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Effects of Environmental Factors on Reproduction of the Sea Urchin *Strongylocentrotus Intermedius*

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Additional information is available at the end of the chapter

Abstract

The results of long-term studies (2003–2015) of the reproductive biology of the sea urchin *Strongylocentrotus intermedius* in wild populations located in the northwestern Sea of Japan along 400 km of the coast of the Primorye region of Russia and differing by the level of anthropogenic pressure are reported. Our analysis showed that since 1970–1980s, the shift in spawning season from autumn to early summer occurred in *S. intermedius* populations inhabiting anthropogenically polluted areas of Peter the Great Bay, resulting in the appearance of three types of populations that differ from each other in the proportions of individuals with early spawning (the end of May–June) and late spawning (September–early October). Our results indicate that neither photoperiod nor temperature may be considered as the primary external factors determining a shift in *S. intermedius* temporal patterns of gonad maturation and the timing of spawning and that phytoplankton concentration is the main factor for initiation of sea urchin spawning activity. We hypothesized that the shift in spawning season from autumn to early summer in *S. intermedius* populations inhabiting polluted areas can be explained by a phenotypic response of this species to environmental changes caused by chronic eutrophication.

Keywords: broadcast spawning, echinoderms, reproductive cycle, phytoplankton, temperature, anthropogenic pollution, eutrophication

1. Introduction

Reproduction of marine poikilothermic animals is a cyclic physiological process. Most marine invertebrate species release eggs and sperm into the water column where fertilization and development into a pelagic larval stage takes place and are called broadcast spawners. Broadcast spawners inhabiting the temperate climate zone mostly possess an annual reproductive cycle

with the spawning period to the season when both abiotic and biotic factors (temperature, salinity, and phytoplankton) are favorable for offspring development and survival [1, 2]. The role of environmental variables in the regulation of the timing of the reproductive cycles and variations among and within populations of the same species is not fully understood. The temperature and photoperiod (either in combination or separately) are generally believed to be the most important environmental factors that control the succession of the reproductive cycle stages and spawning timing and promote the synchronization of the performance of these processes in different individuals within population [3, 4]. Mass synchronous spawning of the individuals of both sexes within a population is very important for reproductive success in broadcast spawners, that is determined by the short longevity of gametes and rapid gamete dilution associated with a decrease in fertilization efficiency [5–7]. It is known, however, that the reproductive cycle of some common species of sea urchins (*Paracentrotus lividus*, *Pseudechinus magellanicus*, *Strongylocentrotus droebachiensis*, *Strongylocentrotus intermedius*) is not synchronized within a population as well as between the populations [8–11]. Some individuals breed under conditions of rising photoperiod and increasing temperature whereas the others breed under opposite conditions. The reasons for such desynchronization of the reproductive cycle remain unclear.

It is well known that appearance of a large number of planktotrophic larvae of bottom invertebrates in the plankton coincides well with the occurrence of microalgae blooms [12–17]. As it was shown in laboratory experiments, the addition of microalgae and their extracts to sea water is able to induce the spawning of sea urchins and bivalve mollusks [18–20], and the combined addition of phytoplankton and sperm causes a synergistic effect on spawning activity [18, 20, 21]. Temperature [17, 22, 23], lunar phases [16, 21], and water salinity [24] can also serve as the stimuli that synchronize or trigger the spawning of bottom invertebrates. In addition, many researchers noted that a combination of several natural factors can contribute to spawning synchronization. Moreover, an analysis of the reproductive cycle of *P. lividus* [17] suggests that in sea urchins possessing fully mature gonads, spawning can begin even in the absence of external stimuli.

Sea urchins are widely distributed in marine coastal waters around the world. Many sea urchin species are both ecologically and economically important; besides, they are used as model organisms to study different aspects of reproductive biology of broadcast spawners both in the laboratory experiments and under field conditions. The sea urchin *S. intermedius* (A. Agassiz, 1863) inhabits hard substrates in the northern regions of the Asian Pacific coastal waters, from the Kamchatka Peninsula in the north to the Korean Peninsula in the south and from the Russian coast in the west to the Japanese Islands in the east [11, 25, 26].

In this paper, we give a review of the results of long-term studies (2003–2015) of the reproductive biology of the sea urchin *S. intermedius* in wild populations of this species located in the northwestern Sea of Japan along 400 km of the coast of the Primorye region of Russia and differing by the level of human activity. The main purposes of our work were: (1) to study the reproductive cycle and spawning schedule of *S. intermedius* populations in the areas with different level of anthropogenic pressure and (2) to clarify the relationships between natural environmental factors (phytoplankton, temperature, salinity, dissolved oxygen, moon cycle, tide level, and anthropogenic pressure) and the timing of spawning in sea urchin populations.

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

2.1. Study area, sea urchin sampling, and examination of gonadal state

Seasonal dynamics of gonadal state and the timing of spawning were studied in *S. intermedius* populations from 23 sites located in the northwestern Sea of Japan in the areas with different levels of anthropogenic pressure (**Figure 1** and **Table 1**). Ten sites in Amursky Bay and seven sites in Ussuriisky Bay, the secondary bays of Peter the Great Bay, are located at a different
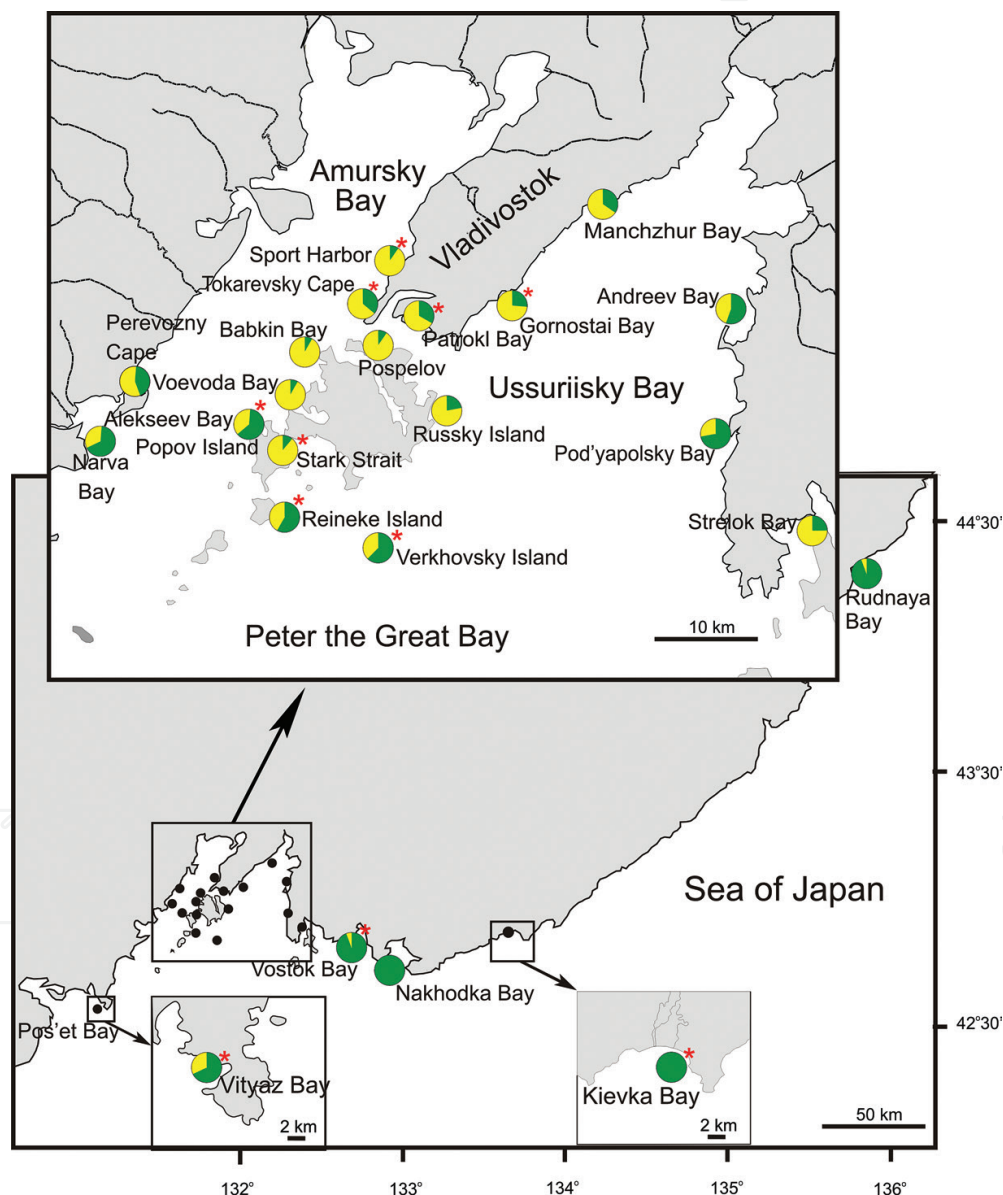
Figure 1. Map of the study area and spatial distribution of the populations of the sea urchin *Strongylocentrotus intermedius* differing in the proportions of individuals with different spawning schedules in the northwestern Sea of Japan. Light grey and dark grey parts of the circles correspond to the proportions of females with early (May–June) and late (September–early October) spawning. Asterisks denote the stations where the studies were conducted during more than one reproductive season.

