The mechanisms influencing the timing, success and failure of spawning in natural populations of the sea urchin *Strongylocentrotus intermedius* in the northwestern Sea of Japan

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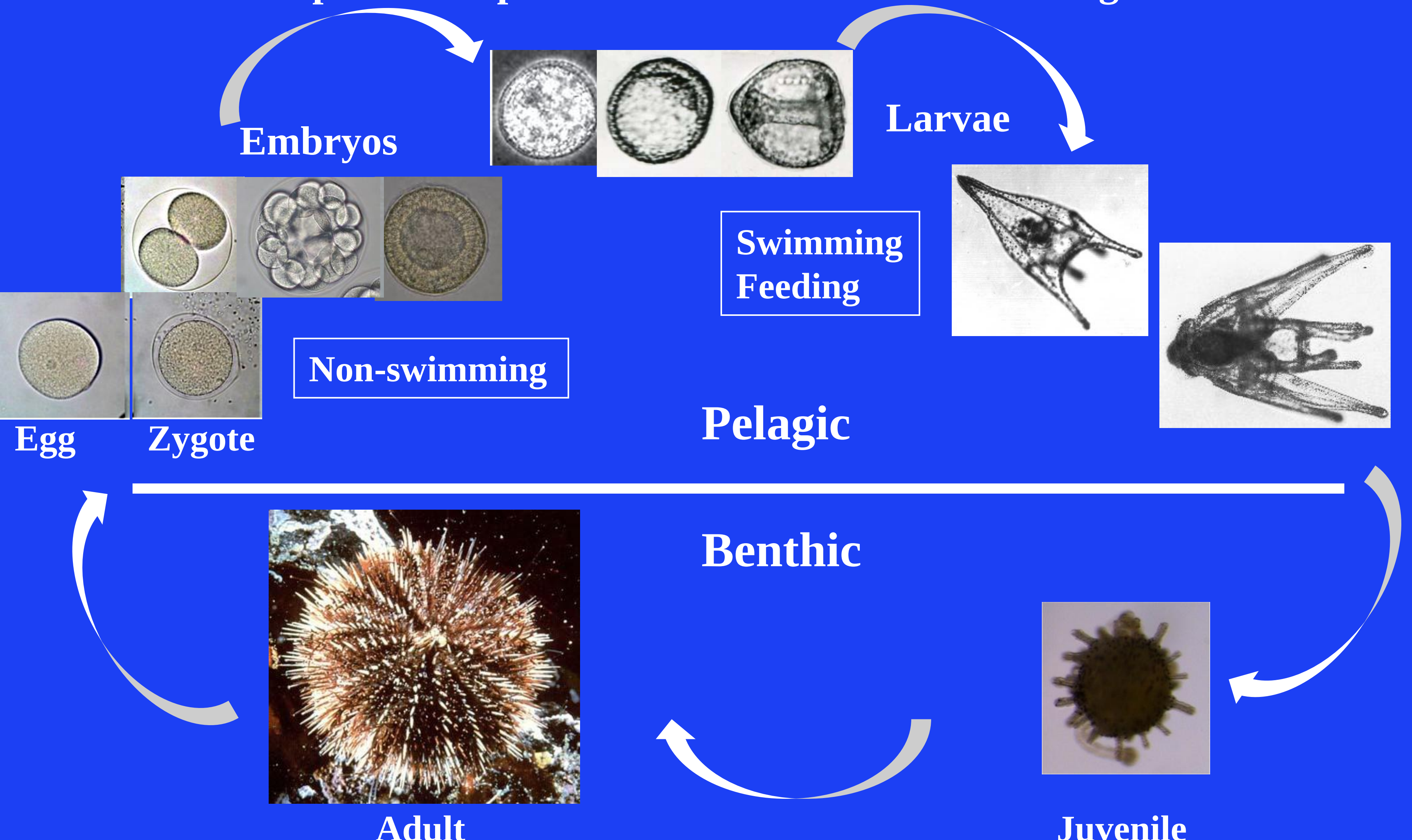
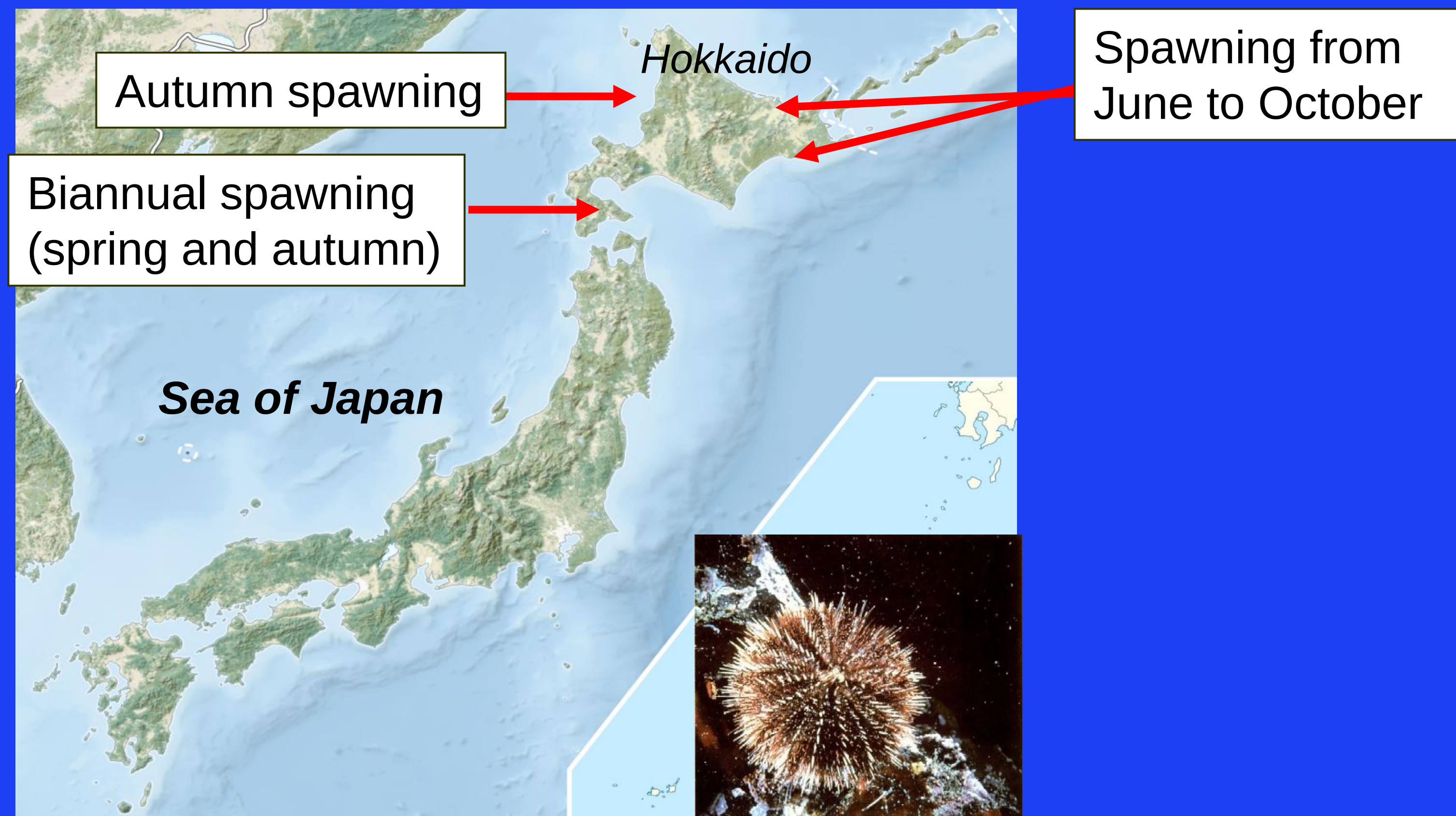
1. Introduction

A biphasic life cycle of broadcast spawner that includes pelagic planktotrophic larval and benthic adult stages



Most sea urchins are typical broadcast spawners with planktotrophic larvae.

In some common species of sea urchins, the reproductive cycle is not synchronized within a population. The reasons for such desynchronization remain unclear.



