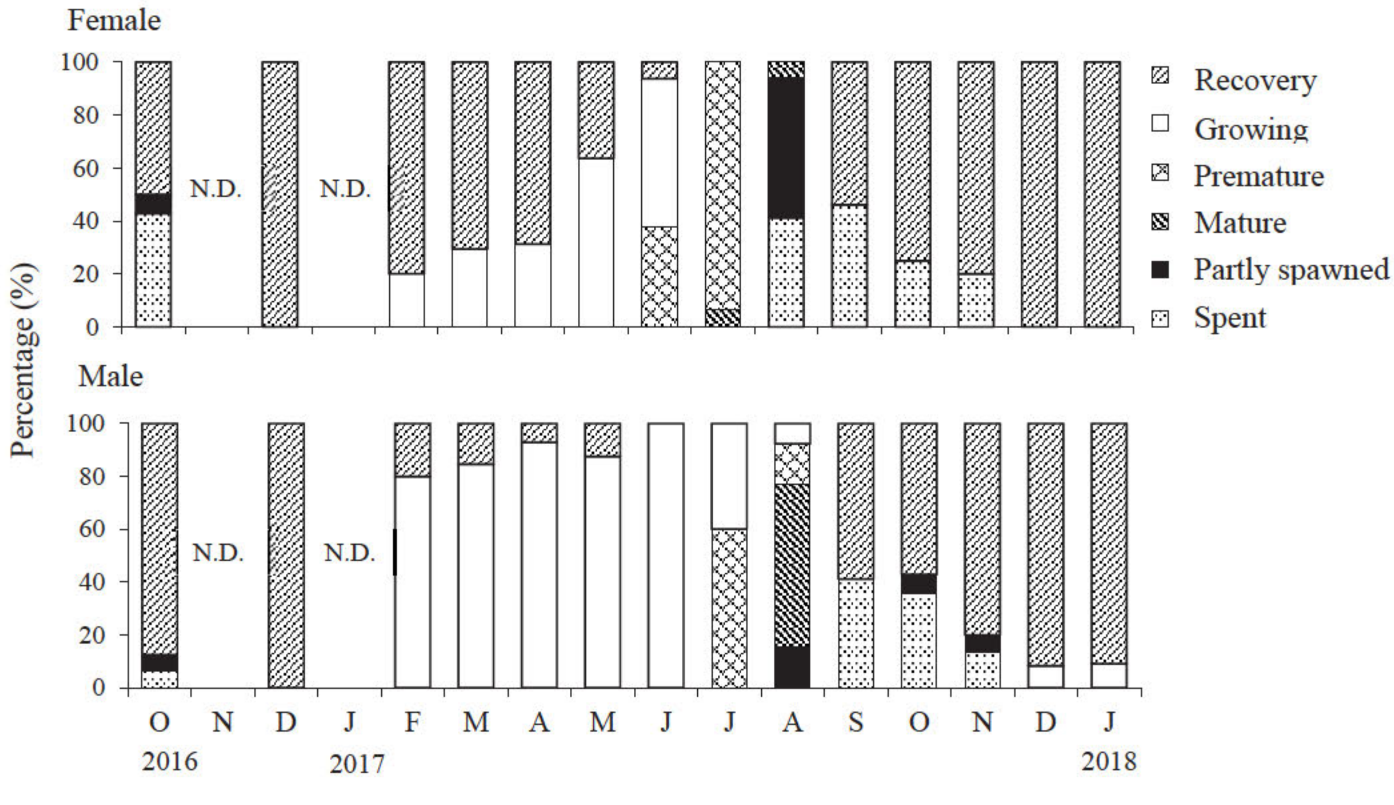

Fig. 2. Monthly changes in the gonad developmental stages of *Heliocidaris crassispina* by sex in Toga Bay along the Oga Peninsula, Akita Prefecture. N.D. indicates no data. Legends indicate gonad developmental stages. Letters from O to J of X axes indicate months.

