

Fine-scale temporal study of the influence of hydrobiological conditions on the spawning of the sea urchin *Strongylocentrotus intermedius*

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ABSTRACT: The role of environmental variables in the triggering and synchronization of spawning in natural populations of broadcast invertebrates is not fully understood. Here, we report the results obtained from a fine-scale temporal study of the timing of spawning of the sea urchin *Strongylocentrotus intermedius* and the associated environmental conditions in the northwestern Sea of Japan. Sea urchins were sampled in August–September of 2011 (3 sites) and 2012 (4 sites) at intervals of 3–12 d (4.2 d on average). Sixteen spawning events were revealed by a significant increase in the number of spawned females and a decrease in the gonad index and/or in the portion of individuals ready for spawning, determined as the percentage of sea urchins releasing gametes after dissection from the gonopores. Depth, temperature, salinity, dissolved oxygen and chl *a* concentrations were measured by sounding of the water column at the sampling sites daily and at 26 other sites in the bay every 5 d in 2011 and every 3 d in 2012. At one of the sampling sites, environmental parameters were recorded every 15 min by a data logger. No apparent relationships were found between temperature, salinity, dissolved oxygen or tidal activity and the timing of spawning, but there was a significant positive relationship between chl *a* concentration and the spawning events. Our results strongly suggest that phytoplankton concentration is the main factor for initiation of *S. intermedius* spawning and that the lunar cycle may serve as an additional factor which is able to affect this process.

KEY WORDS: Broadcast spawning · Echinoderms · Reproductive success · Phytoplankton bloom · Temperature · Upwelling · Downwelling · Moon cycle

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INTRODUCTION

For the successful reproduction of marine invertebrates with external fertilization (so-called broadcast spawners), synchronization of spawning between females and males as well as environmental conditions that are favourable for offspring development appear to be of crucial importance. The necessity of the existence of an extrinsic stimulus to synchronize

spawning is determined by the short longevity of gametes and rapid gamete dilution (Pennington 1985, Levitan & Petersen 1995).

The concurrence of phytoplankton bloom with the spawning season has long been noted for a great number of species of bottom invertebrates (Thorson 1946). Such a coincidence has been observed in many field studies (Himmelman 1975, Smith & Strehlow 1983, Starr et al. 1993, Gaudette et al. 2006,

González-Irusta et al. 2010, Fournier et al. 2012, Zhadan et al. 2015). Moreover, laboratory experiments showed that the addition of various species of microalgae and their extracts to water stimulated the spawning of sea urchins and bivalve molluscs (Breese & Robinson 1981, Starr et al. 1990, 1992) and that phytoplankton in combination with sperm exerted a synergistic effect (Starr et al. 1990, 1992, Reuter & Levitan 2010).

At the same time, many field studies have demonstrated coincident spawning activity of broadcast spawners, and sea urchins in particular, with changes in water temperature (Tsuji et al. 1989, King et al. 1994, Byrne et al. 1998, Guillou & Lumingas 1998, Lamare & Stewart 1998, Himmelman et al. 2008). Moreover, 1 of 2 sea urchin species of the genus *Echinocardium* spawned under conditions of increasing temperature, while the other species spawned under conditions of phytoplankton growth (Egea et al. 2011). The sea urchin *Paracentrotus lividus* from different localities of the Cantabrian Sea spawned in different seasons under temperature increases or phytoplankton growth as well as in the absence of changes in these parameters (González-Irusta et al. 2010). For *P. lividus*, it was concluded that a range of environmental conditions can induce reproductive responses and that sea urchins with completely mature gonads may spawn in the absence of environmental triggers.

Some studies reported the coincidence of echinoderm spawning with certain phases of the moon cycle (Pearse 1975, Iliffe & Pearse 1982, Pearse et al. 1988, Lessios 1991, Mercier et al. 2000, Coppard et al. 2005, Gaudette et al. 2006). The modulating influence of moon phases on the spawning of the sea urchin *Lytechinus variegatus* was shown in laboratory experiments in which sea urchin sperm and phytoplankton were used as stimulating agents (Reuter & Levitan 2010). Sea urchin sensitivity was higher during new and full moon phases. Full moons also seem to stimulate spawning in the holothurian *Isostichopus fuscus* (Mercier et al. 2007). The influence of moon phases on the spawning of echinoderms has mainly been observed in tropical species (see Mercier et al. 2007 for review). For temperate-water echinoderms, the hypotheses that phytoplankton, sperm from conspecific males and temperature changes are the major stimuli inducing synchronous spawning continue to be a central focus for researchers (Mercier & Hamel 2009). However, the exact role of these factors in triggering spawning remains unclear.

Analysis of data obtained from field studies on the relationship between environmental variables and

the timing of broadcast spawning is limited by the low frequency of animal and gonad sampling (usually once per month) and insufficient environmental data. Additionally, there are no data on the time course of *in situ* behavioural response relative to environmental conditions appropriate for spawning. In laboratory experiments with sea urchins and mussels, several tens of minutes to several days were required for spawning initiation after the addition of phytoplankton (Starr et al. 1990, 1992, Reuter & Levitan 2010). Marine coastal waters where most species of broadcast spawners live are characterized by high spatial and temporal variability of physical and biological parameters (salinity, temperature, phytoplankton content, etc.). To study spawning—as distinct from gonadal cycles—the frequency of animal sampling should be more than once per month, and environmental variables should be monitored directly in animal habitats and, if possible, constantly (or, at the least, with a frequency larger than that of animal sampling).

In the present study, we clarify the relationships between environmental factors, such as salinity, dissolved oxygen, temperature, phytoplankton and moon phases, and the timing of spawning in wild populations of the sea urchin *Strongylocentrotus intermedius* (A. Agassiz, 1863) from Kievka Bay located in the northwestern Sea of Japan (the coastal waters of the Primorye region of Russia). The sea urchin *S. intermedius* is widely distributed in the northern regions of Asian Pacific coastal waters, from the Kamchatka Peninsula south to the Korean Peninsula and from the Russian coast east to the Japanese Islands (Bazhin 1998, Kafanov & Pavlyuchkov 2001, Agatsuma 2013). This is a commercially important species; therefore, an investigation of the environmental factors responsible for spawning initiation in *S. intermedius* populations would be of great importance for both fundamental research and aquaculture needs. Our previous studies showed that *S. intermedius* inhabiting Kievka Bay has an annual reproductive cycle (Zaslavskaya et al. 2012, Zhadan et al. 2015). The sea urchin gonads became mature in late July and August, and spawning occurred from August into the beginning of September. However, despite the seasonal maturation of gonads by 100% of individuals, spawning did not occur for 90% of *S. intermedius* individuals in some years (Zhadan et al. 2015). This was evident from the absence of a statistically significant decrease in the gonad index of both females and males at the end of September and a large-scale and prolonged (for approximately 7 mo) cleaning process in the gonads associated with the

resorption of numerous undischarged eggs and spermatozoa by nutritive phagocytes. The phenomenon of spawning failure was also found in other populations of *S. intermedius* in the northwestern Sea of Japan. Based on satellite chl *a* data from the study area, we proposed that spawning failure is attributable to low primary productivity in the water column during the sea urchin spawning season.

Our present observations were conducted at a much higher temporal resolution than our and other previous studies, and environmental parameters were monitored directly in animal habitats, with the goal of testing the hypothesis that phytoplankton and/or other environmental variables (temperature, salinity, moon phases and tide level) induce or fail to induce spawning.

MATERIALS AND METHODS

Study area

Kievka Bay (42° 50' N, 133° 41' E) (Fig. 1) is an open bay typical of the southeastern coast of the Primorye region of Russia (northwestern Sea of Japan), with a width of 8.3 km and a length of 3.3 km. The right and left coasts of the bay are made up of rocks, while the central part is a sandy beach with a length of 5.5 km. The bay is open to the winds from the southeast to the southwest. The Kievka River enters the left corner of the bay. The cold Primorsky Current flows along the coast of Kievka Bay from the northeast to the southwest. In accordance with a scheme of sur-

face currents, the hydrological regime of the shallow waters on the right side of the bay is determined by open ocean waters, while the shallow waters on the left side of the bay are influenced by river runoff. In addition, the wind-induced upwelling associated with the monsoon has a pronounced effect on the hydrological regime and enhances the primary productivity in the study area, especially during the period from April to October (Zhabin et al. 1993, Zvalinskii et al. 2006, Zhadan et al. 2015).

This research work was conducted at the Zapovednoye Marine Biology Field Station of the Far Eastern Federal University (Primorsky Krai, Russia). The studies were carried out in Kievka Bay at Sites 1–3 in 2011 and at Sites 1–4 in 2012 (Fig. 1). Study sites 1, 2 and 4 were bedrock and boulder habitats with *Saccharina japonica* and *Phyllospadix iwataensis* as the dominant plant species. At site 3, the habitat was hard bottom covered by small rubble, with a frequent occurrence of thick aggregations of detached drift seaweed *S. japonica* and sea grass *Zostera marina*. According to the data for 1996–1998, the median *Strongylocentrotus intermedius* density in Kievka Bay at a depth up to 20 m was 2 ind. m⁻² (Borisovetz et al. 2000); this is consistent with our estimation of 2 to 3 ind. m⁻².