The main purposes of our work were:

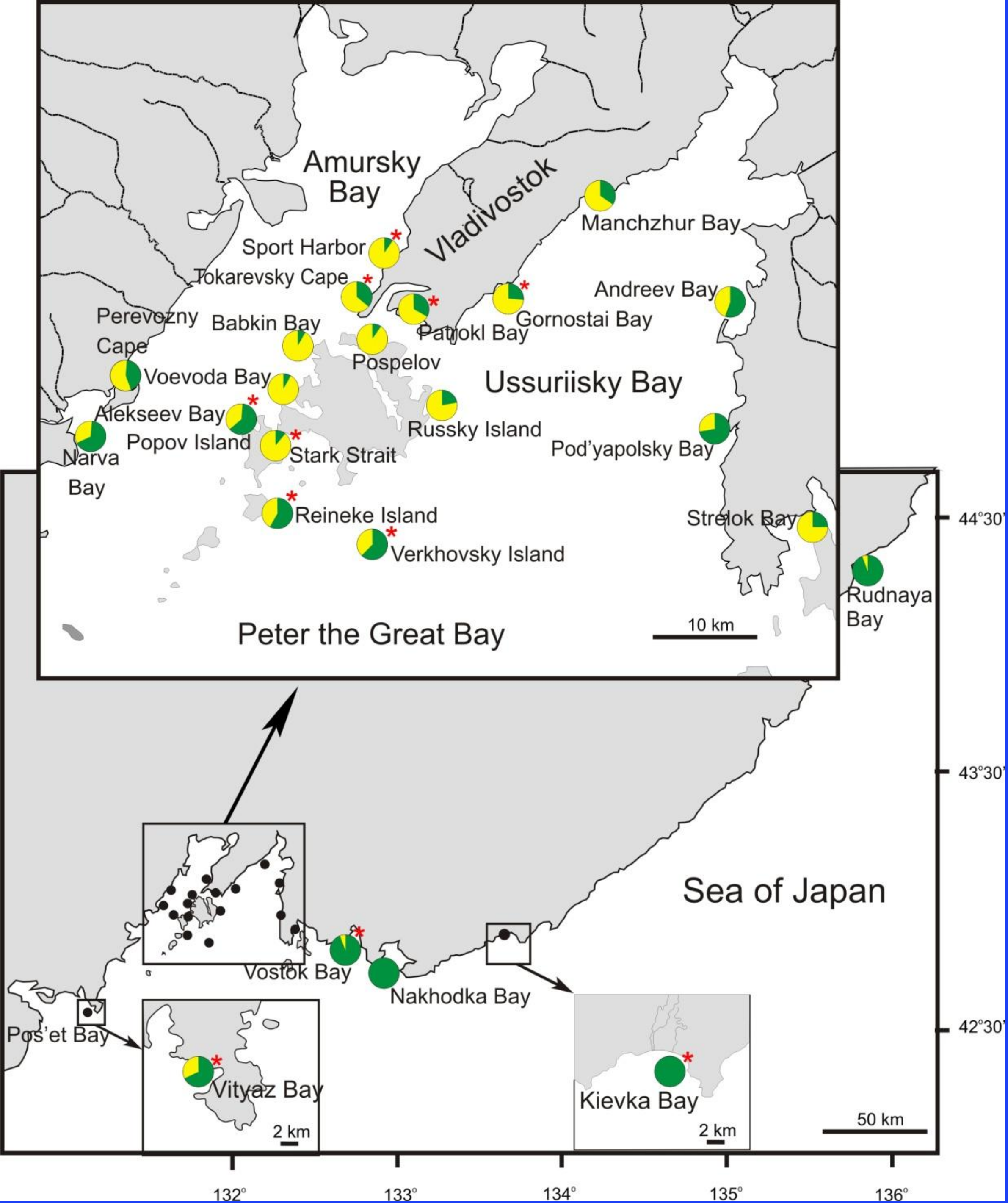
(1) to study the reproductive cycle and spawning schedule of *S. intermedius* populations inhabiting the areas with different level of anthropogenic pressure in the northwestern Sea of Japan (along 400 km of the coast of the Primorye region of Russia);

(2) to clarify the relationships between natural environmental factors (phytoplankton, temperature, salinity, dissolved oxygen, moon cycle, tide level, time of the day and anthropogenic pressure) and the timing of spawning in sea urchin populations.

2. Reproductive cycle and spawning schedule of *S. intermedius* in different populations

In the northwestern Sea of Japan, 3 types of populations of the sea urchin *S. intermedius* were found that differ from each other in the proportions of individuals with different spawning schedules.

- **Sea urchin populations with a pronounced late spawning (autumn) were referred to the first type.**
- **Sea urchin populations with a pronounced early spawning (early summer) were referred to the second type.**
- **The major part of sea urchin populations belongs to the third type, which is characterized by approximately equal proportions of individuals with early and late spawning.**

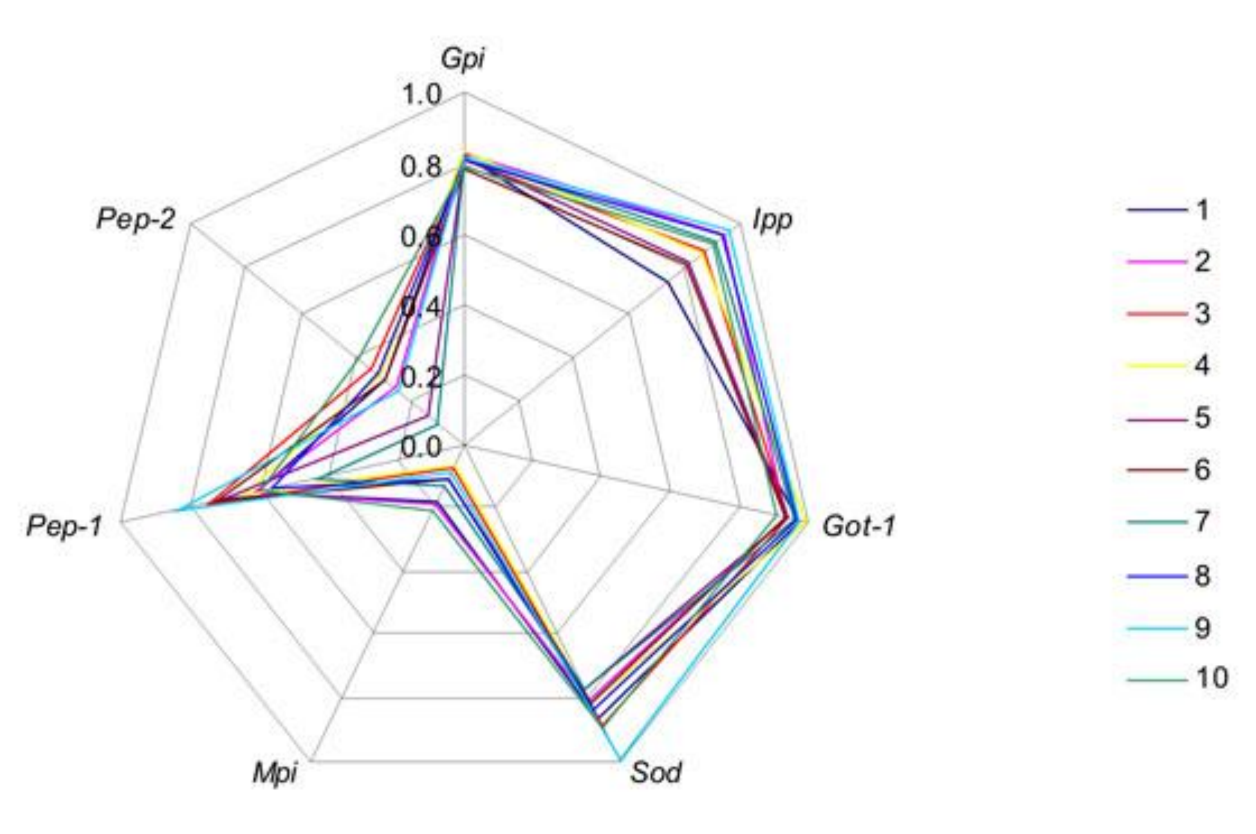


Sea urchin populations with a predominant early spawning (yellow parts of the circles) are located close to Vladivostok city which is the main source of marine pollution in the study area.

The reasons for the regional differences in the reproductive cycle of the same species of marine invertebrates with planktothrophic larvae are still unknown. There are several hypotheses for explanation of this fact.