distance from Vladivostok, the largest port in the Russian Far East of over 600,000 population, which is the main source of marine pollution in Peter the Great Bay. Numerous studies (reviewed in [27–30]) have been undertaken since the 1970s to determine the levels of anthropogenic pollutants in water, sediments, and biota of Peter the Great Bay and to assess the ecological consequences of pollution. Based on the results of these studies, we concluded that coastal waters adjacent to Vladivostok are chronically polluted by heavy metals of anthropogenic origin (Zn, Cu, Ni, and Pb) and organochlorine compounds such as dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls. The eastern coastal area of Amursky Bay where the sites Sport Harbor and Tokarevsky Cape are located and western coastal area of Ussuriisky Bay in the vicinity of a large municipal dump located on the Gornostai Bay coast (**Figure 1**) were considered the most polluted areas of Peter the Great Bay. The open part of Peter the Great Bay (off Russky, Popov, Reineke, and Verkhovsky Islands) was considered a relatively clean zone.

In the 1990s, there was an abrupt recession in industrial production in Primorye region that generated hope for the improvement of the ecological situation in Peter the Great Bay. Actually, according to chemical monitoring, the level of sediment contamination by oil, hydrocarbons, phenols, lead, and copper considerably decreased in the northern part of Amursky Bay [31]. However, our results of long-term monitoring of several sites located in the eastern coastal area of Amursky Bay showed that the contamination of bottom sediments by heavy metals and organochlorine pesticides in the late 1990s and in the early 2000s remained rather high [27]. Moreover, according to our data, during this period silting and contamination of bottoms in the island part of Peter the Great Bay increased in the vicinity of Popov Island (Amursky Bay) and Verkhovsky Islands (Ussuriisky Bay). This was probably related to the removal of thin sediment particles in the form of a suspension by currents from the coastal areas adjoining the city into the open island part of Peter the Great Bay. The ecological risk index SQG-Q, suggested by Long and MacDonald [32], was calculated for several surveyed areas in Amursky and Ussuriisky Bays [27]. It was found that sediments were potentially toxic at all surveyed sites.

Considering all above results, the Vostok Bay is a part of Peter the Great Bay whereas Kievka and Rudnaya Bays are located off Peter the Great Bay (**Figure 1**) were chosen as the reference sites in our studies of the reproductive cycle and spawning schedule in *S. intermedius* populations.

Sea urchins were sampled using scuba in 2003 and 2005–2010 on stony and stony-pebble grounds, at depth of 2–6 m once a month: annually at sites Sport Harbor, Gornostai Bay, Alekseev Bay, and Kievka Bay; from May to October at sites Rudnaya Bay, Patrokl Bay, Vostok Bay, Russky Island, Tokarevsky Cape, Verkhovsky Islands, Stark Straight, Reineke Island, Nakhodka Bay (Novitsky Bay site), and Pos'et Bay (Vityaz Bay site); from May to July at sites Perevozny Cape, Narva Bay, Manchzhur Bay, Andreev Bay, Pod'yapolsky Bay, and Strelok Bay (Abrek Bay site); in May and June, at several sites off Russky Island (Pospelov Beach, Babkin Bay, and Voevoda Bay), at the eastern side of Amursky Bay (**Figure 1** and **Table 1**). At 11 sites, the studies were conducted in sea urchin breeding seasons over more than 1 year (**Figure 1** and **Table 1**). The size of each sample was ≥50 individuals.

The gonads of 30 animals with test diameter of 50–75 mm were weighed to determine the gonad index (GI). The stage of gonad maturity was determined for each individual in accordance with

Station (N, E)	Year	Gonad index (mean ± SD)*	Period of spawning**		Proportion of females/males ready for spawning (%)	
			Early	Late	In the earlier period	In the later period
Rudnaya Bay (44°20′, 145°49′)	2009	24.0 ± 4.9	29.05–07.07	07.08–16.09	3/6	100/100
Kievka Bay(42°50′, 133°41′)	2007	18.6 ± 4.5	No spawning	28.08–10.10	0/0	98/100
	2008	20.0 ± 5.4	No spawning	21.08–25.09	0/0	100/100
	2009	19.5 ± 6.7	02.06–26.06	21.08–15.09	8/67	100/100
	2010	22.0 ± 4.1	No spawning	06.08–8.09	0/0	100/100
	2011	16.6 ± 1.9	No spawning	13.08–21.10	0/0	100/100
	2012	16.1 ± 3.4	No spawning	14.08–17.10	0/0	100/100
	2014	10.7 ± 6.7	No data	28.07–No data	0/0	100/100
	2015	14.1 ± 4.7	No data	13.08–No data	0/0	100/100
Nakhodka Bay						
Novitsky Bay (42°47′7″, 132°54′10″)	2010	24.1 ± 6.8	No spawning	19.07–27.09	0/0	100/100
Vostok Bay(42°53′41″, 132°43′59″)	2008	21.9 ± 5.7	19.06–27.07	12.08–21.09	18/29	95/95
	2009	17.8 ± 5.4	No spawning	02.08-22.10	0/0	90/100
Strelok Bay						
Abrek Bay (42°52′36″, 132°21′58″)	2010	14.1 ± 6.5	04.06–15.07	No data	74/100	No data
Ussuriisky Bay						
Pod'yapolsky Bay (43°0′6″, 132°17′56″)	2010	14.9 ± 6.1	04.06–18.07	No data	25/66	No data
Andreev Bay (43°6′24″, 132°19′20″)	2010	10.4 ± 4.1	04.06–18.07	No data	43/62	No data