Sea urchin sampling and examination of gonadal state

An analysis of sea urchin gonads was performed during the period from the beginning of August to

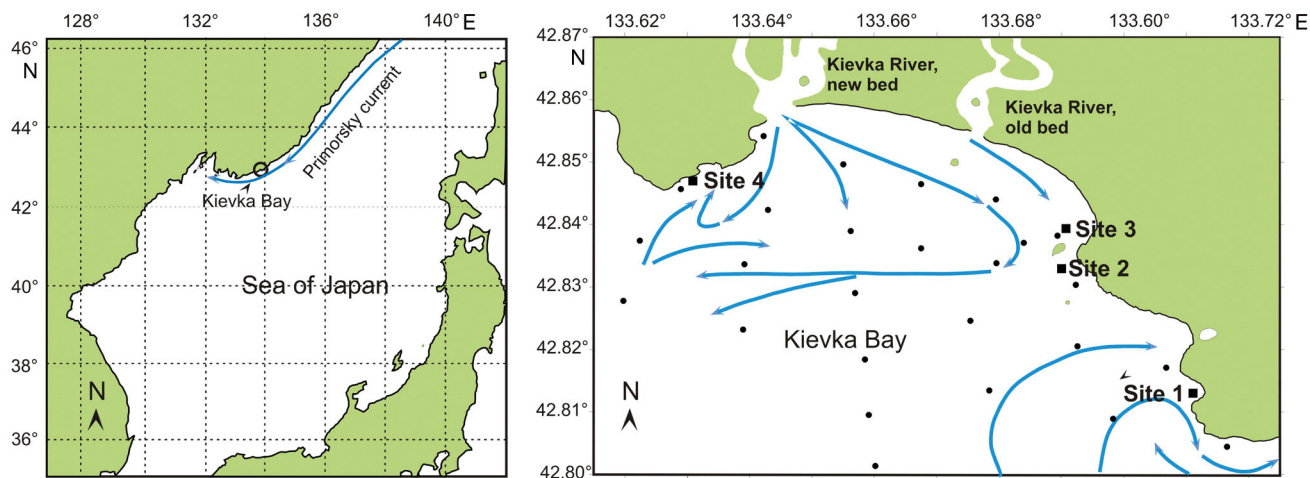


Fig. 1. Kievka Bay (right) in the northwestern Sea of Japan (left). The sites where sea urchins were collected are denoted by squares, and the sites where sounding of the water column was performed are denoted by small circles. Blue arrows show the cold Primorsky Current (left) and surface currents in Kievka Bay in August (right, according to Zuenko & Rachkov 2003)

the end of September (in the pre-spawning, spawning and post-spawning periods [Zhadan et al. 2015]). Depending on the weather (mainly on storm intensity), sea urchin sampling was carried out at intervals of 3 to 12 d (4.2 d on average). To assess overall sea urchin spawning, a final sample from each site was taken in the second half of October. Adult sea urchins were collected at random by SCUBA at depths of 2 to 7 m. The sample size was approximately 50 specimens in 2011 and 40 specimens in 2012 for every collection and all sites. The sea urchins were transported to the laboratory and examined immediately. In total, 1497 males and 1774 females were examined. The diameter of the sea urchins (both sexes) was 62.5 ± 7.9 mm (mean \pm SD).

All specimens were measured to the nearest millimeter by a caliper and weighed to the nearest 0.1 g. All 5 gonads were weighed with an accuracy of 0.01 g. The gonad index (GI) was calculated using the following formula: $GI (\%) = (\text{gonad wet weight} / \text{total wet weight}) \times 100$. The percentage of individuals of each sex that released gametes from the gonopores after dissection was calculated and placed in the group of sea urchins ready for spawning.

For all females in the sample, small pieces of the gonads were used to prepare smears to determine the state of maturation under a compound light microscope. A minimum of 100 reproductive cells from each female were counted and classified as

either oocytes or mature eggs. Oocytes were roughly differentiated by their diameter into 2 classes, small previtellogenic oocytes ($<30 \mu\text{m}$ in diameter) and large vitellogenic oocytes ($>50 \mu\text{m}$ in diameter). The advantage of the gonadal smears analysis was that it was less laborious compared to the histological analysis, which is very important when dealing with a large number of samples. Based on micro- and macroscopic characteristics of the gonads, the following stages of ovarian maturation were determined: (1) immature, (2) premature, (3) partially mature, (4) mature, (5) spent and (6) over-mature (Table 1).

Measurement of environmental variables

The measurement of depth, water temperature, salinity, photosynthetically active radiation (PAR) and concentrations of chl *a* and dissolved oxygen was performed by CTD sounding of the water column at 26 sites (Fig. 1) with a multi-parameter sonde (RBR XRX-620) every 5 d in 2011 and every 3 d in 2012. At the sites of sea urchin sampling (Sites 1–4), measurements were performed daily, except for storm days. The speed of the sonde dipping was adjusted to obtain 7 to 12 measurements per 1 m of depth. In 2012, there was a break in measurements for the period August 22–30 because of a typhoon and malfunctioning of the data logger. In both years at Site 3,

Table 1. Microscopic and macroscopic characteristics of the stages of ovarian maturation of the sea urchin *Strongylocentrotus intermedius* during the reproductive season (August–October)

Stage	Macroscopic characteristics	Microscopic characteristics
(1) Immature (early developing)	Ovaries small or medium sized, dense, yellow to orange	Numerous small oocytes ($<30 \mu\text{m}$ in diameter)
(2) Premature (late developing)	Ovaries large, dense, orange to bright orange	Large vitellogenic oocytes ($>50 \mu\text{m}$ in diameter) predominate, portion of ripe eggs $<50\%$
(3) Partially mature	Ovaries large, somewhat loose, bright orange; bright orange gametes easily released through gonopores after test dissection as well as from gonoducts after ovary dissection	Ripe eggs predominate, portion of large vitellogenic oocytes $<50\%$
(4) Mature	Ovaries large, loose, bright orange; bright orange gametes easily released through gonopores after test dissection as well as from gonoducts after ovary dissection	Ripe eggs, large vitellogenic oocytes may occur ($\leq 1\%$)
(5) Spent	Ovaries very small, highly shrunken, dark brown or almost black	Rare relict ripe eggs and egg fragments
(6) Over-mature	Ovaries large or medium-sized, very loosely packed, bright orange to red-orange and brownish orange; red-orange to brownish orange gametes easily released through gonopores after test dissection as well as from gonoducts after ovary dissection	Numerous morphologically normal and over-ripe (fragmented) eggs, numerous egg fragments

environmental variables (depth, water temperature, salinity, PAR and concentrations of chl *a* and dissolved oxygen) were continuously (every 15 min) recorded by a YSI 6920V2 data logger, which was installed 50 cm above the bottom. In 2012, there was a break in the measurements of chl *a* for the period August 24–30 because of a malfunction of the corresponding sensor. After August 31, chl *a* at Site 3 was measured daily by the RBR XRX-620 sonde.

To calibrate the chl *a* sensors, 1.0 to 1.5 l of sea water was filtered through Whatman GF/F filters (nominal pore size 0.7 μm) immediately after sampling. The filters were preserved frozen (-20°C) until subsequent analysis, which was performed within a month. Pigments were extracted with 90% acetone and measured by spectrophotometry. Chl *a* concentrations were calculated by using the SCORE UNESCO equations (UNESCO 1966). To compensate for the dependence of chl *a* sensor data on illumination intensity, PAR data were used for their correction. Data on Kievka River runoff were obtained from the Far Eastern Regional Hydrometeorological Research Institute. Data on the level of precipitation were obtained from the Preobrazhenie weather station (Russia).

Statistical analysis

For normally distributed data, unpaired *t*-tests, Pearson's *r* analysis and 1-sample *t*-tests were performed. Parametric methods were not employed when the variance homogeneity (Bartlett's test and Fisher's *t*-test, $p < 0.05$) and normal distribution (D'Agostino and Pearson normality tests, $p < 0.05$) of the data were not met. The GI values were transformed using the equation $p' = \arcsin\sqrt{p}$ to normalize their distribution and then analyzed by 1-way ANOVA followed by Tukey's post hoc test. All these statistical analyses were run using GraphPad Prism 6.0 for Windows (GraphPad Software). The numbers of females and males ready for spawning and the numbers of spent females in each sample were analysed using binomial statistics (Zar 2010). The adjusted Wald CIs (95%) for binomial expectations were calculated (Agresti & Coull 1998). In accordance with the theory of CIs (Zar 2010), we assumed that the expectations were significant ($p < 0.05$) when the upper confidence limit for a lesser expectation did not overlap with the lower confidence limit for a larger expectation.