- **<u>Hypothesis of the latitudinal gradient</u> is based on the recognition of temperature and photoperiod as the main environmental factors regulating reproductive cycles of marine invertebrates. It states that in temperate waters, the specimens spawn during a short period of the year while under tropical conditions, the spawning period extends and some species can spawn throughout the year (Mercier&Hamel 2009). However, this hypothesis cannot explain, why *S. intermedius* populations in the northwestern Sea of Japan (our data) as well as populations of this species around Hokkaido (Agatsuma 2007) have different spawning schedules despite they inhabit at same latitude which ensures similar photoperiod and temperature conditions.**

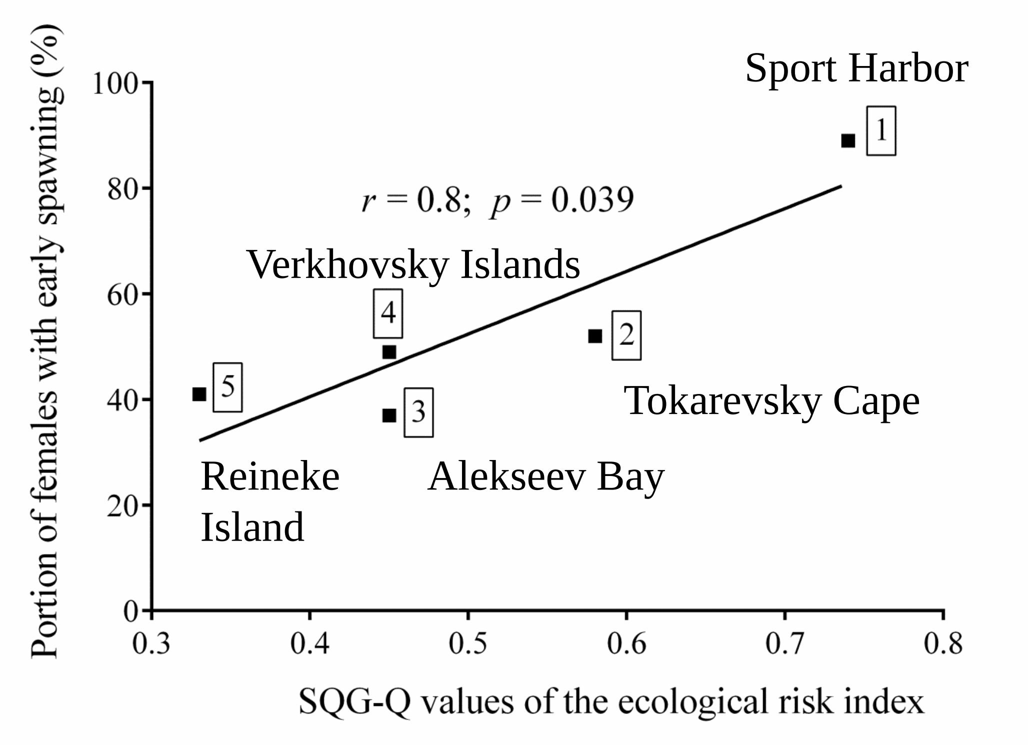
<u>Hypothesis of genetic determination of the reproductive cycle</u> of *S. intermedius* suggested by Japanese scientists (Agatsuma 2007) was not confirmed by our study of genetic population structure of this sea urchin based on the analysis of 7 enzyme polymorphic loci and 12 polymorphic loci of microsatellite DNA which revealed no significant genetic differences between the specimens with different spawning time (Zaslavskaya et al. 2012, Bondar et al. 2013).



Genetic profiles of 10 *S. intermedius* populations with different spawning time by frequencies of the most often occurring alleles in 7 enzyme loci (acording to: Zaslavskaya et al. 2012).

Hypothesis of phenotypic response to changing environmental conditions due to chronic anthropogenic pollution is based on our observations that:

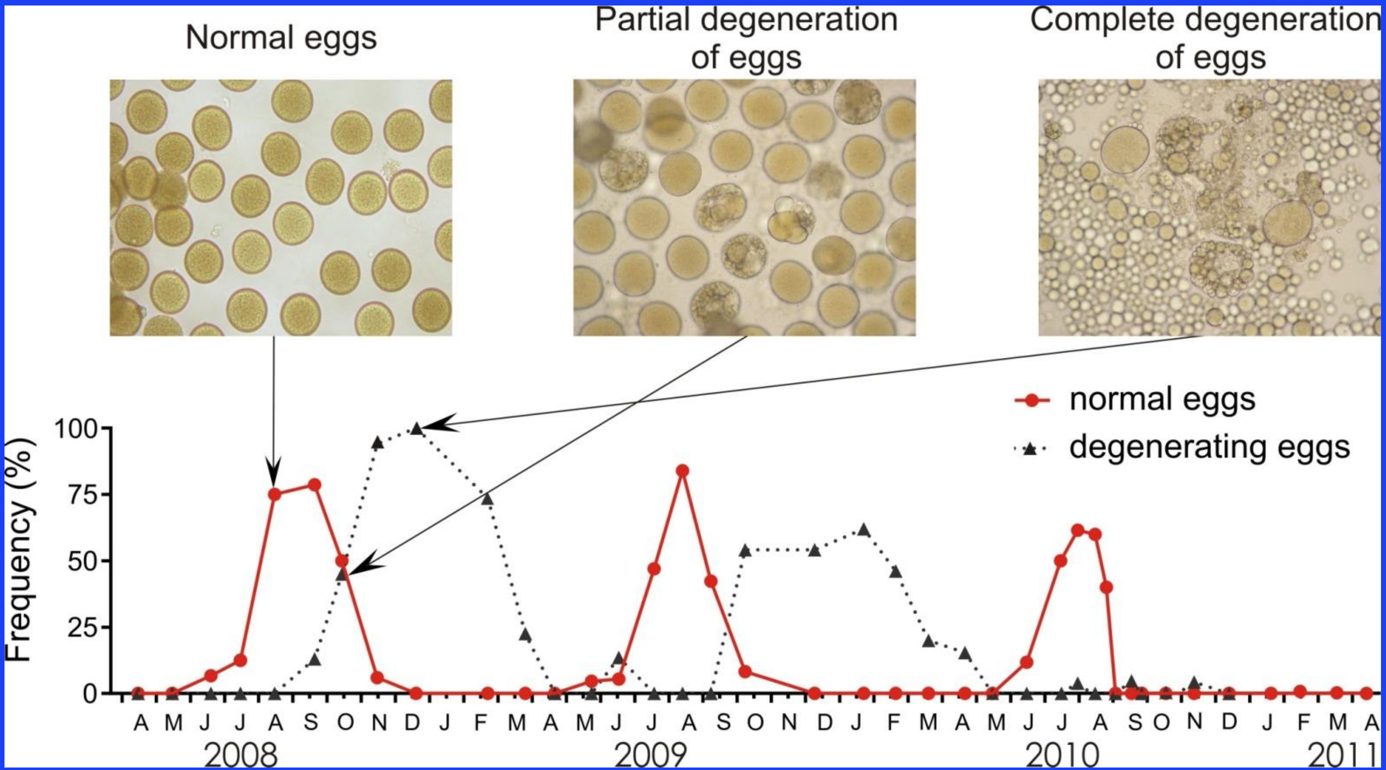
1) **early spawning is mostly pronounced in *S. intermedius* populations located close to sources of pollution;**
2) **during the recent 50–60 years, the shift in spawning season from autumn to early summer happened in some sea urchin populations inhabiting chronically polluted environments in Peter the Great Bay whereas in relatively clean areas, sea urchin populations retained autumn spawning.**
3) **there is correlation between the portions of females with early spawning in populations of *S. intermedius* and potential toxicity of surface bottom sediments.**



Our hypothesis suggests that sea urchins due to their high phenotypic plasticity synchronize their spawning with conditions favorable for food supply for the larvae, that is, availability of phytoplankton.