Station (N, E)	Year	Gonad index (mean ± SD)*	Period of spawning**		Proportion of females/males ready for spawning (%)	
			Early	Late	In the earlier period	In the later period
Manchzhur Bay (43°14′3″, 132°13′51″)	2009	20.7 ± 4.7	11.06–29.07	No data	64/87	No data
Gornostai Bay(43°7′50″, 132°2′36″)	2009	19.4 ± 7.8	11.06–27.07	08.09–13.10	71/88	26/80
	2010	15.7 ± 5.0	15.06–22.07	21.09–20.10	72/73	22/75
Patrokl Bay (43°4′26″, 131°57′1″)	2009	16.5 ± 6.4	02.06–27.07	09.09–29.09	53/61	52/90
	2011	14.5 ± 4.9	25.05–08.07	05.08–06.10	76/75	27/31
Russky Island (43°1′25″, 131°56′8″)	2009	7.8 ± 3.6	25.05–13.06	14.09–26.10	77/59	18/56
Verkhovsky Islands (42°54′22″, 131°50′22″)	2009	14.9 ± 5.6	08.06–19.07	10.09–23.10	39/60	61/85
	2011	14.5 ± 4.7	13.05–14.07	8.09–25.10	40/41	60/59
Amursky Bay						
Sport Harbor(43°7′14″, 131°52′27″)	2003	23.3 ± 5.2	24.05–25.06	06.08–06.10	78/82	10/68
	2005	22.5 ± 4.8	31.03–04.06	02.08–06.09	82/80	10/62
	2006	27.0 ± 4.6	15.05–11.07	25.08–17.10	92/91	17/80
	2009	21.0 ± 4.8	28.05–07.07	06.09–22.10	92/98	43/95
	2010	23.8 ± 5.2	24.05–17.06	26.08–30.09	100/100	8/80
Tokarevsky Cape(43°5′5.10″, 131°50′45″)	2003	14.3 ± 3.2	24.05–25.06	06.08–06.10	54/68	52/79
	2013	13.3 ± 2.3	21.05–25.06	17.08–29.09	80/95	17/41
Pospelov Beach (43°03′30″, 131°52′34″)	2013	14.3 ± 3.3	16.05–21.06	No data	93/100	No data
Babkin Bay (43°03′08″, 131°47′27″)	2013	11.7 ± 5.7	16.05–21.06	No data	93/95	No data

Station (N, E)	Year	Gonad index (mean ± SD)*	Period of spawning**		Proportion of females/males ready for spawning (%)	
			Early	Late	In the earlier period	In the later period
Voevoda Bay (43°00′39″, 131°46′14″)	2013	14.2 ± 2.3	16.05–20.06	No data	93/100	No data
Alekseev Bay(42°59′10″, 131°42′58″)	2003	7.2 ± 4.1	10.06–2.07	22.08–27.09	15/30	70/63
	2005	12.5 ± 4.7	17.04–10.06	01.09–12.10	44/60	51/45
	2006	13.6 ± 6.5	16.05–02.07	26.08–06.10	50/38	44/60
	2009	9.6 ± 3.9	27.05–16.06	11.09–13.10	38/47	54/44
	2010	11.1 ± 3.7	05.05–16.06	30.08–10.09	39/54	43/52
	2016	10.1 ± 4.9	22.05–10.06	17.08–12.09	58/70	33/45
Stark Straight(42°58′4″, 131°45′8″)	2009	14.4 ± 5.5	07.06–15.07	11.09–13.10	81/83	17/77
	2010	16.1 ± 6.4	05.05–18.06	10.09–22.10	96/75	8/73
Reineke Island(42°54′53″, 131°44′48″)	2003	12.5 ± 4.3	24.05–11.06	21.08–27.09	44/74	55/58
	2009	9.1 ± 3.8	08.06–19.07	12.08–23.10	38/56	50/70
Perevozny Cape (43°3′25″, 131°35′51″)	2010	27.4 ± 5.0	31.05–15.07	No data	55/97	No data
Narva Bay (42°57′49″, 131°30′19″)	2010	11.9 ± 5.3	31.05–15.07	No data	32/32	No data
Pos'et Bay						
Vityaz Bay(42°35′5″, 131°9′55″)	2007	15.3 ± 5.6	15.05–4.06	05.08–03.10	35/88	56/61
	2009	13.7 ± 6.3	25.05–6.07	No data	28/56	No data

*The gonad index (GI) data for sea urchin sample with maximum mean GI value are presented.

**The date of the spawning beginning corresponds to the time when the portion of spawned individuals was ≤10%. The date of the spawning ending corresponds to the time when the portion of spawned individuals was >90%.

Table 1. Reproductive characteristics of the populations of the sea urchin *Strongylocentrotus intermedius* in the northwestern Sea of Japan.

the slightly modified classification which was suggested for the sea urchin *S. intermedius* [33, 34]. Five stages of gonad maturity for both males and females were distinguished: (1) recovering, (2) growing, (3) premature, (4) mature, and (5) spent. Spawning was revealed by a significant increase in the number of spent individuals of both sexes and decrease in the GI.

2.2. Determination of temporal spawning patterns of different *S. intermedius* populations

An analysis of seasonal dynamics of gonadal condition showed the timing of spawning to be different in different *S. intermedius* populations of the northwestern Sea of Japan. We distinguished three types of sea urchin populations that differed from each other in the proportions of individuals with different spawning schedules (**Table 1** and **Figure 1**).