The data on chl *a* obtained by the YSI 6920V2 data logger were edited to remove spikes and filtered

using a 5-point equally weighted running mean. To evaluate the relationships between temperature, chl *a* concentration, salinity and the probability of sea urchin spawning, generalized linear models for binomial data (logistic regression) were used. The result of the registration of spawning events was used as a binary dependent variable, i.e. a value of 1 was assigned to each inter-sample time interval when spawning occurred (spawning windows, $n = 16$), and a value of 0 was assigned to each inter-sample time interval when spawning did not occur (non-spawning windows, $n = 55$). The mean chl *a* concentration for a window, maximum chl *a* concentration for a window, ratios of mean and maximum chl *a* concentrations for a window to the mean seasonal chl *a* concentration at the site, mean temperature for a window, maximum and minimum temperature for a window, maximum negative and positive temperature deviations from the mean window value, mean salinity for a window, maximum salinity for a window, ratio of the mean salinity for a window to the mean seasonal salinity at the site, and ratio of the maximum salinity for a window to the mean seasonal salinity at the site were used as continuous independent variables (predictors). The year of study or the site was used as a categorical independent variable. Both separate and combined influences of these predictors were analyzed. The best model was chosen based on Akaike's information criterion (AIC) values. The significance of the relationships between the spawning events and the predictors was evaluated by dispersion analysis (1-way ANOVA) using a chi-squared test (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m550p147_supp/ for AIC and *p*-values). Logistic regression procedures were implemented using free and open-source R software (R Development Core Team 2010).

Circular statistics for axially distributed data (Zar 2010) was performed to analyze a relationship between the spawning time and the lunar phases.

RESULTS

Sex ratio

The overall mean male/female ratio from all sea urchin samples was 1.28, which differed significantly from 1 (1-sample *t*-test, $df = 78$, $t = 4.504$, $p < 0.0001$). There were no significant differences in the mean sex ratios between the sites in both years (1-way ANOVA, $df = 6$, $MS = 0.461$, $F = 1.796$, $p = 0.113$). No hermaphrodites were observed.

Gonad index

As no significant difference in the GI between sexes was detected (unpaired *t*-test and ANOVA, all $p > 0.05$), the GI data from both sexes were pooled within each sample (Figs. 2 & 3). In both years of the study and at all the sites, the GI values significantly

depended on the sampling time (ANOVA, all $p < 0.0001$); the highest GIs were registered at the beginning of the study, and at the end of the study, they were the lowest.

In 2011, a significant decrease in the GI at Site 1 was registered between August 13 and 25 and September 24 and 28 (ANOVA, Fig. 2, brown bars). The analysis

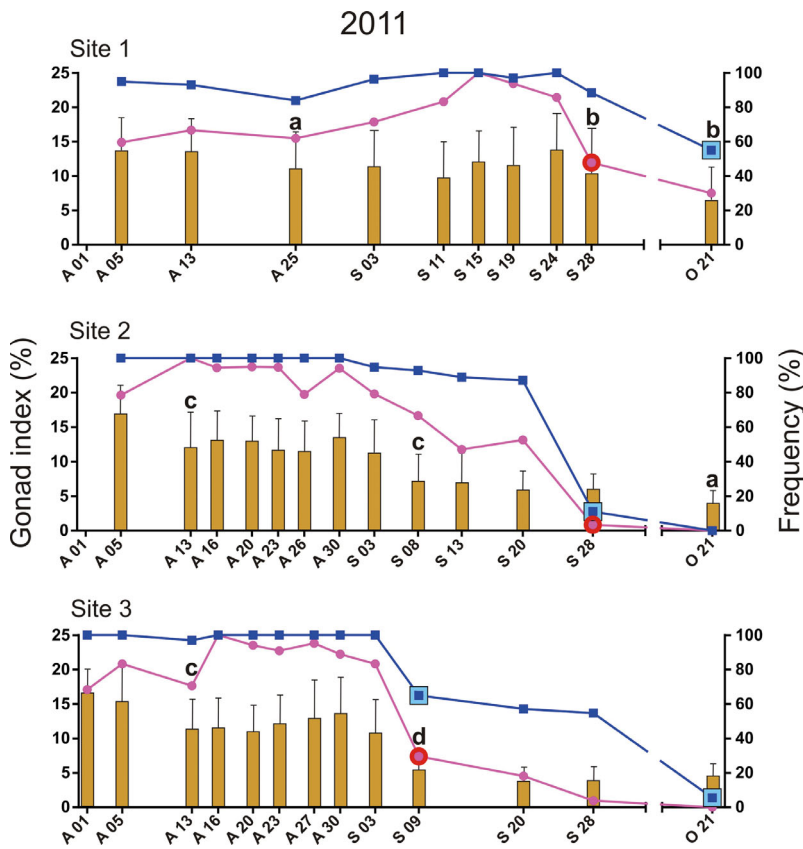


Fig. 2. Temporal dynamics of the gonad index (brown bars: mean \pm SD) and the percentages of males (blue lines with squares) and females (red lines with circles) ready for spawning in sea urchins *Strongylocentrotus intermedius* sampled at 3 sites in Kievka Bay (northwestern Sea of Japan) in 2011. Different letters above the columns denote significant differences between 2 consequent samplings (a: $p < 0.05$, b: $p < 0.01$, c: $p < 0.001$, d: $p < 0.0001$; 1-way ANOVA, Tukey's multiple comparison test). Large squares and circles indicate significant differences between 2 consequent samplings ($p < 0.05$, binominal statistics). x-axis: month and date

of the final samples taken on October 21 showed that approximately 40% of individuals of both sexes from Site 1 remained ready for spawning (Fig. 2, Site 1). The GIs of these individuals did not differ significantly from the GI values at the beginning of the study (unpaired *t*-test, $df = 69$, $t = 0.7435$, $p = 0.4597$). At Sites 2 and 3, the GI dynamics were significantly different. A significant decrease in the GIs was registered in the periods between August 5 and 13 and September 3 and 8 (ANOVA, Fig. 2, Sites 2 and 3). There were no significant correlations between the GIs at Site 1 and those at the 2 other sites ($r_{1,2} = 0.234$, $p = 0.244$; $r_{1,3} = 0.433$, $p = 0.576$). The distribution of the GI values in the final sample from Site 1 was bimodal (Fig. 3, Site 1), while in the samples from Sites 2 and 3, it was unimodal (Fig. 3, Sites 2 and 3).

In 2012, the seasonal dynamics of the GIs at all 4 sites were synchronous (Fig. 4, Sites 1–4). The correlation between the GIs at different sites was high (r ranged from 0.96 to 0.99, p ranged from <0.002 to <0.0001). More than a 50% decrease in the GI (ANOVA, p -values for all the sites <0.0001) occurred at Site 1 between August 14 and 18, at Site 2 between August 11 and 17, at Site 3 between August 14 and 17, and at Site 4 between August 15 and 18 (Fig. 4). Then, the GIs gradually decreased until October 17; however, these latter decreases were not significant (ANOVA, $p > 0.05$, Fig. 4, Sites 1–4).

October 21, 2011

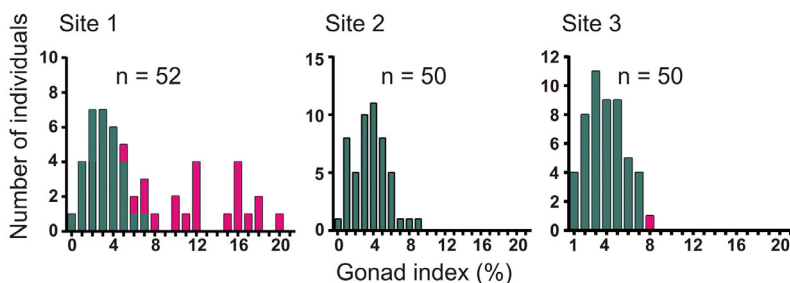


Fig. 3. Frequency distribution of the gonad index in sea urchins *Strongylocentrotus intermedius* sampled at 3 sites in Kievka Bay (northwestern Sea of Japan) on October 21, 2011. Note that the majority of the individuals from Site 1, which were ready for spawning (red parts of the columns), had a high gonad index