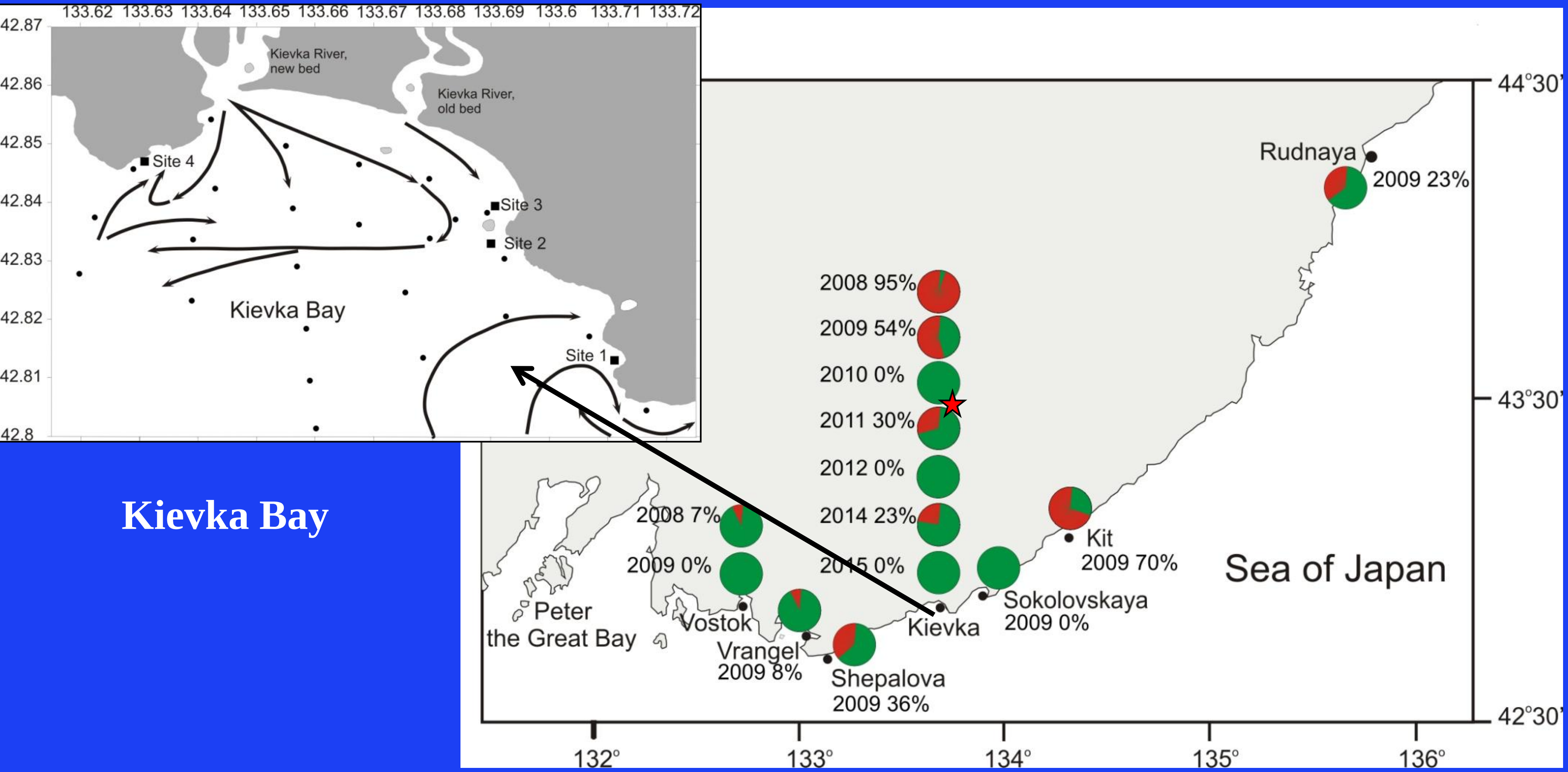
3. Environmental variables influencing *S. intermedius* spawning
3.1. Spawning failure in *S. intermedius* populations

A very important finding was that sea urchins from Kievka Bay failed to end their reproductive cycle with complete spawning in some years. Instead of normal spawning, a mass fragmentation of sea urchin eggs occurred. The studies of this phenomenon helped us to understand the mechanisms influencing the timing of the reproductive cycle of *S. intermedius*.



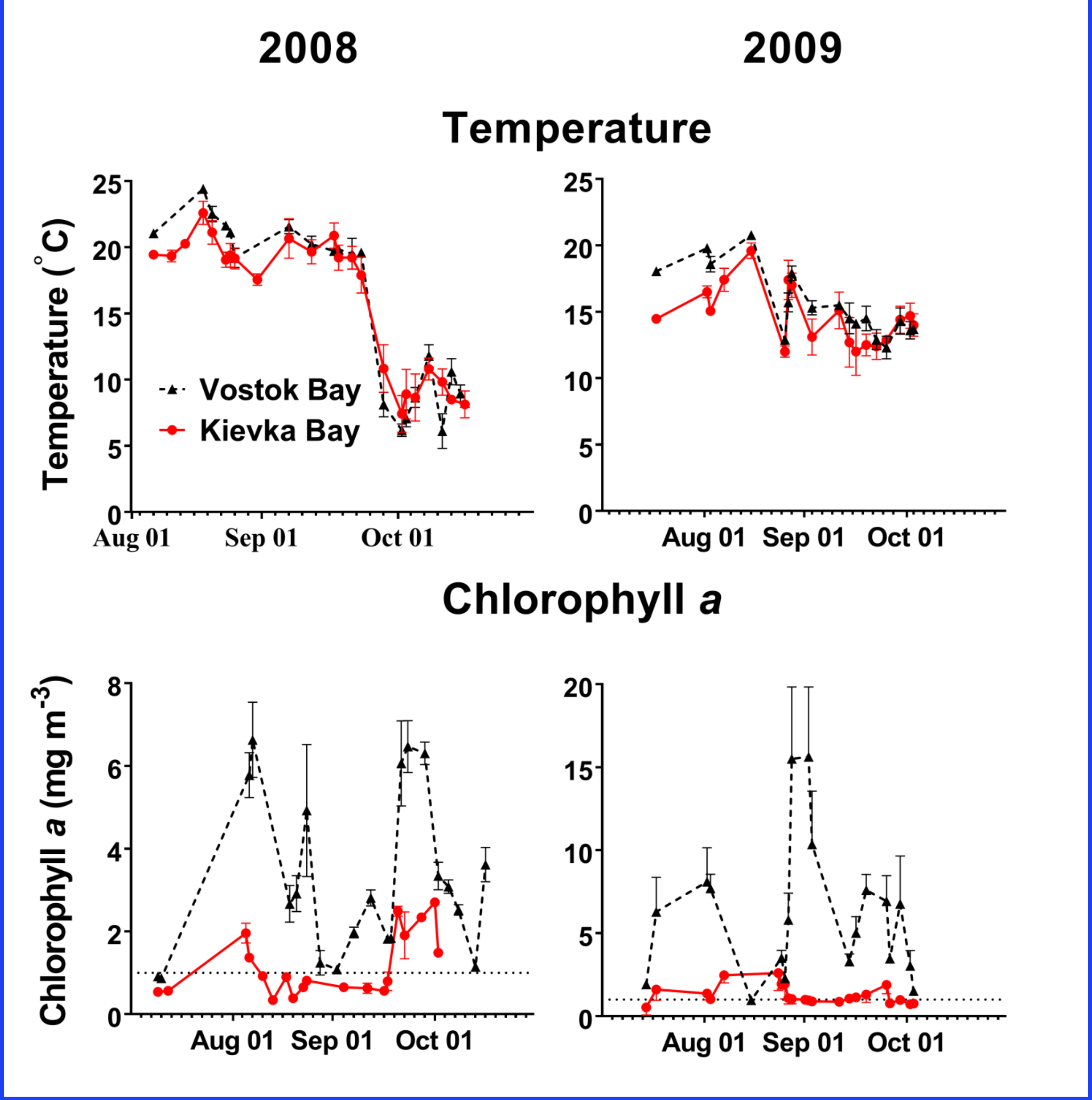
Temporal dynamics of the percentages of *S. intermedius* females from Kievka Bay releasing normal eggs (red lines) and fragmented eggs (black dashed lines) in 2008–2011.

Occurrence of spawning failure in *S. intermedius* populations along the coastline of the Primorye region (northwestern Sea of Japan). Red parts of the circles denote the percentages of unspawned females.



Kievka Bay

According to satellite data, the spawning failure (up to 95%) takes place in the areas with low chlorophyll concentration during the sea urchin spawning season.