Sea urchin populations with pronounced late (autumn) spawning were referred to the first type. Sea urchin GIs in these populations were the highest during summer months and sharply decreased in September–October. The portion of females with mature gonads exhibiting egg release reached maximum in August to the beginning of September and sharply decreased in the second half of September–October, whereas the percentage of spent females in this period was 90–100% (**Table 1** and **Figure 2A**). Such populations are located from Vostok Bay north to Rudnaya Bay (**Figure 1**).
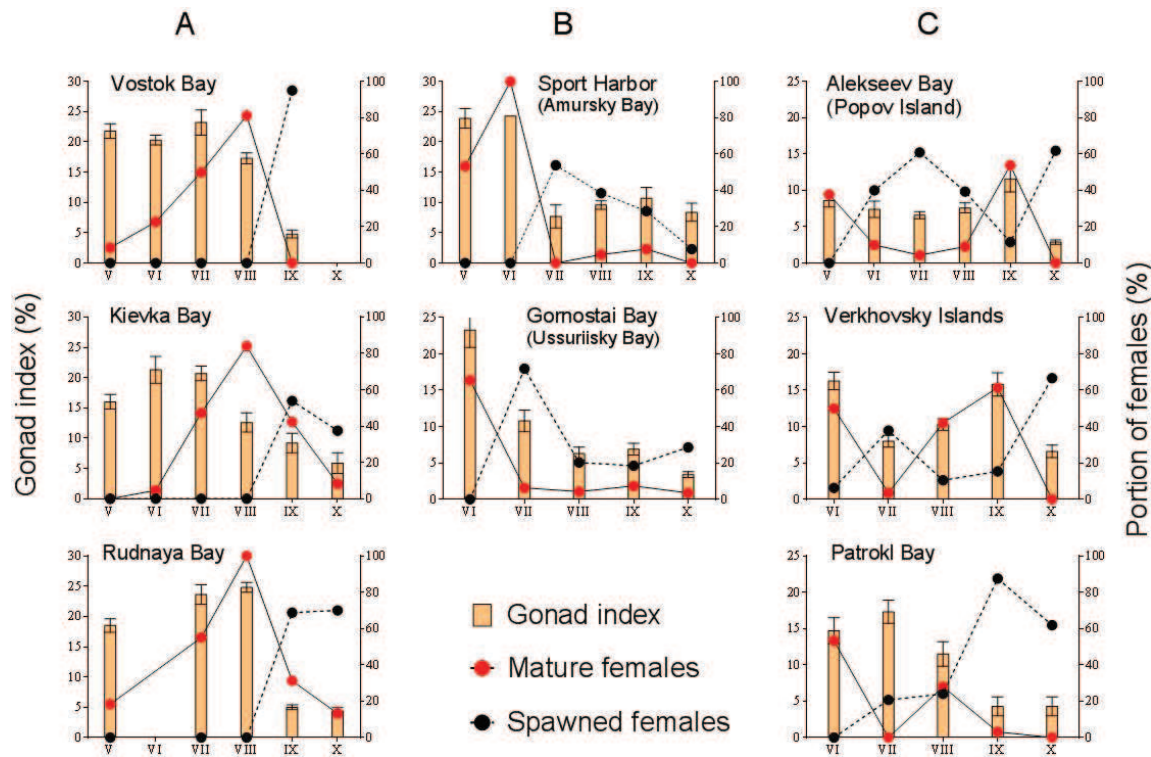
Figure 2. Seasonal dynamics of the gonad index (mean ± SE) and portions of mature and spawned females in populations of the sea urchin *Strongylocentrotus intermedius* with different spawning timing. (A) Populations of the first type (individuals with autumn spawning prevail); (B) populations of the second type (individuals with early summer spawning prevail); (C) populations of the third type (approximately equal proportions of individuals with early and late spawning).

Sea urchin populations with pronounced early spawning (early summer) were referred to the second type. Sea urchin GIs and the portion of mature females in these populations significantly decreased in June–July (**Figure 2B**). The portion of females that spawned in the end of May to the beginning of June varied from 71 to 100% in different years (**Table 1**). Such populations inhabit Sport Harbor and Gornostai Bay sites which are located in coastal waters of Amursky and Ussuriisky Bays adjacent to Vladivostok, as well as Stark Straight between Russky and Popov islands, Russky Island site at the western side of Ussuriisky Bay where heavily contaminated sediments dredged from Vladivostok harbor were dumped, and Abrek Bay located in Strelok Bay where Navy ships were based (**Figure 1**). In 2013, the second type populations were found at Pospelov Beach, Babkin Bay, and Voevoda Bay sites located in Amursky Bay off the Russky Island (**Figure 1** and **Table 1**).

The major part of *S. intermedius* populations belongs to the third type, which is characterized by approximately equal proportions of individuals with early and late spawning (**Figure 1**). In these populations, sea urchin GIs remained almost unchanged during summer and decreased in September–October, and the portion of mature females exhibited two picks, in May and August (**Figure 2C**). Such populations are located in the insular zone of Peter the Great Bay (Popov Island, Reineke Island, and Verkhovsky Islands) as well as at the sites near the western shore of Amursky Bay (Narva Bay and Perevozny Cape) and near the eastern shore of Ussuriisky Bay (Andreev and Pod'yapolsky Bays). Sea urchin populations from Manchzhur Bay, Patrokl Bay, and Tokarevsky Cape sites also can be referred to the third type; it should be noted, however, that at Tokarevsky Cape site in 2013, 80% of females and 95% of males with early spawning pattern were found (**Table 1**). At Vityaz Bay site located in Pos'et Bay, approximately 30% of females were ready for spawning in May–June (**Table 1**).

In most of *S. intermedius* populations studied, the individuals both with early and late spawning occurred, and their ratio was different in different locations (**Figure 1** and **Table 1**). Analysis of the dynamics of gonadal maturation and spawning in *S. intermedius* populations with different spawning schedules showed that the total proportions of females and males that spawned over the entire spawning season, including early and late periods, were 99 ± 9 and 126 ± 30%, respectively (mean $\pm$ SD, the differences were significant at $p < 0.0001$). This indicates that females participated in the spawning only ones, whereas significant proportion of males took part in both spawning periods. An exception was the total sample of sea urchins from Sport Harbor site collected in 2009 and comprised 92% females that had spawned in early summer and 43% females that had spawned in autumn period. However, the data on the timing of gonad maturation and spawning in females allowed us to determine the type of spawning (early-, late-, or double-peak) for *S. intermedius* populations [35].

The spawning schedule of *S. intermedius* did not depend on the relative weight of gonads. The largest GIs (>20%) were registered in sea urchins from sites Rudnaya Bay, Kievka Bay, Vostok Bay, and Sport Harbor (**Table 1**); however, late spawning was characteristic of the first three populations, whereas at the fourth site, early spawning was observed. In 2014, in Kievka Bay, sea urchins had relatively low GI of approximately 10% but retained the late spawning pattern. Significant variations in the GIs were found among *S. intermedius* populations with a double-peak (early and late) in spawning activity (**Table 1**).

Comparison of our results (**Table 1**) with literature data suggests that, at present, the spawning schedule of *S. intermedius* in the northwestern Sea of Japan differs from that described for this species in the end of 1960s, in 1970s, and the beginning of 1980s. The first studies of the reproductive cycle of *S. intermedius* in Russian waters of the Sea of Japan were performed in several secondary bays of Peter the Great Bay in 1970–1980s [36–39]. It was concluded that the spawning period of *S. intermedius* in Ussuriisky Bay and Vostok Bay usually falls on late summer–early autumn [38, 39]. In the 1970s, in *S. intermedius* populations inhabiting Pos'et Bay (at Pos'et Village and in Troitsa Bay) autumn spawning was also clearly pronounced; however, in 20–30% of sea urchin females the ripe eggs were also found in May [40]. In the end of 1970–1980s, approximately 5% of females with mature ovaries could be found in May in *S. intermedius* populations inhabiting Vityaz Bay (Pos'et Bay), whereas sea urchin mass spawning occurred from the end of August to October (Durkina V.B., NSCMB FEB RAS, personal communication).