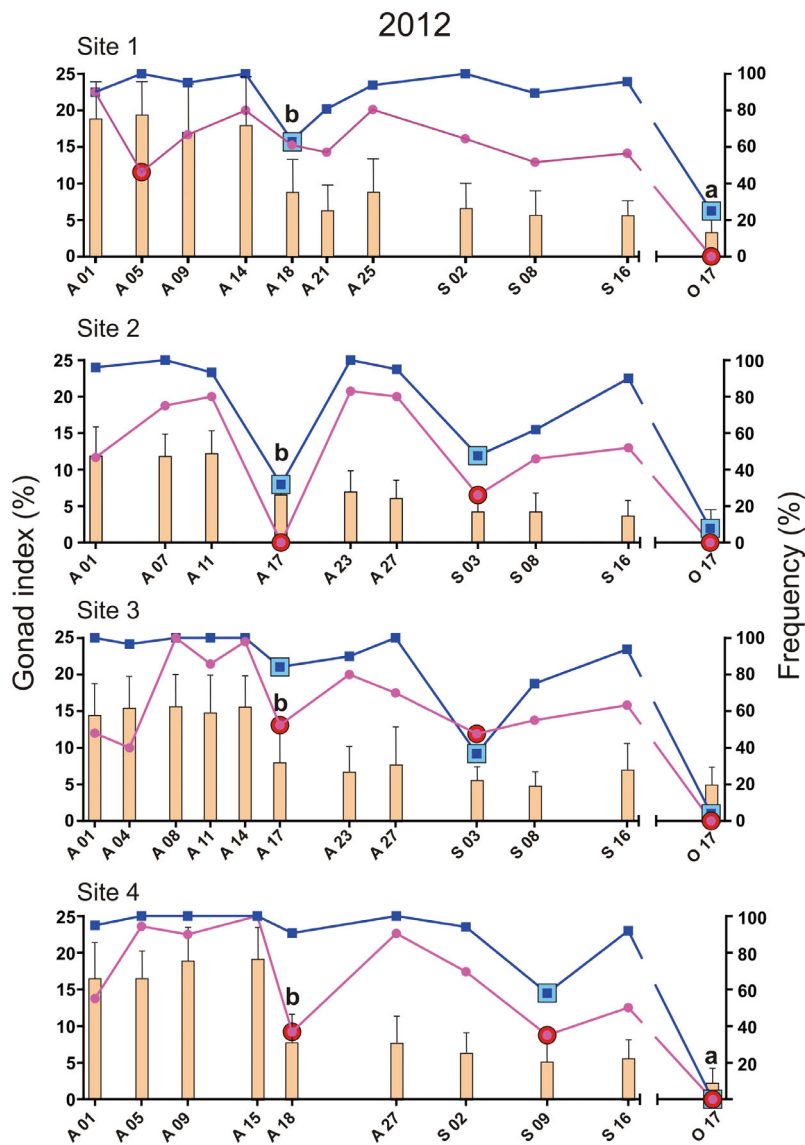


Fig. 4. Temporal dynamics of the gonad index (brown bars: mean \pm SD) and the percentages of males (blue lines with squares) and females (red lines with circles) ready for spawning in sea urchins *Strongylocentrotus intermedius* sampled at 4 sites in Kievka Bay (northwestern Sea of Japan) in 2012. Different letters above the columns denote significant differences between 2 consequent samplings (a: $p < 0.05$, b: $p < 0.0001$; 1-way ANOVA, Tukey's multiple comparison test). Large squares and circles indicate significant differences between 2 consequent samplings ($p < 0.05$, binominal statistics). x-axis: month and date

Readiness for spawning

For the total study period, the proportions of male sea urchins ready for spawning were higher than those of females (red and blue lines on Figs. 2 & 4). For all the sites, there was a positive correlation between the sexes for this parameter (r ranged from 0.72 to 0.92, p ranged from 0.0012 to < 0.0001).

As a general pattern, the seasonal dynamics of the proportions of females and males ready for spawning showed significant drops coincident with significant decreases in the GIs (Figs. 2 & 4). However, in a number of the cases, the decreases in both parameters were not confirmed statistically (binomial statistics, $p > 0.05$). Moreover, significant decreases in the proportions of sea urchins ready for spawning were observed in the absence of changes in the GI, particularly in September and October (binomial statistics, $p > 0.05$, Figs. 2 & 4).

Ovarian maturity and spawning events

In the first half of August of both years, the vast majority of females at all the sites were at the premature (2), partially mature (3) and mature (4) stages of maturation (Figs. 5 & 6). As evident from the comparison of Figs. 2 & 4 and Figs. 5 & 6, a significant drop in the GI in August was rarely followed by the appearance of fully spent females (Stage (5) of ovarian maturation). This allows us to conclude that in August, the partial spawning of *Strongylocentrotus intermedius* populations took place.

In both years, the first spent females at all the sites (from 5 to 20%) appeared between August 13 and 27 (Figs. 5 & 6). In September 2011, the proportions of spent females at Site 1 ranged from 10 to 40% (Fig. 5). The analysis of the final sample taken on October 21 revealed that 30% of females did not complete their reproductive cycle by spawning (Fig. 5, Site 1). They had large gonads (GI did

not differ from that of the females sampled on August 5, unpaired t -test, $df = 27$, $t = 0.731$, $p = 0.34$) which released both morphologically normal and also over-mature fragmented eggs. This state of the ovaries was referred to as the over-mature (6) stage of ovarian maturity. Recently, we described this phenomenon in *S. intermedius* populations as spawning failure (Zhadan et al. 2015). The proportions of spent

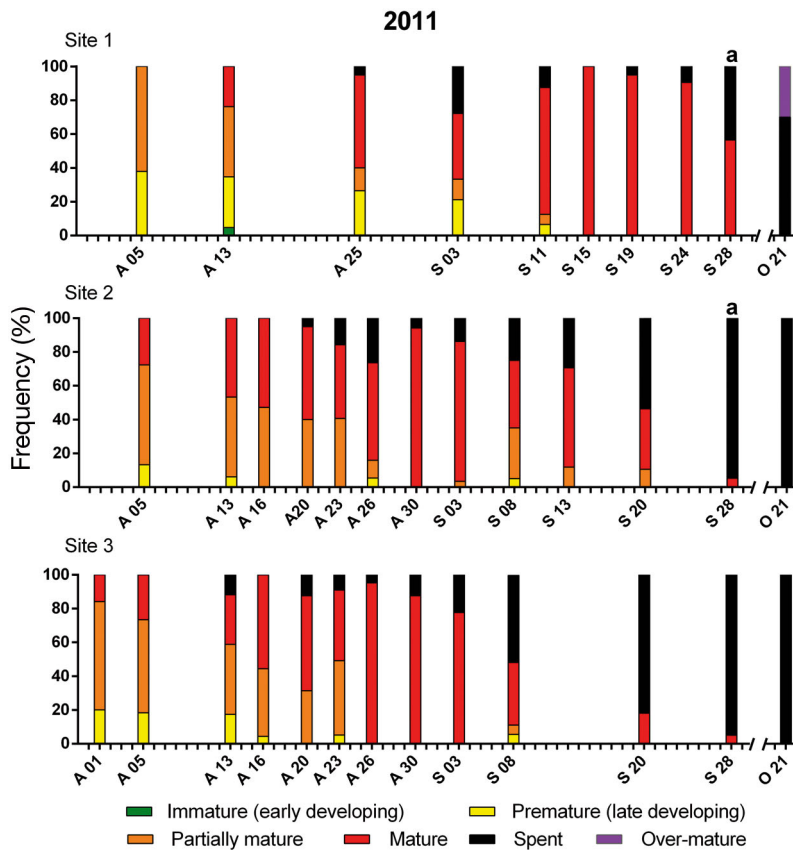


Fig. 5. Temporal dynamics of the percentages of ovarian maturity stages in *Strongylocentrotus intermedius* females sampled at 3 sites in Kievka Bay (northwestern Sea of Japan) in 2011. Letter 'a' above the columns denotes a significant decrease in the portion of spent females ($p < 0.05$, binominal statistics). x-axis: month and date

females at Sites 2 and 3 gradually increased and reached 95% by September 28 (Fig. 5, Sites 2 and 3). On October 21, all the females from these sites were spent.

In September 2012, the proportions of spent females at different sites ranged from 25 to 55% (Fig. 6). By October 17, 2012, all 4 populations were completely spawned out.

In sum, 16 sea urchin spawning events were revealed by a significant increase in the number of spawned females and significant decreases in the GI and/or the percentage of females and males ready for spawning (see Figs. 7D & 8D, shaded areas).

Relationship between the dynamics of environmental variables and spawning events

The summer–autumn seasons of 2011 and 2012 differed in weather conditions. In August–September, the quantity of precipitation was 115 and 405 mm

in 2011 and 2012, respectively. This was responsible for the inter-year variations in terrigenous input into Kievka Bay followed by a difference in the dynamics of the bottom layer salinity (Figs. 7B & 8B). Seasonal wind-driven upwelling was more pronounced in 2011 than in 2012; this was responsible for the higher spatial and temporal variability in distribution of temperature and salinity in the water column in 2011 (Figs. 7D & 8D, see also Animations S1–S4 in the Supplement at www.int-res.com/articles/suppl/m550p147_supp/). The oxygen content in the waters of Kievka Bay was close to saturation and constituted $104 \pm 6\%$ in the surface waters and $97 \pm 11\%$ in the bottom water layer (mean \pm SD, data not plotted).

Salinity

Salinity in the bottom layer of water was not lower than 32 psu at all the sites in 2011 (Fig. 7D, Sites 1–3) and at Sites 1 to 3 in 2012 (Fig. 8D, Sites 1–3). A maximum decrease in the salinity of up to 29 psu was registered at Site 4 in 2012 on August 31 (Fig. 8D, Site 4) after the typhoon that caused a significant

increase in river runoff (Fig. 8C). However, as is evident from the data presented in Fig. 8D, sea urchin spawning at Site 4 occurred both before and after this event.

Temperature

In 2011, the development of upwelling started on August 8 (see Animation S1 in the Supplement) and led to 2 incomings of cold water into the study area and thus led to strong temperature decreases during 2 periods, August 12–21 and August 26–September 2, which reached 14 and 12°C, respectively, for 1 d (Fig. 7D, Sites 1–3). Each period of temperature decrease was followed by a period of temperature increase (Fig. 7D, Sites 1–3) caused by downwelling of warm surface water (see Animation S1 in the Supplement).