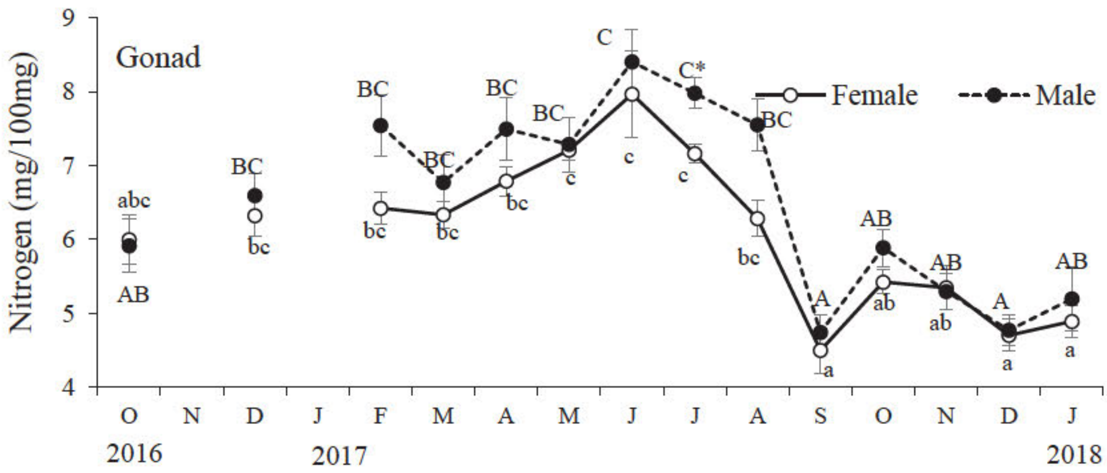

Fig. 3. Seasonal changes in nitrogen contents of gonad in female and male *Heliocidaris crassispina* (means ± SE). Upper and lower alphabetical letters indicate significant differences in male and female gonads, guts and gut contents among months, respectively. Asterisks indicate sexual differences ($p < 0.05$).

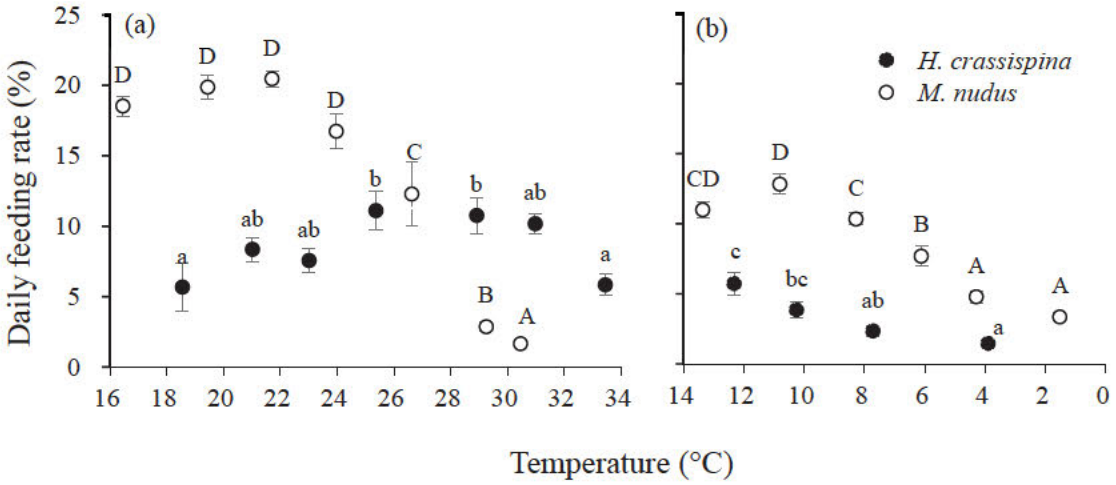

Fig. 4. Changes in daily feeding rate of *Heliocidaris crassispina* and *Mesocentrotus nudus* along with temperature change in high (a) and low (b) temperature tolerance experiments (means ± SE). Lower and upper alphabetical letters indicate significant differences in daily feeding rate of *H. crassispina* and *M. nudus*, respectively, among different temperatures, respectively ($p < 0.05$).