According to our results, in 2007 and 2009 in Vityaz Bay, the portion of *S. intermedius* females which spawned in the early summer was on average 31.5%, and more than 50% of females spawned during late August to the beginning of October (**Table 1** and **Figure 1**). The reproductive cycle of *S. intermedius* inhabiting Amursky Bay has not been previously studied. However, in the mid-1980s to the beginning of 2000s, we observed an extremely low level of the gonad maturity in sea urchins from several sites in Amursky Bay in August, just before an anticipated *S. intermedius* mass spawning, and suggested another timing of spawning of this species [41, 42]. Indeed, our further studies revealed the peaks of early spawning (late May–June) in several *S. intermedius* populations from Amursky Bay (**Table 1** and **Figure 1**). The highest percentages of females participating in early spawning were found at sites Sport Harbor (88.8 ± 8.8%, 5 years mean ± SD, min = 78%, max = 100%) and Stark Straight (88.5 ± 10.6%, 2 years mean ± SD, min = 81%, max = 96%). This parameter tended to decrease along the western and eastern coasts of the bay, in the direction of its open part; however, several *S. intermedius* populations with more than 90% of females with the early spawning pattern were found in the vicinity of Russky Island (**Figure 1**).

In Ussuriisky Bay, the spawning schedule of *S. intermedius* has changed significantly in the last 40–50 years. In the late 1960s and early 1980s, autumn spawning was registered here [36, 38], while our studies revealed predominantly early spawning in most of *S. intermedius* populations. Thus, at Gornostai Bay site located close to municipal waste landfill, early spawning was registered in 71.8 ± 6.3% females (3 year mean ± SD, min = 65.5%, max = 78%). At Russky Island located close to dumping, 77% of females spawned during early summer (1 year observations). In Ussuriisky Bay, a tendency to decreasing percentages of females with early spawning along the western and eastern coasts of the bay was also observed, up to 30 and 40% at Pod'yapolsky Bay and Verkhovsky Islands sites, respectively (**Figure 1**). This tendency was disrupted in Strelok Bay adjacent to Ussuriisky Bay on the east, Abrek Bay is a part of Strelok Bay (area of Navy base), the portion of females with early spawning was 74%.

S. intermedius populations inhabiting Vostok Bay vicinal to the Strelok Bay and the bays located east and northeast of Vostok Bay exhibited predominantly late spawning (August–September). In May–June of 2009, the ripe eggs were found in the ovaries of only a small portion of sea

urchin females in the samples from Vostok Bay (18%), Kievka Bay (8%), and Rudnaya Bay (3%) sites. It should be noted that the studies of the reproductive cycle of *S. intermedius* conducted in 1971–1975 in Vostok Bay revealed only late spawning of *S. intermedius* [37].

Thus, our results showed that the reproductive cycle of *S. intermedius* in the study area is desynchronized both within one population (the individuals spawn in different seasons, in early summer and autumn) and between the populations (there are three types of populations differing in the proportions of individuals with early or late spawning).

2.3. Hypotheses to explain regional differences in sea urchin spawning schedule

The studies of the reproductive cycle of *S. intermedius* from different population in the Hokkaido area along the Sea of Japan, Sea of Okhotsk, and Eastern Pacific sides conducted over the past 50 years have also revealed different spawning schedules in this species (see for review [11]). Three patterns of *S. intermedius* reproductive cycle were distinguished, as follows: (1) "Sea of Japan cycle" with autumn spawning peak (September–October); (2) "Sea of Okhotsk-Eastern Pacific cycle" with extended spawning period (June–October), and (3) a cycle with two pronounced spawning peaks, in spring (April–May) and in autumn (August–October) that was characteristic of sea urchins inhabiting the northern part of Tsugaru Strait and Funka Bay (southern coast of Hokkaido) [11]. It is important to note, however, that Fuji [33] studied the reproductive cycle of *S. intermedius* in the eastern Tsugaru Strait and Funka Bay in 1950s and revealed only autumn spawning peak (September–October).

There is evidence that the populations of other sea urchin species, in some areas of their geographic range, can spawn in different seasons [8–10]. In most detail, the reproductive biology has been studied in the sea urchin *P. lividus* distributed along the western coast of the Atlantic, from Ireland to the southern extremity of Morocco and in the Mediterranean. The data from 52 publications were involved into a statistical analysis to reveal spatial and temporal patterns of the reproductive processes of this species over the entire geographical range [43]. It was shown, that Atlantic populations of *P. lividus* spawned once a year, in spring–early summer. Mediterranean populations of this species, according to most authors, spawned twice (in spring and autumn), although some authors reported either a single spring spawning or multiple summer spawning peaks [44, 45].

The reasons for such desynchronization of the reproductive cycle of the same sea urchin species remain unclear. There are several hypotheses that have been proposed to explain regional differences in spawning schedule of sea urchins.

1. Hypothesis of the latitudinal gradient is based on the recognition of temperature and photoperiod as the main environmental factors regulating reproductive cycles of marine invertebrates and 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 (see for review [46]). However, this hypothesis cannot explain, why *P. lividus* populations located along the western coast of the Atlantic, from Ireland to the southern extremity of Morocco, that is, for approximately 2000 km from

south to north, are characterized by a single spawning [43], whereas the populations of the same species inhabiting the Mediterranean and located at the same latitude have several spawning peaks that mostly fall on spring and autumn [44, 45]. This hypothesis also cannot explain different spawning schedules of the sea urchin *S. intermedius* in Peter the Great Bay (Sea of Japan) because all the examined populations of this species were located at approximately the same latitude (**Figure 1** and **Table 1**). The populations of this species around Hokkaido are also located at approximately the same latitude but have different spawning seasons (see for review [11]).

2. Hypothesis of genetic determination of temporal patterns of gonad maturation and timing of spawning of *S. intermedius* is based on the results of the experiments on transplantation of the offspring obtained from sea urchins with different spawning schedules [47, 48]. In these experiments, the offspring of sea urchins inhabiting the Sea of Japan side of Hokkaido was transplanted into the region of the eastern Pacific coast and *vice versa*, the offspring of sea urchins from the oceanic region into the Sea of Japan. The results showed that in both cases sea urchins retained the parental pattern of the reproductive cycle. It was suggested that these two populations are isolated. However, from this point of view, it is difficult to explain the changes in spawning schedules of some *S. intermedius* populations happened during the recent 50–60 years. For example, in the 1950s the spawning in sea urchin populations of southern Hokkaido occurred from September to November [33], whereas studies of 1980–1990s revealed double spawning (spring and autumn) in this area [11]. This disagreement was explained by changes in the warm Tsushima Current and Oyashio Current, which provided prerequisites for transfer of sea urchin larvae from other regions of the Pacific. In the northwestern Sea of Japan, in the late 1960s and early 1980s autumn spawning was also registered in *S. intermedius* from Ussuriisky Bay [38], whereas in the 2000s we revealed double spawning in this species [35]; moreover, in the population at Gornostai Bay site, most sea urchins spawned during early summer (**Table 1**). From point of view of the hypothesis mentioned above [47, 48], it should be concluded that genetically isolated populations of *S. intermedius* exist in Peter the Great Bay at distances of a few tens kilometers from each other. However, taking into account active hydrodynamics of this area owing to monsoon climate, such a conclusion appears unlikely. Summer winds of mostly southern directions and northerly autumn winds initiate pronounced wind currents mixing the water and transferring planktonic invertebrate larvae from the open part of Peter the Great Bay to its inner areas and *vice versa*. Also, no information is available about any changes in hydrological regime of the bay for the recent 50 years, which could benefit the transfer of *S. intermedius* larvae from remote areas, like, for example, the Sea of Okhotsk.