In 2012, the strength of upwelling was less pronounced than in 2011 (see Animation S2 in the

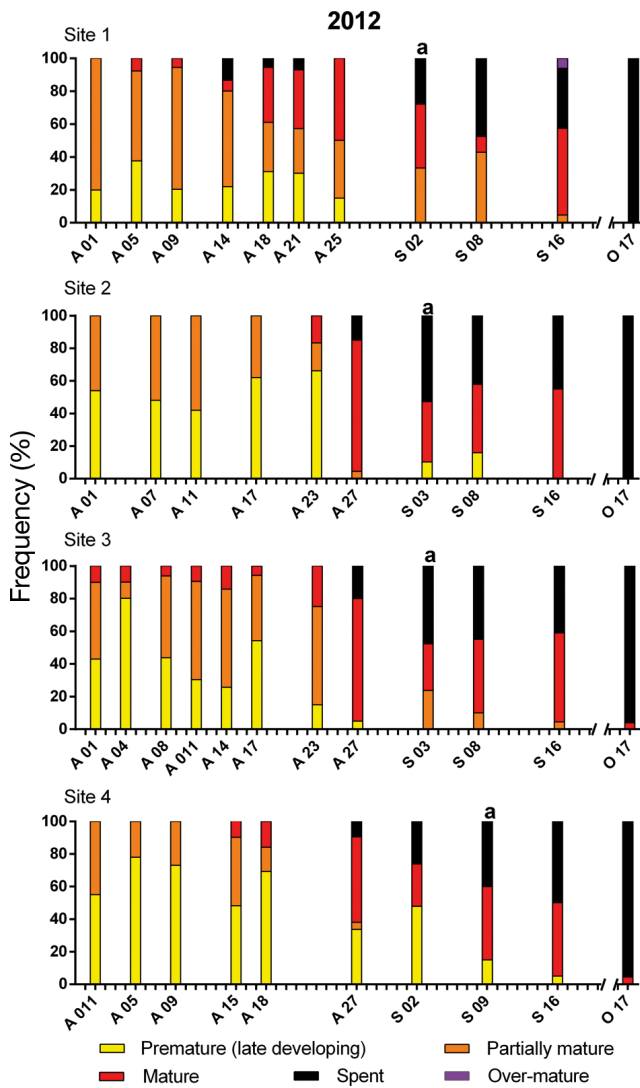


Fig. 6. Temporal dynamics of the percentages of ovarian maturity stages in *Strongylocentrotus intermedius* females sampled at 4 sites in Kievka Bay (northwestern Sea of Japan) in 2012. Letter 'a' above the columns denotes a significant decrease in the portion of spent females ($p < 0.05$, binominal statistics). x-axis: month and date

Supplement). During the first period of temperature decrease (August 12–15), inter-daily temperature fluctuations were not higher than 4°C (Fig. 8D, Sites 1–4), and diurnal temperature variations only once reached 6°C (Fig. 8D, Site 3). During the second period of temperature decrease (August 25–September 1), inter-daily temperature fluctuations reached 10°C, and diurnal temperature variations were up to 14°C (Fig. 8D, Site 3). After sharp temperature decreases, rapid temperature increases up to approximately 18°C were registered (Fig. 8D, Site 3).

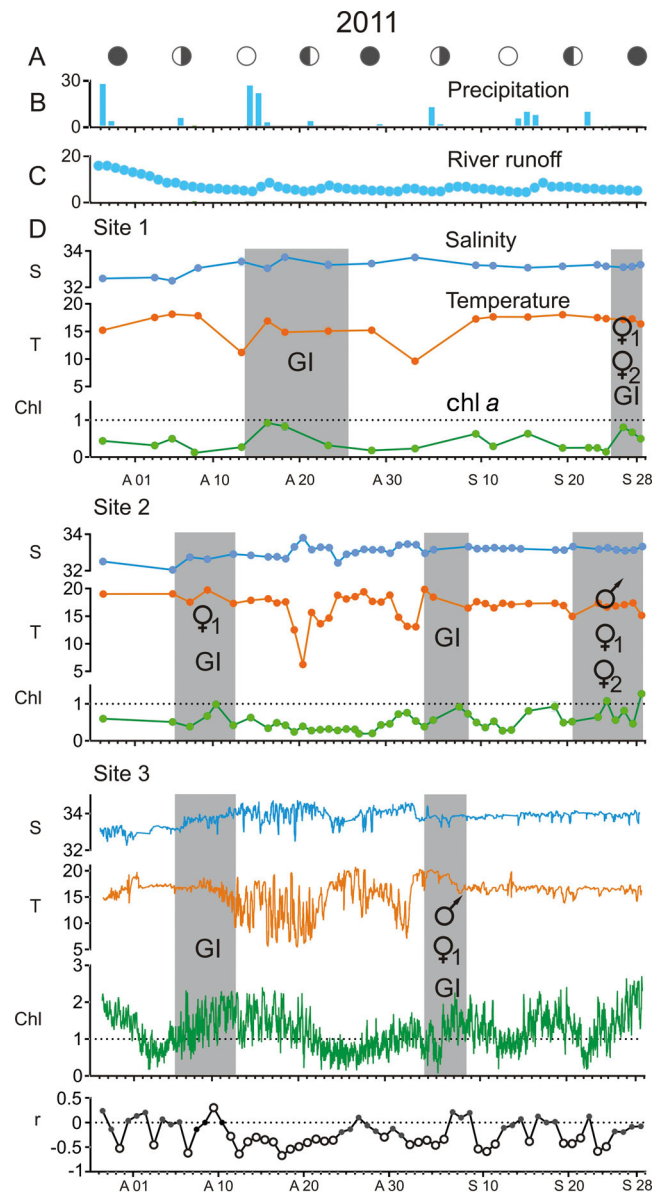
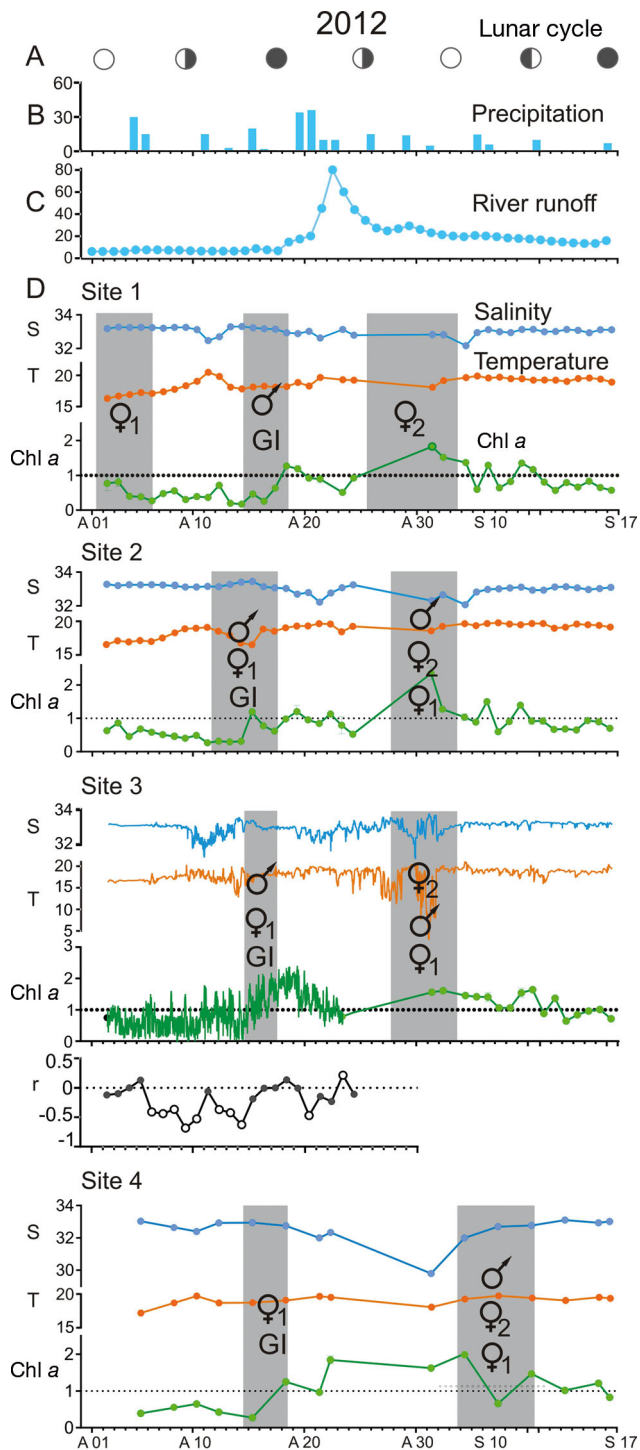