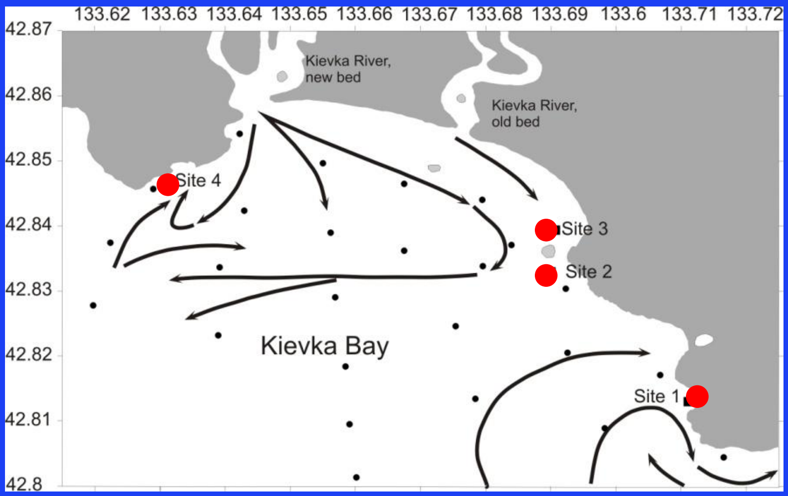


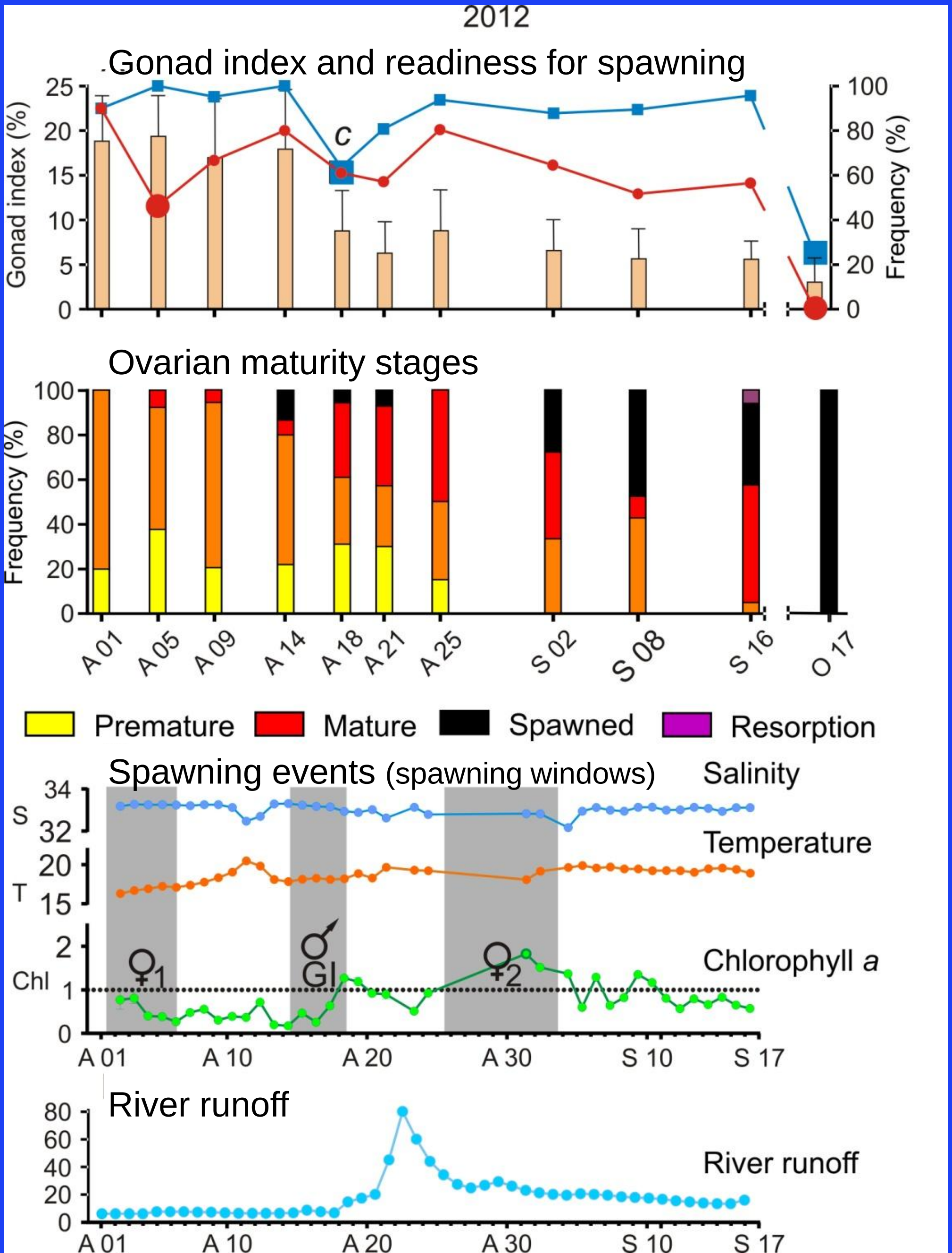
The data obtained indicate that:

1) **sea urchin spawning does not occur automatically after gamete maturation and needs for appropriate environmental conditions;**
2) **a link appeared to be between spawning success in *S. intermedius* populations and the phytoplankton level in the study area;**
3) **it is necessary to conduct the monitoring of environmental parameters directly in sea urchin habitats.**

- **To test the hypothesis that phytoplankton induces or fails to induce spawning in *S. intermedius* populations, we conducted a detailed analysis of environmental parameters (concentrations of dissolved oxygen and chlorophyll *a*, temperature, salinity, moon phases and tide level) which were monitored directly in the animal habitats by a multi-parameter sonde, along with examination of gonadal state of sea urchins sampled at a fine temporal scale (3–12 days, on average 4.2 days). The studies were carried out in August–September of 2011 and 2012 in Kievka Bay at 4 sites.**

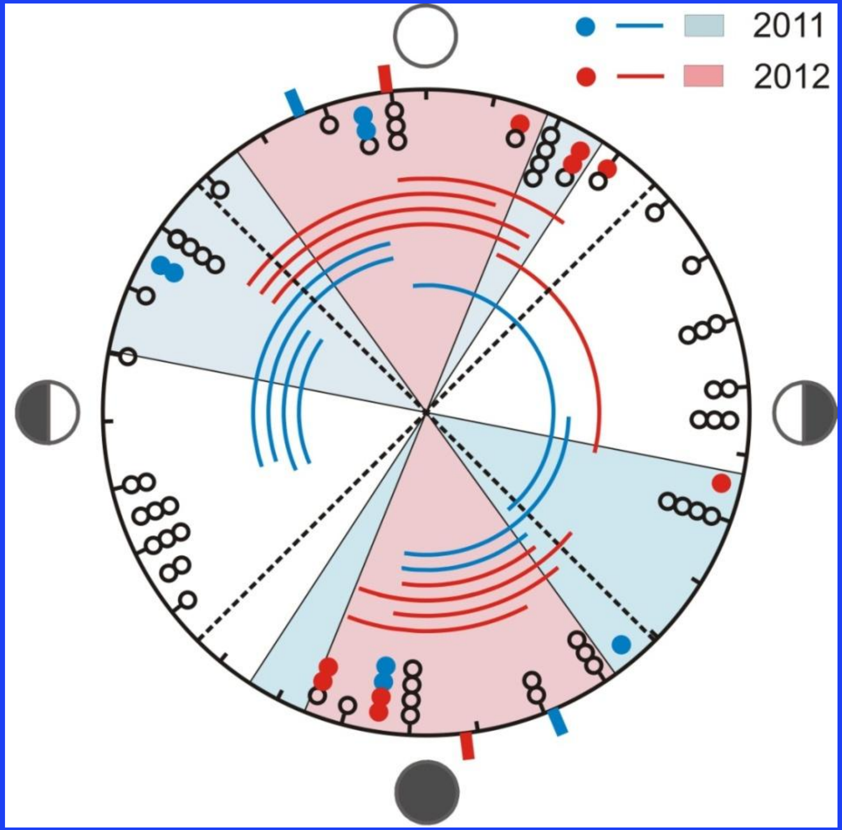
3.2. Relationship between environmental variables and spawning events





Logistic regression analysis revealed no significant relationships between temperature, salinity, dissolved oxygen or tidal activity and the timing of spawning, but there was a significant positive relationship between chlorophyll concentration and the probability of spawning events.

Additionally it was shown that the majority of spawning events coincided with the phases of new and full moon. However, the differently pronounced influence of the moon cycle on sea urchin spawning in different years and even at different sites indicates that the lunar cycle may serve as an additional factor which is able to affect spawning process.