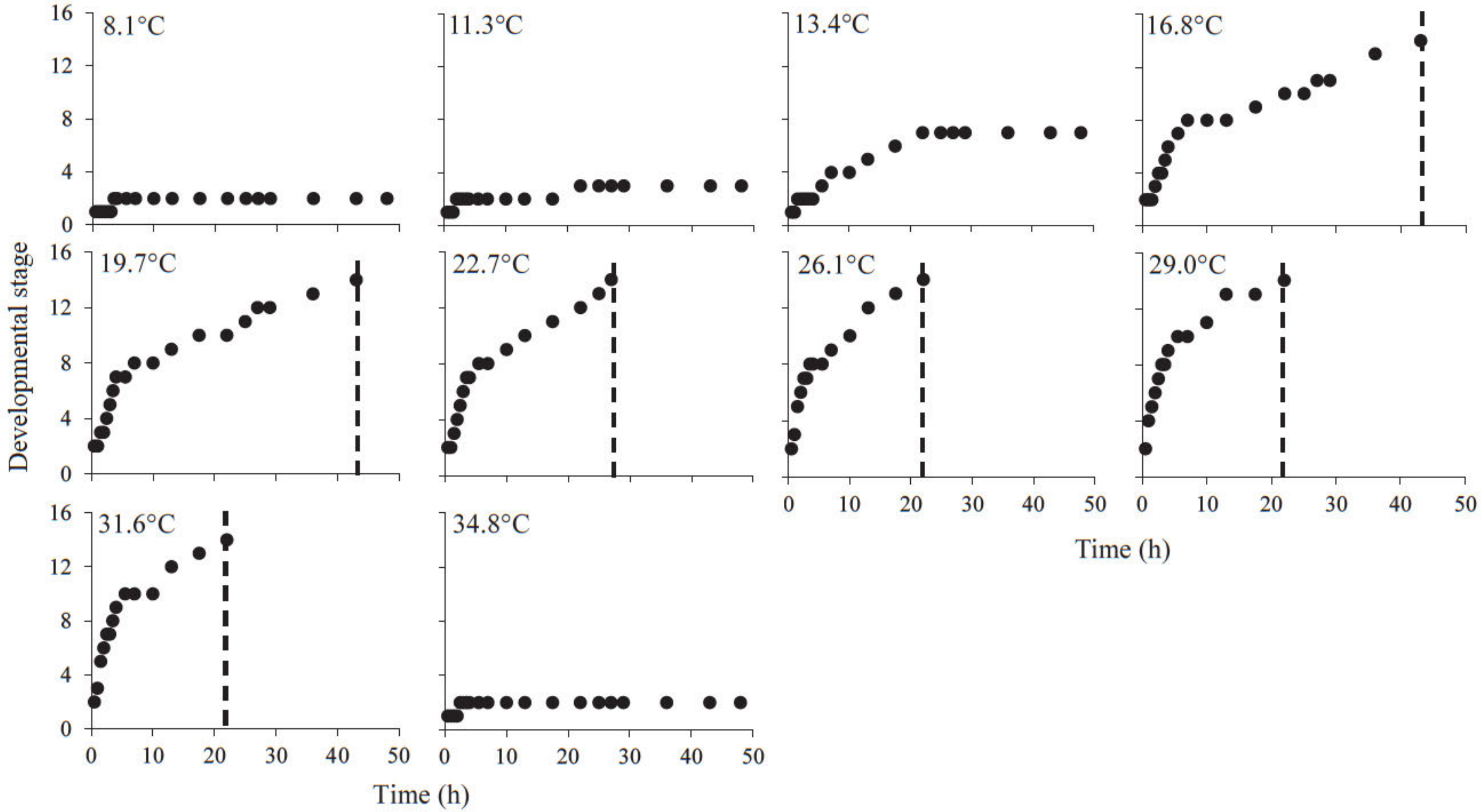

Fig. 5. Temperature-dependent deve`opment rates of *Heliocidaris crassispina* reared at 8.1–34.8°C. Stages of embryo development: Stage 1. unfertilized egg; 2. fertilized egg; 3. 2-cell; 4. 4-cell; 5. 8-cell; 6. 16-cell; 7. 32-cell; 8. early blastula; 9. hatched blastula; 10. early gastrula; 11. Gastrula; 12. early prism; 13. prism; 14. early pluteus. Broken vertical lines indicate the minimum time required to reach early pluteus.

Table 1.

Warming trends in average seawater temperature off Unosaki near Toga Bay by month and annual average for 33 years (1986–2018) (n = 33).

	Annual avg.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
r	0.531	0.343	0.044	0.089	0.332	0.706	0.706	0.856	0.636	0.102	0.297	0.324	0.307
p	**0.002**	0.063	0.817	0.639	0.073	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.591	0.112	0.081	0.082

Statistically significant differences ($p < 0.05$) are shown in bold.