Fig. 7. Timing of spawning of the sea urchin *Strongylocentrotus intermedius* and associated environmental conditions in August–September of 2011 in Kievka Bay (northwestern Sea of Japan). (A) Lunar cycle phase. (B) Daily precipitation (mm). (C) Daily runoff of the Lazovka River, the main tributary of the Kievka River that flows into Kievka Bay ($m^3 s^{-1}$). (D) Spawning events and dynamics of environmental variables at Sites 1–3. Chl a ($\mu g l^{-1}$); S: salinity (psu); T: temperature ($^{\circ}C$); r: Pearson r coefficient reflecting the relationship between the temperature and chl a concentration recorded at 15 min intervals at Site 3 (open circles denote significant correlation, $p < 0.05$). Data on Site 3 are presented as continuous (every 15 min) measurements. Horizontal dashed line indicates the concentration of chl a corresponding to $1 \mu g l^{-1}$. Shaded areas indicate the spawning windows determined by different parameters. GI: significant decrease in the gonad index; ♀₁: significant decrease in the portion of females ready for spawning; ♂: significant decrease in the portion of males ready for spawning; ♀₂: significant increase in the portion of spent females. x-axis: month and date

Chl a

Both vertical and horizontal bottom distributions of chl a during both survey seasons varied greatly (see Animations S5 & S6 in the Supplement). The places at which chl a concentrations were elevated may or



may not coincide with the zones of increased temperature. The highest chl a concentration (approximately 5 µg l⁻¹) was registered out of the area where the sampling sites were located. At all the sampling sites, chl a concentration rarely exceeded 1.0 µg l⁻¹ (Figs. 7D & 8D). The exceptions were comparatively high chl a concentrations at Site 3 in 2011 (approximately 1.5 µg l⁻¹, see Fig. 7D) and at all the sites in September 2012 after a long rainy period (1.0–2.5 µg l⁻¹, see Fig. 8D).

Relationship between temperature, chl a concentration, salinity and spawning events

As shown by the gray bars in Figs. 7D & 8D, 14 of 16 sea urchin spawning events occurred at the beginning of seasonal upwelling and after its end under conditions of stable temperature (diurnal temperature spread less than 1°C) or weak temperature variations (inter-daily and diurnal temperature spread up to 4°C). Two spawning events occurred in the periods when severe temperature fluctuations took place, with inter-daily temperature variations up to 6°C (Fig. 7D, Site 1, first spawning event) and 14°C (Fig. 8D, Site 3, second spawning event). However, in both cases, there were several days with relatively stable temperatures between sea urchin samplings. At the same time, very strong temperature fluctuations recorded in 2011 at Sites 2 and 3 during upwelling/downwelling (inter-daily and diurnal temperature spread more than 14°C, see Fig. 7D) did not cause mass spawning in sea urchin populations despite a high readiness for spawning (approximately 100%, see Fig. 2).

Fig. 8. Timing of the spawning of the sea urchin *Strongylocentrotus intermedius* and associated environmental conditions in August–September of 2012 in Kievka Bay (northwestern Sea of Japan). (A) Lunar cycle phase. (B) Daily precipitation (mm). (C) Daily runoff of the Lazovka River, the main tributary of the Kievka River that flows into Kievka Bay (m³ s⁻¹). (D) Spawning events and the dynamics of environmental variables at Sites 1–4. Chl a (µg l⁻¹); S: salinity (psu); T: temperature (°C); r: Pearson r coefficient reflecting the relationship between the temperature and chl a concentration recorded at 15 min intervals at Site 3 (open circles denote significant correlation, $p < 0.05$). Data on Site 3 are presented as continuous (every 15 min) measurements. Horizontal dashed line indicates the concentration of chl a corresponding to 1 µg l⁻¹. Shaded areas indicate the spawning windows determined by different parameters. GI: significant decrease in the gonad index; ♀₁: significant decrease in the portion of females ready for spawning; ♂: significant decrease in the portion of males ready for spawning; ♀₂: significant increase in the portion of spent females. x-axis: month and date

Comparison of the timing of sea urchin spawning with the dynamics of chl *a* concentration in the bottom water layer showed that all 16 spawning events coincided with an increase in chl *a* concentration (see Figs. 7D & 8D). Logistic regression analysis revealed significant relationships between the probability of sea urchin spawning and all the predictors based on chl *a* concentration (p-values for different models ranged from <0.05 to <0.001, see Table S1 in the Supplement) except for the mean chl *a* for a window (AIC = 74.585, $p = 0.237$). The best results were obtained with the use of the ratio of maximum chl *a* for a window to the mean seasonal chl *a* for the site (AIC = 55.728, $p < 0.001$). Analysis of the relationship between temperature and chl *a* concentration recorded at 15 min intervals at Site 3 revealed mainly negative correlations (see Figs. 7D & 8D for r coefficient). In several cases, however, an increase in chl *a* concentration coincided with increasing temperature.

Logistic regression analysis did not reveal any significant relationship between spawning events and different parameters of salinity and temperature (see Table S1 in the Supplement for AIC and p-values). There were no significant models which used different paired combinations of temperature, salinity and chl *a* as predictors except for a significant interaction between the ratio of maximum chl *a* for a window to the mean seasonal chl *a* for the site and maximum salinity for a window (AIC = 62.189, $p < 0.001$). However, the AIC of this model is higher than the AIC of the best model for chl *a* (AIC = 55.728, $p < 0.001$).

Correlation analysis of the data of continuous registration of salinity and chl *a* at Site 3 revealed a significant positive correlation between these environmental variables for both survey seasons ($r = 0.216$, $p < 0.0001$ and $r = 0.263$, $p < 0.0001$ for 2011 and 2012, respectively). This indicates that significant interaction between salinity and chl *a* is likely because phytoplankton enters the study area with waters of higher salinity. This is evident from the data of CTD sounding of the bay waters at 26 sites: significant positive correlation between salinity and chl *a* took place at the depth from 10 to 35 m (r ranged from 0.421 to 0.857, $p < 0.0001$).

Lunar phases

As is evident from Figs. 7A,D & 8A,D, 13 of 16 dates of registration of sea urchin spawning events coincide with the phases of new and full moons. The means of mean angles of arcs which correspond to

the spawning windows were placed close to the new and full moons (Fig. 9, blue and red ticks for 2011 and 2012, respectively). The values of standard deviations and 95% CIs for the mean of mean angles (spawning windows) were 23 ± 56 and $23 \pm 90^\circ$, respectively, in 2011 (Fig. 9, gray-blue sectors for SD) and 7 ± 29 and $7 \pm 34^\circ$ in 2012 (Fig. 9, pink sectors for SD). This indicates that in 2012, sea urchin spawning was more synchronized during the phases of new and full moons than in 2011.

The semi-diurnal tide amplitude in Kievka Bay during the study period varied from 0.31 to 0.54 m. There were no differences in the tide amplitude between different lunar phases (ANOVA, $p > 0.05$).

DISCUSSION

Examination of gonadal development (GI dynamics, ovarian maturity and dynamics in the proportion of sea urchins ready for spawning) in *Strongylocentrotus intermedius* at different sites in Kievka Bay throughout 2 reproductive seasons (August–October

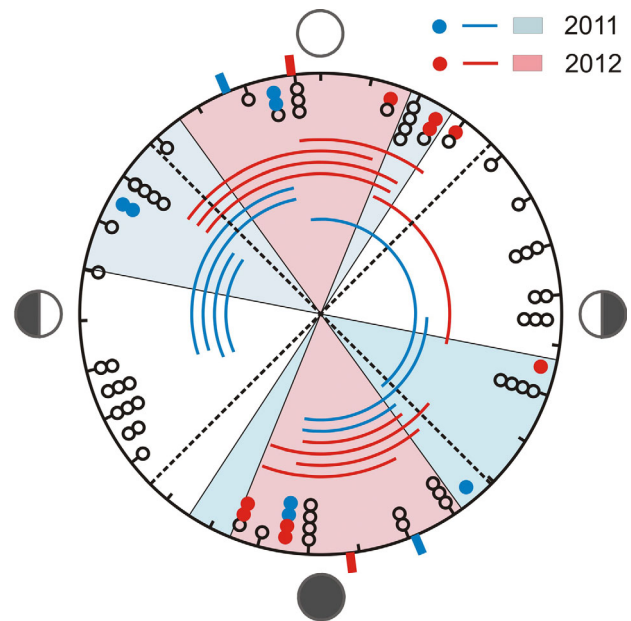


Fig. 9. Summary of spawning events for *Strongylocentrotus intermedius* in Kievka Bay (northwestern Sea of Japan) in 2011 and 2012 in relation to the lunar phases. Blue and red arcs and circles indicate the time intervals and dates over which a significant change ($p < 0.05$) in the gonad index and/or the portions of spent females and individuals ready for spawning was recorded in 2011 and 2012, respectively. Open circles correspond to the sampling dates when no spawning events were recorded. Gray-blue and pink sectors indicate standard angular deviation of the mean of mean angles for spawning windows in 2011 and 2012, respectively

of 2011 and 2012) showed that the timing of spawning (spawning windows) in different *S. intermedius* populations may be different within a comparatively small area. Additionally, the annual reproductive cycle of some sea urchin populations may not end by fully completed spawning. All this indicates that the exact timing of spawning during the spawning period depends on the local properties of the environment, i.e. certain external stimuli are necessary for spawning. The dynamics of the proportions of sea urchins ready for spawning indicate that in all the populations studied, *S. intermedius* has the potential to spawn at any time in August and most of September. The temporal patterns of spawning events indicate that in 2011 and 2012, complete spawning occurred at different times in all study sites except for Site 1 in 2011, where 30% of females and approximately 60% of males failed to spawn. Previously, we described that in 2008 and 2009, up to 95% of *S. intermedius* females from the locality, which corresponds to Site 2 in the present study, did not complete their reproductive cycle by spawning (Zhadan et al. 2015).