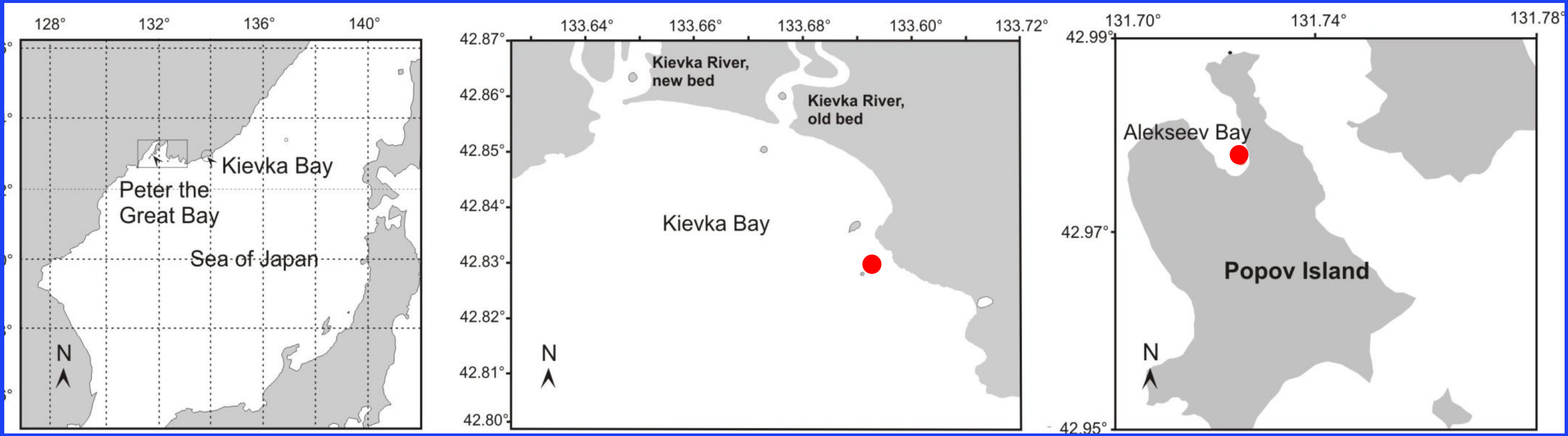
Summary of spawning events (blue and red circles) for *Strongylocentrotus intermedius* in Kievka Bay (northwestern Sea of Japan) in 2011 and 2012 in relation to the lunar phases. Open circles correspond to the sampling dates when no spawning events were recorded.

However, despite the data obtained strongly suggest that phytoplankton concentration is the main factor driving the initiation of spawning in *S. intermedius* populations, the methodological approach applied in these studies does not allow for an exact correlation between spawning events and the dynamics of environmental variables. To achieve this goal, we used a complex methodological approach that included both traditional methods of sea urchin gonad examination (with a sampling frequency of 3–7 days) and video recording of sea urchin spawning behaviour (at 1-min intervals) in combination with automatic datalogging of environmental variables (at 10-min intervals) in the habitats of sea urchins.

3.3. Assessing the effect of environmental factors on sea urchin spawning activity through video recording observations

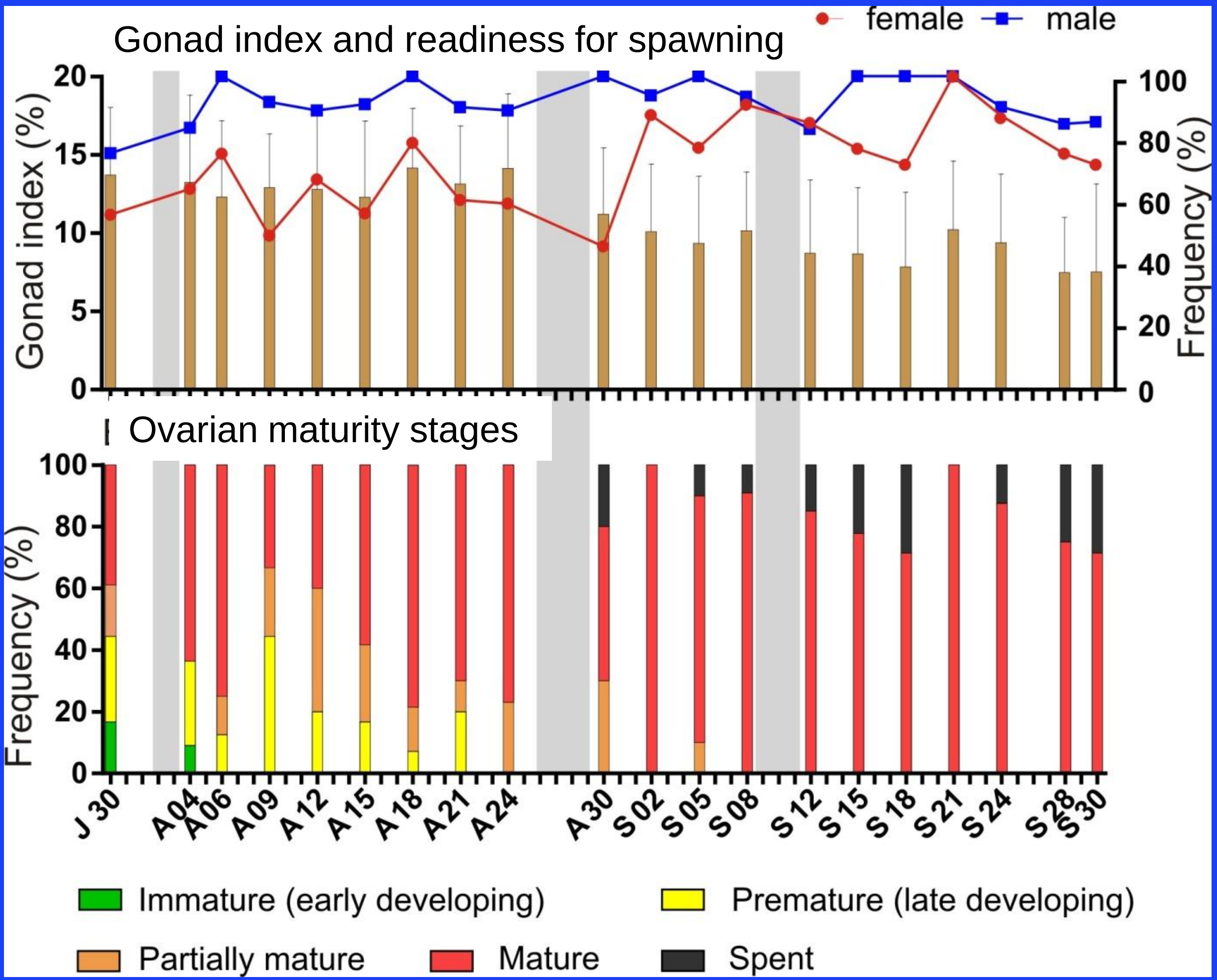
The installation for video recording of sea urchin behaviour and measurement of environmental variables which included from 2 to 6 video cameras and a multi-parameter RBRXRX-620 sonde.

The studies on video recording of sea urchin spawning behaviour were conducted in two bays, Kievka Bay and Alekseev Bay, differing by levels of primary production. Three sets of observations were performed, two in Kievka Bay (August–September of 2014 and 2015) and one in Alekseev Bay (May–June of 2016).