Moreover, the study of the genetic structure of 10 *S. intermedius* populations in the northwestern Sea of Japan that differ in the proportion of individuals with different spawning seasonality was conducted using seven allozyme loci as genetic markers [49]. No significant genetic differences between specimens of *S. intermedius* with different timing of spawning were revealed (genetic similarity was 0.988–0.991). A similar conclusion was drawn from the study of population genetic structure of 8 *S. intermedius* populations with different spawning schedule based on the analysis of 12 polymorphic loci of microsatellite DNA [50]. The results of these studies led us to suggest that the shift of spawning period of

S. intermedius in Peter the Great Bay from autumn to early summer is a phenotypic response of sea urchin populations to changes in environmental conditions.

3. Hypothesis of phenotypic response of *S. intermedius* populations to changing environmental conditions due to chronic anthropogenic pollution is based on our observations that: (1) early spawning is the most characteristic of sea urchin populations located close to sources of pollution and (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 [49].

2.4. Marine pollution, eutrophication, and sea urchin reproductive cycle

Sea urchins are characterized by high level of phenotypic plasticity; their morphological and physiological characteristics (growth rate, maximum sizes of body and gonads, and morphology of the body) are prone to changes during the adaptation to particular environmental conditions [51]. Boudouresque and Verlaque [52] explained different spawning schedules in different populations of the sea urchin *P. lividus* in terms of an adaptive response of this species aimed to increase the chances of the offspring for survival. The individuals of *P. lividus* are supposed to synchronize their spawning with conditions favorable for food supply for the larvae (availability of phytoplankton) and preventing their evacuation with currents into the open ocean. An analysis of genetic variability using fragments of mitochondrial and nuclear DNA as markers in two cohorts of *P. lividus* recruits enriching the populations of this species in the Mediterranean after spring and autumn spawning peaks did not reveal any significant differences between the cohorts [53]. The results of our study, which showed the absence of significant genetic differences between the individuals of *S. intermedius* from Peter the Great Bay that spawn in early summer and in autumn [49, 50], are consistent with these data and greatly support the hypothesis that different spawning timing in different populations of the same sea urchin species can be result of their adaptation to environmental changes caused by chronic anthropogenic pollution of marine environment.

Significant positive correlation was found between the portions of females with early spawning in *S. intermedius* populations from several localities in Peter the Great Bay and potential toxicity of surface bottom sediments from these localities assessed by the calculation of the ecological risk index (**Figure 3**) that supports this hypothesis.

It is important to note that marine environment pollution is associated with eutrophication of waters; thus, the effects of anthropogenic pollution on the reproductive cycle of the sea urchin are likely to be indirect and conditioned by great concentrations of phytoplankton and its metabolites in seawater. Based on the analysis of the structural and quantitative characteristics of phytoplankton of Peter the Great Bay during the period from 1996 to 2009, the waters adjacent to Vladivostok in the Amursky Bay were characterized as extremely eutrophic ($>3 \times 10^6$ cells/l) whereas the open waters of Amursky Bay and Ussuriisky Bay as well as Vostok Bay were attributed to the eutrophic type (from 3×10^4 to 3×10^6 cells/l) [54]. The changes in phytoplankton composition and biomass in Amursky Bay are attributed, to a significant degree, to anthropogenic eutrophication [55].
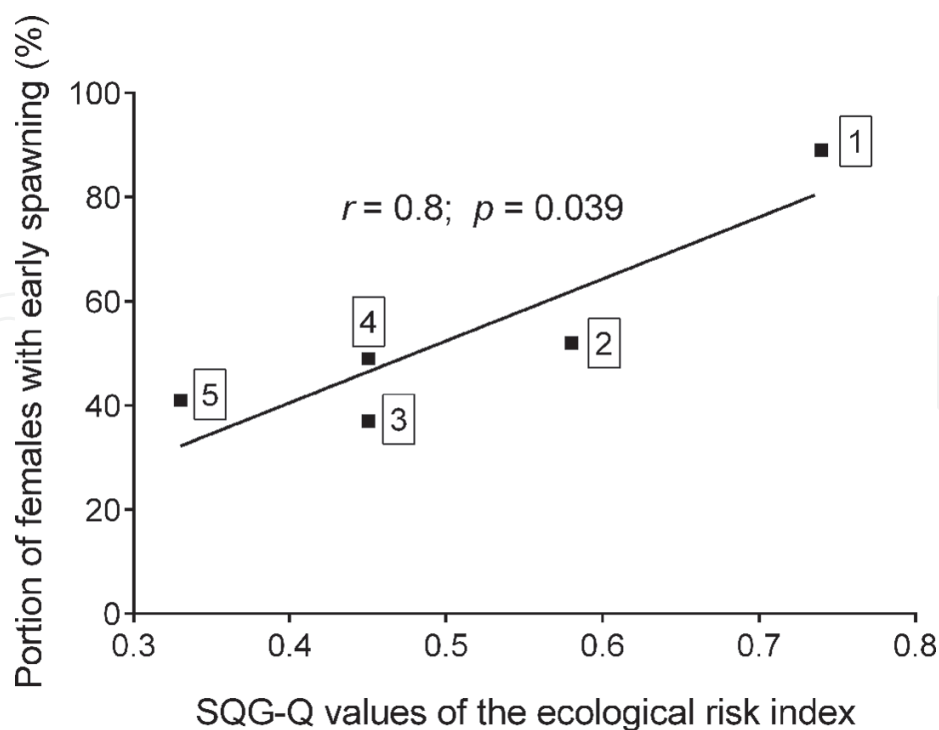
Figure 3. Correlation between the portions of females with early spawning in populations of *Strongylocentrotus intermedius* inhabiting several localities in Peter the Great Bay and potential toxicity of surface bottom sediments from these localities assessed by the calculation of the ecological risk index SQG-Q. 1—Sport Harbor, 2—Tokarevsky Cape, 3—Alekseev Bay, 4—Verkhovsky Islands, 5—Reineke Island. The SQG-Q values are given according to Ref. [27].

To address whether the timing of spawning in *S. intermedius* populations is associated with the level of anthropogenic pressure in the study area, we examined in more details the reproductive cycle of this species in Kievka Bay. This bay is located in sparsely populated area adjoining the State Reserve of Laso, one of the largest forest reserve in Russian Far East; therefore, there is no any significant anthropogenic impact on the marine environment in the chosen area. The wind-induced upwelling associated with the monsoon has a pronounced effect on the hydrological regime and enhances the primary productivity in this bay, especially during the period from April to October [56–59].