In all other cases, multiple spawning of different intensities intermittently occurred during August–September so that by October of both years, all the females were spent. In August, partial spawning took place when, despite significant declines in the GIs, fully spent females rarely occurred. In a number of cases, along with a drop in the GI, a significant decrease in the proportions of females and males ready for spawning was registered. This was evidently associated with partial spawning—a release of mature gametes from partially mature gonads. A subsequent increase in the proportion of sea urchins ready for spawning was apparently associated with further maturation of both female and male gametes.

Partial spawning is a common phenomenon for sea urchins. It takes place when spawning occurs from partially mature ovaries (Byrne 1990, Brewin et al. 2000, Kelly 2001, Lamare et al. 2002, Williamson & Steinberg 2002, Marzinelli et al. 2006, González-Irusta et al. 2010). Judging from the loss of ovary weight in August, *S. intermedius* females released most of their eggs from partially mature ovaries. In September, changes in the GIs were less prominent, while the proportion of fully spent females increased sharply; this confirms the repeated spawning events that could not be determined through GI changes. The low indicative value of GI as the spawning parameter in the case of repeated spawning events may be due to small changes in GI values that could not overlap with their intra-population variability,

which is characteristic for sea urchin species and depends on the quantity of nutrients accumulated by the gonad (Fuji 1960, Conor 1972, Crapp & Willis 1975, Byrne 1990).

At present, only a few field studies have been conducted with a temporal resolution close to that in our study and with direct measurements of environmental parameters in the habitats of broadcasting invertebrates. Mass spawning of the holothurian *Cucumaria frondosa* with lecithotrophic larvae was recorded under the conditions of an increase in temperature and a decrease in chl *a* concentration (Hamel & Mercier 1995). The spawning of the bivalve mollusc *Placopecten magellanicus* with planktotrophic larvae was observed under increasing or sharply fluctuating temperature conditions caused by a downwelling of warm surface water and did not coincide with increases in phytoplankton or organic substances (Bonardelli et al. 1996).

Our study revealed no apparent relationship between temperature change and the timing of spawning in *S. intermedius* natural populations. The absence of such a relationship is further evidenced by the fact that, despite the season from the beginning of August to the end of September in the study area being characterized by prolonged periods of stable warmest water temperature followed by periods of rapid, sharp change due to upwelling/downwelling events (Zvalinskii et al. 2006, Zhadan et al. 2015), the reproductive cycle of *S. intermedius* in some populations did not end in complete spawning during 5 reproductive seasons between 2008 and 2015 (Zhadan et al. 2015, the present study, P. Zhadan' unpubl. data). From all these data, we concluded that the changes in temperature are unlikely to be the primary stimulus necessary to induce spawning in the sea urchin *S. intermedius*.

Field observations of spawning events in a number of echinoids, both tropical (2 species of the genus *Diadema* and 2 species of the genus *Echinothrix* [Coppard et al. 2005]) and temperate (*S. droebachiensis* [Gaudette et al. 2006]), as well as laboratory experiments on *Lytechinus variegatus* (Reuter & Levitan 2010) provide evidence of lunar periodicity in some species. Our study revealed a coincidence between the majority of *S. intermedius* spawning events and new and full moons. However, the influence of the moon cycle on the spawning of *S. intermedius* was differently pronounced in 2011 and 2012. This, as well as the phenomenon of spawning failure (Zhadan et al. 2015, the present study), may be explained from the point of view that the lunar phases seem unlikely to be a proximal environmental

cue for triggering *S. intermedius* spawning activity. Most likely, the lunar cycle may serve as an additional factor enhancing sea urchin sensitivity to other natural stimuli as proposed for *L. variegatus* (Reuter & Levitan 2010).

Our results showed a significant positive relationship between chl *a* concentration and spawning events in *S. intermedius* natural populations. These results provide strong support for the hypothesis that an increase in the concentration of phytoplankton may serve as the primary stimulus to trigger sea urchin spawning (Himmelman 1975, Starr et al. 1993, Levitan 2002, Gaudette et al. 2006, González-Irusta et al. 2010). The laboratory experiments demonstrated that various species of microalgae (both combined with sperm and in the absence of it) induced the spawning of sea urchins (Starr et al. 1990, 1992, Gaudette et al. 2006, Reuter & Levitan 2010). It was proposed that phytoplankton stimulates the spawning of the most sensitive males, whose sperm promotes synchronous mass spawning. However, there are some contradictory results on the role of phytoplankton and sperm in inducing spawning. For example, Reuter & Levitan (2010) did not detect a consistent reaction to phytoplankton in experiments with the sea urchin *L. variegatus*, though the introduction of phytoplankton 75 min before sperm greatly accelerated the initiation of male spawning followed by female spawning. McCarthy & Young (2004) did not observe a reaction to sperm in the *L. variegatus* population in the field. The phenomenon of spawning failure repeatedly registered in *S. intermedius* populations from Kievka Bay (Zhadan et al. 2015, the present study) indicates that *S. intermedius* males with mature gonads are not able to induce spawning in the absence of external stimuli.

Our study showed that patterns of the spatial distribution of chl *a* and temperature in the bottom water layer of Kievka Bay were markedly different (compare Animations S1 & S5 and S2 & S6 in the Supplement at www.int-res.com/articles/suppl/m550p147_supp/). As a result, an increase in phytoplankton concentration, as judged from measured chl *a*, occurred under increasing, decreasing or almost constant temperatures. It is well known that warmer temperature promotes the acceleration of larval development of temperate sea urchin species, thereby decreasing the time when the larvae are susceptible to predation. It seems that the coincidence of increasing phytoplankton concentration and a stable warm or rising temperature would benefit the reproductive success of *S. intermedius*. However, according to our data, such a combination of environmental

factors occurs rather rarely in the study area. Our field observations showed that the spawning process in natural populations of *S. intermedius* lasted for approximately 1 h (see Video S1 at www.int-res.com/articles/suppl/m550p147_supp/). For this species, the time from fertilization to the formation of swimming blastulae over a temperature range of 15–20°C varied from 14 to 9.5 h (Buznikov & Podmarev 1990). A mechanism involving a rapid response of *S. intermedius* parental individuals to increasing phytoplankton density may be suggested. Such a mechanism promotes transportation of the sea urchin swimming larvae by advection together with warm water masses enriched with phytoplankton and thereby food supply for the offspring, even under conditions of the short-time oncoming of phytoplankton-rich warm water during a half-day tidal advection.

The results of the present study suggest that the spawning of the sea urchin *S. intermedius* depends not only on the degree of gonad maturation but also on the local properties of the environment. Phytoplankton concentration exceeding a certain threshold value seems to be the main environmental factor to trigger the sea urchin spawning. Our results do not support the hypothesis on the key role of temperature changes in triggering the sea urchin spawning. The new and full moon phases may most likely serve as additional factors which are able to affect this process.

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LITERATURE CITED

- Agatsuma Y (2013) *Strongylocentrotus intermedius*. In: Lawrence JM (ed) Sea urchins: biology and ecology, 3rd edn. Academic Press, San Diego, CA, p 437–447
- Agresti A, Coull BA (1998) Approximate is better than 'exact' for interval estimation of binomial proportions. *Am Stat* 52:119–126
- Bazhin AG (1998) The sea urchin genus *Strongylocentrotus* in the seas of Russia: taxonomy and ranges. In: Mooi R, Telford M (eds) Proc 9th Int Echinoderm Conf. Balkema, Rotterdam, p 563–566
- Bonardelli JC, Himmelman JH, Drinkwater K (1996) Relation of spawning of the giant scallop, *Placopecten magellanicus*, to temperature fluctuations during downwelling events. *Mar Biol* 124:637–649
- Borisovetz EE, Bregman YE, Viktorovskaya GI, Kalinina MV (2000) Biology of the sea urchin *Strongylocentrotus intermedius* (A. Agassiz) in the northwestern coastal waters of the Sea of Japan. Distribution and size composition of the