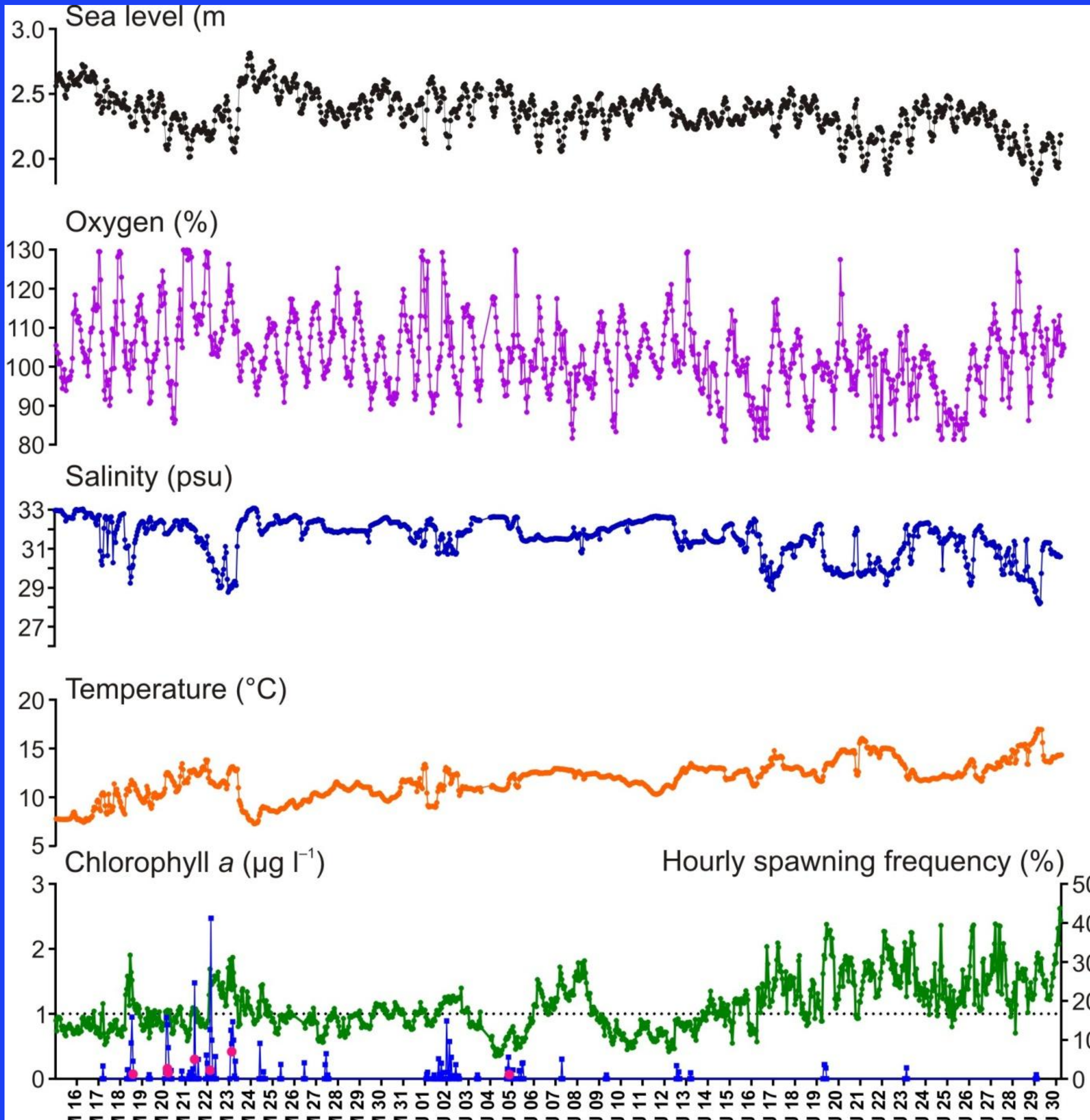
During these studies, the following data were obtained:

- **The data on the gonad state of sea urchins (gonad index and maturity condition, readiness for spawning);**



Temporal dynamics of the gonad index (brown bars), the percentages of males (blue lines with squares) and females (red lines with circles) ready for spawning and the percentages of ovarian maturity stages in *S. intermedius* from Kievka Bay (northwestern Sea of Japan) in 2015. Shaded areas denote the storm periods. x-axis: month and date.

The data on the environmental conditions;

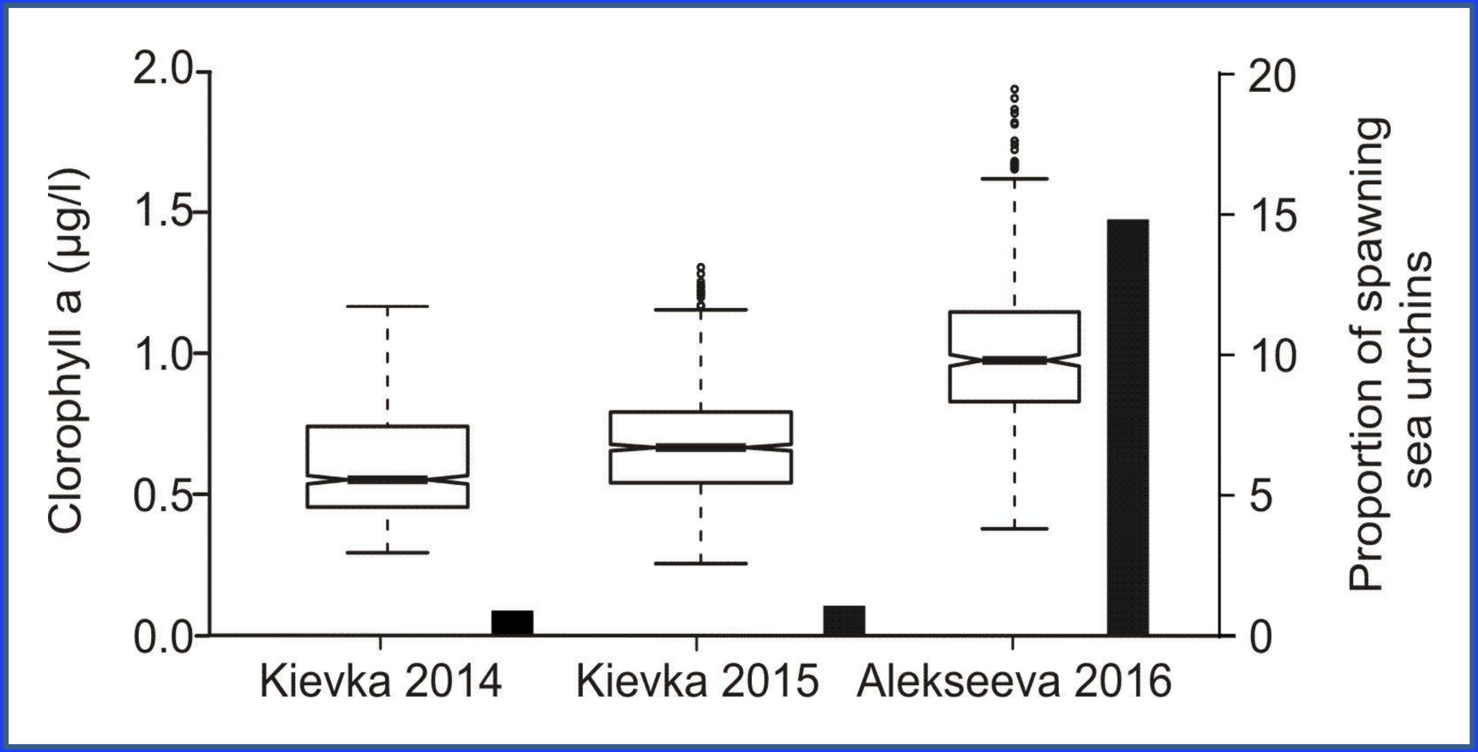


Timing of spawning of *S. intermedius* assessed through video recording and the associated environmental conditions in May–June of 2016 in Alekseev Bay (northwestern Sea of Japan).

● The data on the spawning behaviour of sea urchins.

The results of analysis of these data showed that:

- The significantly higher chlorophyll concentration (box plots) in Alekseev Bay corresponded to a higher average daily frequency of sea urchin spawning events (black columns), compared to that in Kievka Bay;