In the course of our research, we have faced an unexpected phenomenon. In 2008, more than 90% of sea urchin specimens seemed to have not completed their reproductive cycle by spawning [59]. To the best of our knowledge, this was the first case when the spawning failure in wild sea urchin population was revealed. Below we give a brief review of this phenomenon and discuss potential environmental causes.

3. Spawning failure in *S. intermedius* populations

Sea urchins were sampled in Kievka Bay from 2 to 6 m depths monthly from April 2008 to April 2011, that is, across three reproductive cycles. Methods for GI determination and gonad histology are described in Ref. [59]. In addition to five stages of gonad maturity (1) recovering, (2) growing, (3) premature, (4) mature, and (5) spent, we distinguished one more stage for

unspawned females, in which the gonadal cleaning process was highly prolonged. Therefore, we referred to this period of the reproductive cycle as the stage of prolonged oocyte resorption (6) [59]. In unspawned males, the gonad acini contained a large number of spermatozoa across two reproductive cycles, therefore, we referred to this period as the mature stage (4). The percentage of ready for spawning individuals that released gametes through gonopores during sampling or after dissection was calculated and referred to a group of sea urchins ready for spawning, independently whether they released normal or degenerating gametes.

3.1. Peculiarities of gonadal temporal dynamics in spawned and unspawned *S. intermedius*

Examination of *S. intermedius* gonadal development throughout three reproductive cycles showed that in all the years, the GI of *S. intermedius* reached its maximum in the period from April to July (**Figure 4**) which corresponds to growing (2) and premature (3) stages of gonadal development. In August, the gonads of both sexes were at the premature (3) and mature (4) stages of the reproductive cycle (see **Figure 3** in Ref. [59] for temporal dynamics of the percentages of the reproductive cycle stages). However, despite the seasonal ripening of gonads
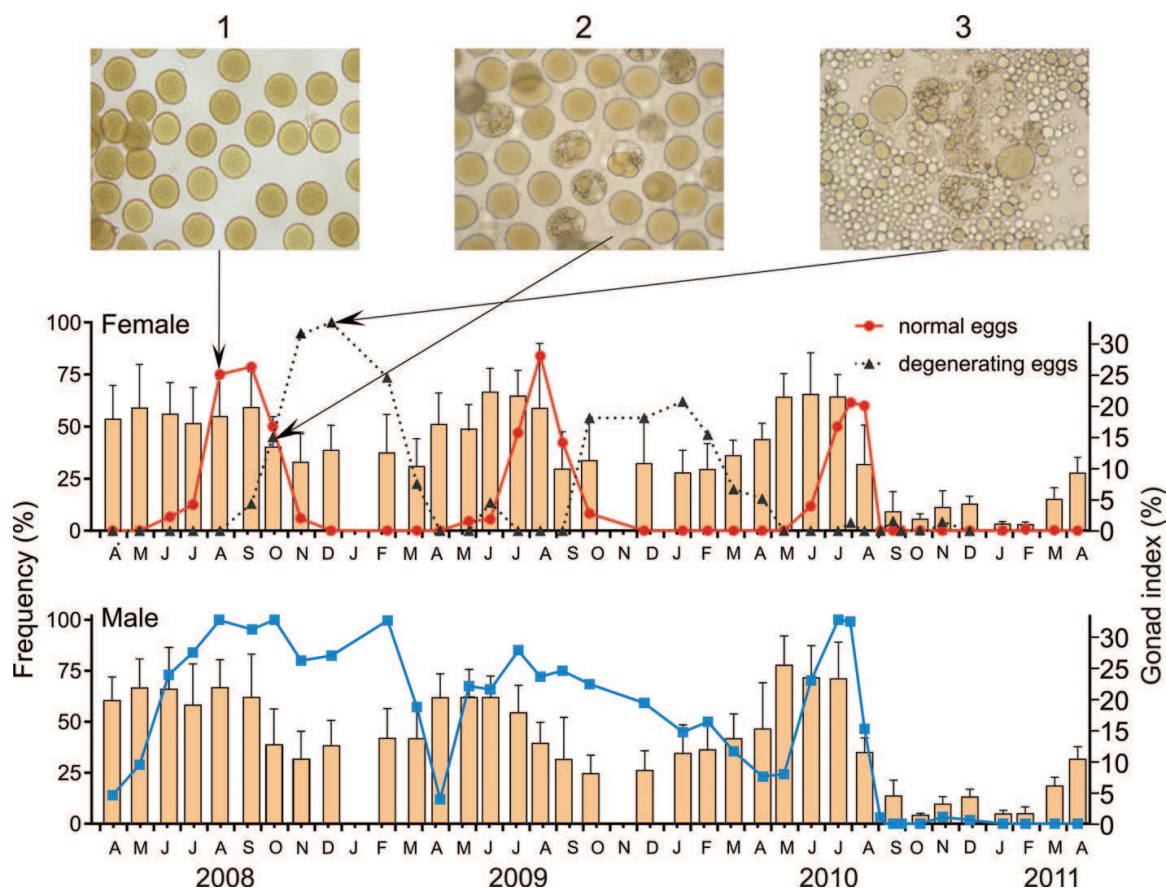
Figure 4. Temporal dynamics of the gonad index (bars, mean ±SE) and the percentages of *Strongylocentrotus intermedius* females (lines with circles and triangles) and males (lines with squares) ready for spawning from Kievka Bay (northwestern Sea of Japan) in 2008–2011. Photograph 1 shows normal eggs, photograph 2 and 3 show degenerating eggs at different stages of their fragmentation. The data shown are extracted from **Figures 3** and **5** and the graphical abstract in Ref. [59].

by all individuals in *S. intermedius* population, the dynamics of the GIs and percentages of females and males ready for spawning as well as gonad histology throughout April 2008 to April 2011 indicate that spawning may not occur for a most individuals in a population in some years. In two of three reproductive seasons, a nearly complete absence (95% unspawned females in 2008) or a partial absence (53% unspawned females in 2009) of spawning was observed. The males also did not spawn completely. In September, the destruction of undischarged eggs had begun in unspawned females. Undischarged eggs disintegrate into numerous spherical fragments (**Figure 4**). The stage of prolonged oocyte resorption (6) as well as the cleaning process associated with the resorption of numerous undischarged sperm by testicular nutritive phagocytes lasted for approximately 7 months [59].

The seasonal dynamics of the percentages of sea urchins ready for spawning generally corresponded to the data obtained from the histological analysis and reflected well the level of gonadal maturity [59]. In August 2008, approximately 80% of females released normal eggs; in September, the percentage of females ready for spawning reached 91% but 13% of females released a mixture of degenerating and normal eggs (**Figure 4**). In December, the percentage of females ready for spawning reached 100%; however, only degenerating eggs and egg fragments were released. The females which released degenerating eggs were observed through March 2009 (**Figure 4**). In 2009, the dynamics of females ready for spawning was similar to that in 2008; however, the percentage of females releasing degenerating eggs during autumn–winter seasons was two times lower.

Judging from the 3 month shift in the line reflecting the percentage of females which released fragmented eggs compared with that reflecting the percentage of females which released normal eggs (**Figure 4**) and from significant positive correlation between these dynamics ($r = 0.966$, $p = 0.0073$), it may be proposed that a life-time of mature eggs in sea urchin ovary lasts approximately 3 months.