- populations. Proc Pac Res Inst Fish Oceanogr (TINRO) 127:416–439 (in Russian with English summary)
- Breese WP, Robinson A (1981) Razor clams, *Siliqua patula* (Dixon): gonadal development, induced spawning and larval rearing. Aquaculture 22:27–33
- Brewin PE, Lamare MD, Keog JA, Mladenov PV (2000) Reproductive variability over a four-year period in the sea urchin *Evechinus chloroticus* (Echinoidea: Echinodermata) from differing habitats in New Zealand. Mar Biol 137:543–557
- Buznikov GA, Podmarev VK (1990) Sea urchins *Strongylocentrotus droebachiensis*, *S. nudus*, and *S. intermedius*. In: Dettlaff TA, Vassetzky SG (eds) Animal species for developmental studies. Vol 1, Invertebrates. Consultants Bureau, New York, NY, p 253–285
- Byrne M (1990) Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland. Mar Biol 104:275–289
- Byrne M, Andrew NL, Worthington DG, Brett PA (1998) Reproduction in the diadematoid sea urchin *Centrostephanus rogersii* in contrasting habitats along the coast of New South Wales, Australia. Mar Biol 132:305–318
- Coppard SE, Andrew O, Campbell C (2005) Lunar periodicities of diadematid echinoids breeding in Fiji. Coral Reefs 24:324–332
- Crapp GB, Willis ME (1975) Age determination in the sea urchin, *Paracentrotus lividus* (Lamarck), with notes on the reproductive cycle. J Exp Mar Biol Ecol 20:157–178
- Egea E, Merigot B, Mahe-Bezac C, Feral JP, Chenuil A (2011) Differential reproductive timing in *Echinocardium* spp.: the first Mediterranean survey allows interoceanic and interspecific comparisons. C R Biol 334:13–23
- Fournier J, Levesque E, Pouvreau S, Le Pennec M, Le Moulac G (2012) Influence of plankton concentration on gametogenesis and spawning of the black lip pearl oyster *Pinctada margaritifera* in Ahe atoll lagoon (Tuamotu Archipelago, French Polynesia). Mar Pollut Bull 65:463–470
- Fuji A (1960) Studies on the biology of the sea urchin. III. Reproductive cycle of two sea urchins, *Strongylocentrotus nudus* and *S. intermedius*, in southern Hokkaido. Bull Fac Fish Hokkaido Univ 11:43–48
- Gaudette J, Wahle RA, Himmelman JH (2006) Spawning events in small and large populations of the green sea urchin *Strongylocentrotus droebachiensis* as recorded using fertilization assays. Limnol Oceanogr 51:1485–1496
- Conor JJ (1972) Gonad growth in the sea urchin, *Strongylocentrotus purpuratus* (Stimpson) (Echinodermata: Echinoidea) and the assumptions of gonad index methods. J Exp Mar Biol Ecol 10:89–103
- González-Irusta JM, De Cerio FG, Canteras JC (2010) Reproductive cycle of the sea urchin *Paracentrotus lividus* in the Cantabrian Sea (northern Spain): environmental effects. J Mar Biol Assoc UK 90:699–709
- Guillou M, Lumingas LJJ (1998) The reproductive cycle of the 'blunt' sea urchin. Aquacult Int 6:147–160
- Hamel JF, Mercier A (1995) Spawning of the sea cucumber *Cucumaria frondosa* in the St. Lawrence Estuary, eastern Canada. SPC Beche-de-mer Inf Bull 7:12–18
- Himmelman JH (1975) Phytoplankton as a stimulus for spawning in three marine invertebrates. J Exp Mar Biol Ecol 20:199–214
- Himmelman JH, Dumont CP, Gaymer CF, Vallières C, Drolet D (2008) Spawning synchrony and aggregative behaviour of cold-water echinoderms during multi-species mass spawnings. Mar Ecol Prog Ser 361:161–168
- Iliffe TM, Pearse JS (1982) Annual and lunar reproductive rhythms of the sea urchin *Diadema antillarum* (Philippi) in Bermuda. Int J Invert Reprod 5:139–148
- Kafanov AI, Pavlyuchkov VA (2001) Ecology of the commercial sea urchins (genus *Strongylocentrotus*) of continental Japan Sea. Proc Pac Res Inst Fish Oceanogr (TINRO) 128:349–373 (in Russian with English summary)
- Kelly MS (2001) Environmental parameters controlling gametogenesis in the echinoid *Psammechinus miliaris*. J Exp Mar Biol Ecol 266:67–80
- King CK, Hoegh-Guldberg O, Byrne M (1994) Reproductive cycle of *Centrostephanus rogersii* (Echinoidea), with recommendations for the establishment of a sea urchin fishery in New South Wales. Mar Biol 120:95–106
- Lamare MD, Stewart BG (1998) Mass spawning by the sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea) in a New Zealand fiord. Mar Biol 132:135–140
- Lamare MD, Brewin PE, Barker MF, Stephen RW (2002) Reproduction of the sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea) in a New Zealand fiord. N Z J Mar Freshw Res 36:719–732
- Lessios HA (1991) Presence and absence of monthly reproductive rhythms among eight Caribbean echinoids off the coast of Panama. J Exp Mar Biol Ecol 153:27–47
- Levitan DR (2002) Density-dependent selection on gamete traits in three congeneric sea urchins. Ecology 83:464–479
- Levitan DR, Petersen C (1995) Sperm limitation in the sea. Trends Ecol Evol 10:228–231
- Marzinelli E, Bigatti G, Gimenez J, Penchaszadeh P (2006) Reproduction of the sea urchin *Pseudechinus magellanicus* (Echinoidea: Temnopleuridae) from Golfo Nuevo, Argentina. Bull Mar Sci 79:127–136
- McCarthy DA, Young CM (2004) Effects of water-borne gametes on the aggregation behavior of *Lytechinus variegatus*. Mar Ecol Prog Ser 283:191–198
- Mercier A, Hamel JF (2009) Endogenous and exogenous control of gametogenesis and spawning in echinoderms. Adv Mar Biol 55:1–302
- Mercier A, Battaglione SC, Hamel JF (2000) Periodic movement, recruitment and size-related distribution of sea cucumbers *Holothuria scabra* in the Solomon Islands. Hydrobiologia 440:81–100
- Mercier A, Ycaza RH, Hamel JF (2007) Long-term study of gamete release in a broadcast-spawning holothurian: predictable lunar and diel periodicities. Mar Ecol Prog Ser 329:179–189
- Pearse JS (1975) Lunar reproductive rhythms in sea urchins. J Interdiscipl Cycle Res 6:47–52
- Pearse JS, McClary DJ, Sewell MA, Austin WC, Perez-Ruzafa A, Byrne M (1988) Simultaneous spawning of six species of echinoderms in Barkley Sound, British Columbia. Int J Invert Reprod 14:279–288
- Pennington JT (1985) The ecology of fertilization of echinoid eggs: the consequences of sperm dilution, adult aggregation, and synchronous spawning. Biol Bull 169:417–430
- R Development Core Team (2010) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. www.R-project.org

- Reuter KE, Levitan DR (2010) Influence of sperm and phytoplankton on spawning in the echinoid *Lytechinus variegatus*. Biol Bull 219:198–206
- Smith JR, Strehlow DR (1983) Algal-induced spawning in the marine mussel *Mytilus californianus*. Int J Invert Reprod 6:129–133
- Starr M, Himmelman JH, Therriault JC (1990) Direct coupling of marine invertebrate spawning with phytoplankton blooms. Science 247:1071–1074
- Starr M, Himmelman JH, Therriault JC (1992) Isolation and properties of a substance from the diatom *Phaeodactylum tricornutum* which induces spawning in the sea urchin *Strongylocentrotus droebachiensis*. Mar Ecol Prog Ser 79:275–287
- Starr M, Himmelman JH, Therriault JC (1993) Environmental control of green sea urchin, *Strongylocentrotus droebachiensis*, spawning in the St. Lawrence Estuary. Can J Fish Aquat Sci 50:894–901
- Thorson G (1946) Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae of the Sound (Øresund). Medd Kom Dan Fisk Havunders Plankton 4:1–523
- Tsuji S, Yoshiya M, Tanaka M, Kuwahara A, Uchino K (1989) Seasonal changes in distribution and ripeness of gonad of a sea urchin *Strongylocentrotus nudus* in the western part of Wakasa Bay. Bull Kyoto Inst Oceanogr Fish Sci 12: 15–21
- UNESCO (1966) Determinations of photosynthetic pigments in sea-water. UNESCO Monogr Oceanogr Methodol 1: 11–18
- Williamson JE, Steinberg PD (2002) Reproductive cycle of the sea urchin *Holopneustes purpurascens* (Temno-pleuridae: Echinodermata). Mar Biol 140:519–532
- Zar JH (2010) Biostatistical analysis, 5th edn. Prentice-Hall, Englewood Cliffs, NJ
- Zaslavskaya NI, Vashchenko MA, Zhadan PM (2012) The genetic structure of populations of the sea urchin *Strongylocentrotus intermedius* from the northwestern Sea of Japan in connection with a shift in spawning time. Russ J Mar Biol 38:325–338
- Zhabin IA, Gramm-Osipova OL, Yurasov GI (1993) Wind-induced upwelling off the northwestern coast of the Japan Sea. Russ Meteorol Hydrol 10:82–86 (in Russian with English summary)
- Zhadan PM, Vaschenko MA, Almyashova TN (2015) Spawning failure in the sea urchin *Strongylocentrotus intermedius* in the northwestern Sea of Japan: potential environmental causes. J Exp Mar Biol Ecol 465:11–23
- Zuenko YI, Rachkov VI (2003) General features of oceanographic and hydrochemical regime of the Kiyevka Bay (Japan Sea). Proc Pac Res Inst Fish Oceanogr (TINRO) 133:303–312 (in Russian with English summary)
- Zvalinskii VI, Lobanov VB, Zakharkov SP, Tishchenko PYa (2006) Chlorophyll, delayed fluorescence, and primary production in the northwestern part of the Sea of Japan. Oceanology (Mosc) 46:23–32

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