論文審査の結果の要旨及び担当者

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論　文　審　査　の　結　果　の　要　旨

近年、海洋の高水温化の進行にともない、海洋生物の高緯度への分布の拡大がみられている。2014年8月に秋田県男鹿半島戸賀湾の潮下帯浅所において、数年前まで優占して分布していたキタムラサキウニがみられず、ムラサキウニが優占して分布していることが確認された。本研究はキタムラサキウニからムラサキウニへの種の交替がもたらされた要因と交替したムラサキウニの再生産への寄与について生理生態学的に明らかにする。

ムラサキウニの産卵期に相当する8月に、戸賀湾水深3-5mのヨレモク群落および地理的分布の中心に位置する福井県小浜市沿岸水深3mの無節サンゴモ群落で、各約100個体を無作為に採集し、雌雄別に生殖巣の発達段階と年齢を調べた。年級群組成は両地区とも稚仔の毎年の加入を示した。性比は戸賀湾では有意に雌へ偏り、小浜では雌雄の偏りはなかった。両地区で卵巣は一部放出期と放出期であった。精巣は戸賀湾で成長期と成熟前期、小浜では成熟前期と成熟後期と両地区で精巣の発達は卵巣よりも遅れ、戸賀湾は小浜に比べてより遅れていた。雄は雌に比べてより低水温（戸賀湾）と食物の低保障（小浜）に影響をされやすいと示唆された。長期の水温変化から戸賀湾のムラサキウニの分布拡大は夏季の産卵期の水温上昇に起因すると考えられた。戸賀湾で精巣の成熟が遅れる要因を検証するため、雌雄の季節的な生殖巣の発達過程および生殖巣、消化管、消化管内容物の体重重量比と、水温、生殖巣、消化管、消化管内容物のCN含有量とC/N比の関係を調べた。本種は雌雄で年1回産卵する生殖周期を示した。卵巣は6-7月に成熟前期、8-9月に放出期に移行した。これに対して、精巣は6月に成長期、7月に成熟前期、9-10月に放出期に移行し、精子形成は卵形成よりも1ヶ月遅れ、産卵は雌雄で明瞭に同調しなかった。過去33年間で5-8月の各月の平均水温は有意に上昇したのに対して、冬季間は上昇しなかった。回復期から成長期に生殖巣に含有されるタンパク質は雄が雌よりも多い既往の研究に対して、戸賀湾のムラサキウニ生殖巣のN含有量に雌雄差はなく、冬季の低水温下で精巣へのタンパク質の蓄積が不十分であることを示した。一方、低水温が減数分裂による細胞増殖を阻害して、精子形成に負の影響を与えるのかもしれない。

2018年6月と12月に戸賀湾と宮城県志津川湾において、それぞれムラサキウニとキタムラサキウニの稚仔を採集し、室内循環水槽により水温の上昇と下降（2.5℃/週）が“起き上がり”と“口器の反射”行動および摂餌に及ぼす影響を調べた。ムラサキウニ稚仔の行動と摂餌はキタムラサキウニ稚仔より高水温で耐性があり、より低水温で耐性はなかった。受精から幼生に至る発育速度を8–35℃の異なる10水温下で調べた結果、至適水温は16.8-29.0℃であり、その時期の戸賀湾の水温範囲にあった。

戸賀湾のムラサキウニ成体の雌に偏った性比、雄の成熟の遅れ、そして同調しない雌雄の産卵は再生産への寄与が低いことを示唆する。受精から幼生に至る現場水温への適応と連続した稚仔の加入は南部の個体群からの幼生の輸送によってもたらされている可能性が考えられる。日本海中部海域は過去100年間で日本周辺海域の中で1.7℃と水温がもっとも上昇した。特に、産卵期の夏季の水温の上昇は、さらに高緯度への分布の拡大をもたらすと考えられる。藻場を形成する海藻はコンブ目からヒバマタ目へ交替しており、ムラサキウニには良好な食物環境になると想定される。高水温化により分布を拡大した場所での環境への適応の初期過程における重要な生理生態学的な研究成果であることから、審査員一同、博士（農学）の学位を授与に値すると判断した。