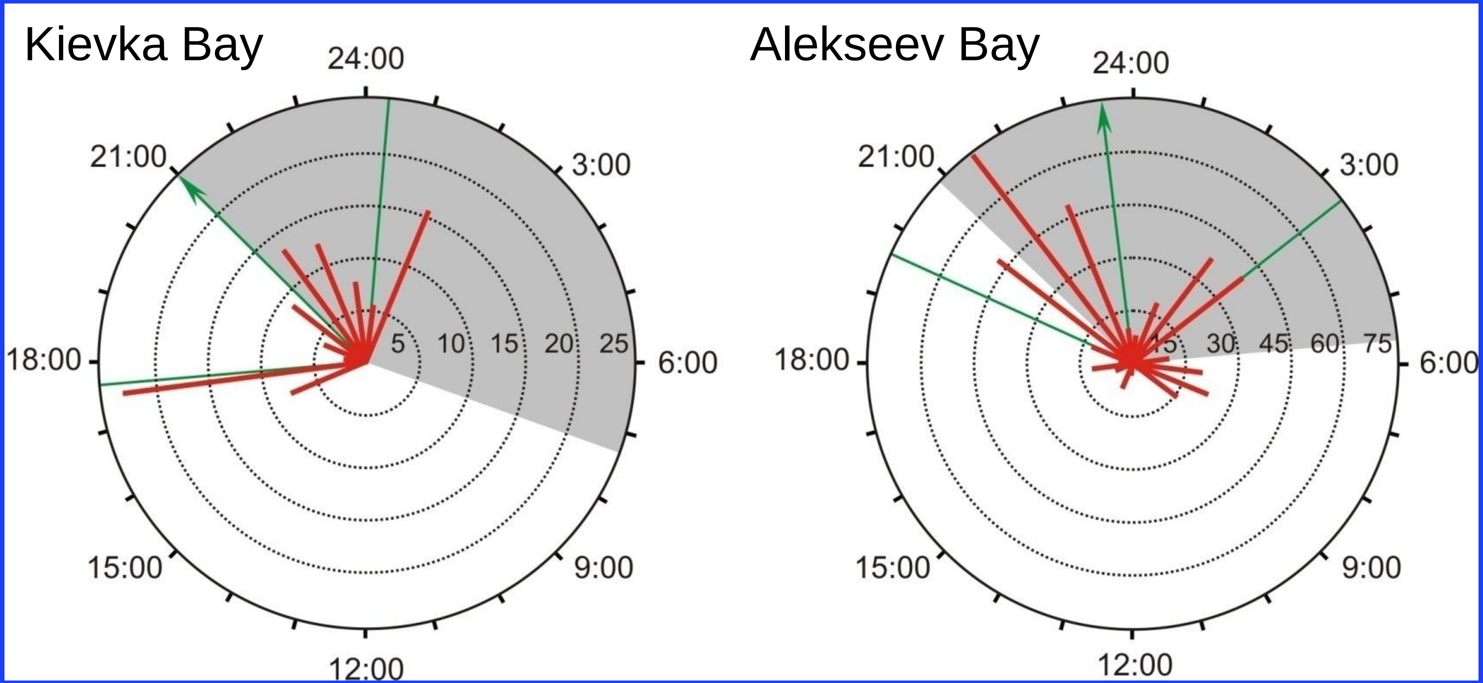


- Spearman's correlation analysis revealed no significant relationships between the hourly frequency of spawning events in sea urchin populations from both study areas and temperature, salinity and oxygen saturation. The only significant positive relationship was between the hourly frequency of spawning events and chlorophyll concentration;

- Linear regression analysis revealed that sea urchin spawning was initiated when a certain chlorophyll concentration (the threshold value) was attained. For Alekseev Bay, this threshold value was 0.8 µg/ l whereas for Kievka Bay, it was lower: 0.5 µg/ l;

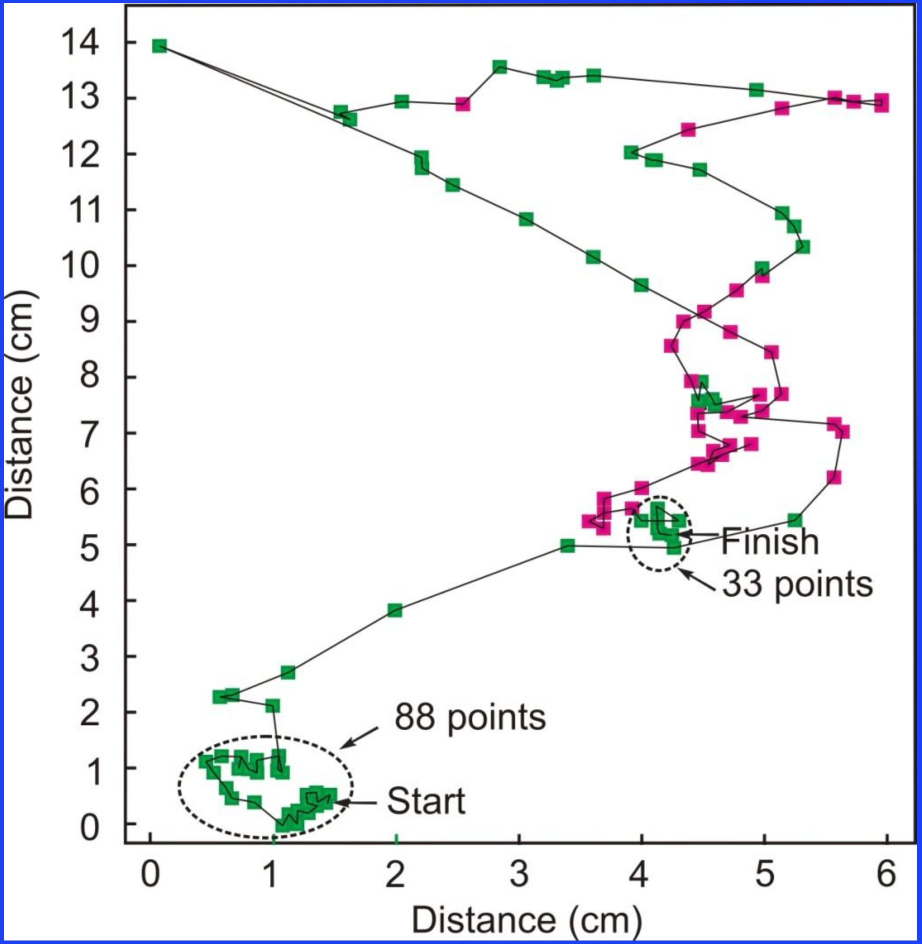
- It was repeatedly shown that the moon cycle influences spawning activity. In Alekseev Bay, the peak of spawning activity was centred around the full moon between May 20 and 24, when 55% of males and 88% of females spawned;

- **In both bays, sea urchin spawning events were not uniformly distributed over the day. Because sea urchins predominantly spawned during the night and spawning coincided with elevated concentration of phytoplankton, we can suggest a synergistic effect of these two factors on *S. intermedius* spawning.**.



The diagrams of distribution of spawning events of the sea urchin *S. intermedius* over a 24-h period in Kievka Bay and Alekseev Bay.

Analysis of sea urchin spawning behaviour showed that males became to spawn first. Spawning activity of males began from an increase in motion activity. Females began to spawn only when there were several spawning males nearby.



The distance passed by the male for a period beginning 10 min before and ending 5 min after spawning. Green line denotes the distance passed by nonspawning male, and red line denotes the distance passed by the male during spawning.

4. Conclusion

1. **Phytoplankton is the most important factor because under conditions of low phytoplankton abundance, spawning failure occurs in natural *S. intermedius* populations.**
2. **Increasing the concentration of phytoplankton induces moving activity and spawning in male sea urchins.**
3. **Both an elevated phytoplankton level and sperm are necessary to stimulate spawning in females.**
4. **The night time and the new and full lunar phases increase the probability of *S. intermedius* spawning, probably by enhancing the sensitivity of sea urchins to phytoplankton.**

5. **We can propose that there are at least two mechanisms responsible for the shift in S. intermedius timing of spawning from autumn to the early summer: (1) the seawater eutrophication promotes the probability of early spawning followed by the earlier beginning of a new wave of gametogenesis and gonad maturation and (2) sea urchin offspring from parents with early spawning pattern attains sexual maturity earlier than that from parents with late spawning pattern, thereby increasing the number of the individuals in sea urchin population which are able to spawn in late spring – early summer.**

6. **Desynchronization of the reproductive cycle can substantially decrease the reproductive success because of so-called 'the Allee effect' when the probability of egg fertilization is sharply reduced due to insufficient concentration of gametes in sea water. In addition, sea urchin offspring in polluted environment has low chances for survival because of low quality of parental gametes. In ecologically favorable areas, a risk for sea urchin reproduction is connected with spawning failure because of low concentration of phytoplankton insufficient to trigger the spawning.**

5. Directions for future research

In the present work, we have shown the hierarchy of exogenous (phytoplankton, moon cycle) and endogenous (circadian rhythm, sperm) factors driving the spawning process in natural populations of the sea urchin *Strongylocentrotus intermedius*. We used this species as the model object for broadcast spawners with planktotrophic larvae and proved a primary role of phytoplankton in triggering the spawning. Considering an important role of marine invertebrates with planktotrophic larvae in coastal communities, it seems necessary to clarify a role of phytoplankton as a stimulus triggering the spawning in other species.

Our results allowed to hypothesise that phytoplankton may not only regulate the spawning process in broadcast spawners with planktotrophic larvae but also participate in the regulation of the reproductive cycle as a whole. It seems to be important to continue the studies in this direction.

Nowadays there is only a scarce information regarding the factors regulating the vital activity, and reproduction in particular, of deep sea invertebrates. The use of the devices similar to that we used in the present study may contribute to this field in the future.

Acknowledgements

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Thank you very much for your attention!