The males ready for spawning were found in all sea urchin samples taken in 2008 and 2009 (**Figure 4**). During the period from August 2008 to March 2009, the portion of males ready for spawning was approximately 100%, and during the period from July 2009 to December 2010, it was from 87 to 57%. In July–August 2010, the percentage of males ready for spawning increased up to 100%.

In 2010, the GIs decreased stepwise from August to October up to zero in both sexes, and the percentage of sea urchins ready for spawning dropped to zero in September (**Figure 4**). It indicates that spawning in *S. intermedius* population was synchronous in females and males and was completed by October. The 3-year minimum for the GI was observed during the period from October 2010 to February 2011 (**Figure 4**), when the GIs were 5.8 and 4.5 times lower than the corresponding GI values recorded in 2008 and 2009, respectively.

The data on the size frequency distribution of female reproductive cells (oocytes and eggs) provided valuable information regarding the dynamics of oocyte development over three reproductive cycles, in relation to the presence or absence of spawning in *S. intermedius* population (**Figure 5**). Generally, the changes in the oocyte size frequency distribution between March and August were similar for the 3 years, reflecting a single cohort of growing oocytes
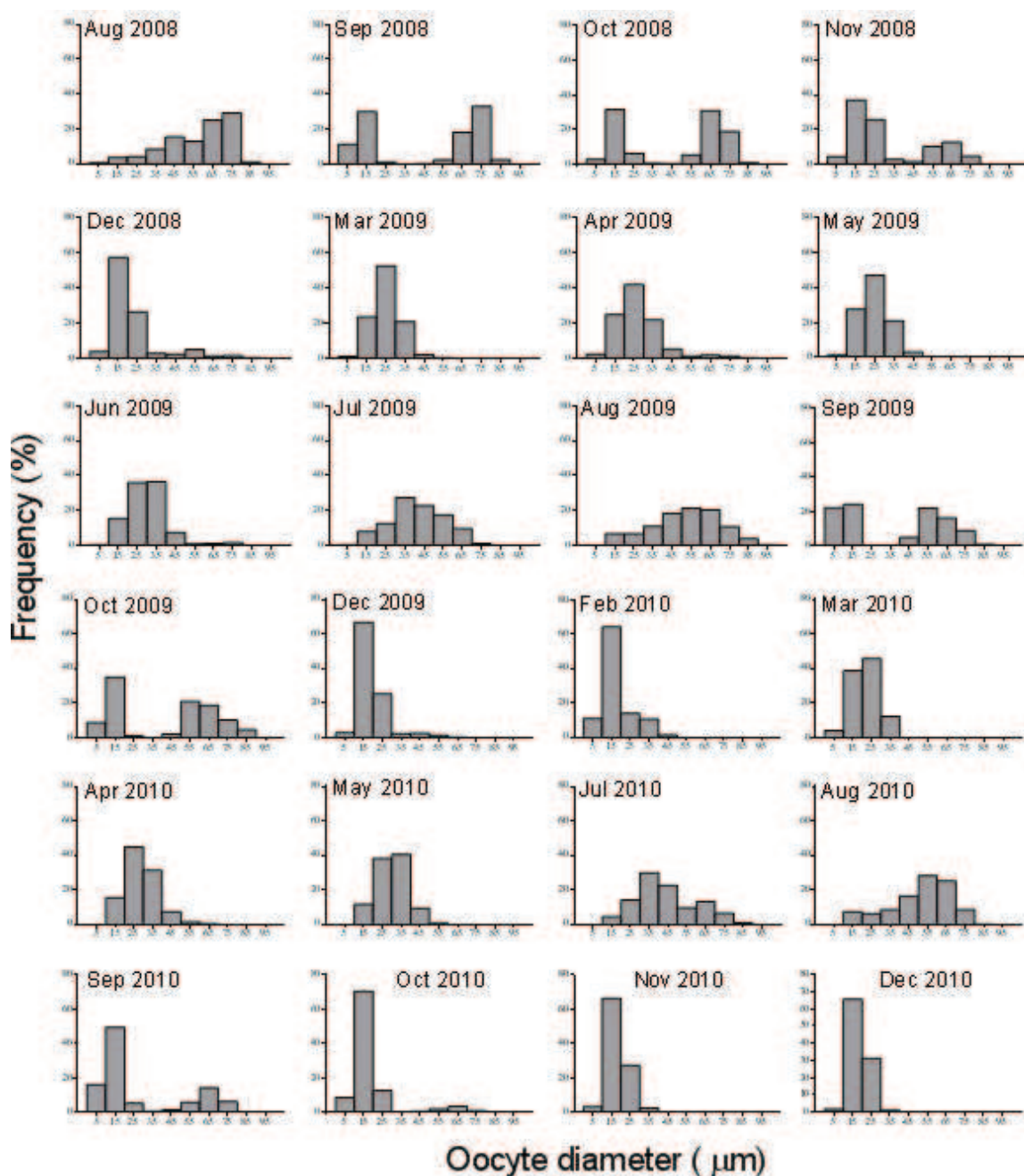
Figure 5. The size frequency distribution of the reproductive cells in *Strongylocentrotus intermedius* females sampled in Kievka Bay (northwestern Sea of Japan) in 2008–2010.

which was present in the ovaries, and the oocyte size distribution was approximately uni-modal. However, some peculiarities were observed in the period from August to December. It is important to note that in all the 3 years, a fraction of the smallest oocytes that just began to grow (a diameter of approximately 10 µm) was absent in June–July and appeared in September, independent of whether spawning in *S. intermedius* population took place or not. In September and October of 2008 and 2009, the oocyte size distribution was bimodal, reflecting approximately equal shares of small growing oocytes with a diameter <30 µm and

large vitellogenous oocytes with a diameter >50 μm and undischarged eggs (**Figure 5**). In November, when no morphologically normal eggs were present in the gonads (fragmented eggs were not taken into account), a fraction of large oocytes decreased sharply, and in December, it was negligible. In September and October of 2010, after complete spawning, spent ovaries presented 70–80% of growing oocytes and less than 20% of residual eggs which disappeared by November (**Figure 5**).

Thus, our study revealed the unique characteristics of the reproductive cycle of *S. intermedius* individuals displaying spawning failure. First, in the case of normal spawning, a more than 10-fold decrease in the GI occurred in September–October whereas in the case of absence of complete spawning, the decrease in the GI was about half of its maximum value, and these intermediate GI values remained stable (no significant differences between subsequent samples) until May of the next year. Second, the cleaning process associated with the resorption of numerous undischarged eggs and spermatozoa was unusually long, lasting for approximately 7 months.

It is well known that after spawning, the acini of sea urchin gonad still contain small quantity of undischarged gametes. These gametes subsequently undergo resorption, which involves nutritive phagocytes [33, 60–64]. Our data indicate that this mechanism is triggered regardless of whether spawning occurs. The ovaries and testes of unspawned sea urchins contain a tremendous quantity of undischarged eggs and sperm which have to be subjected to phagocytosis. Due to the differences in size of female and male gametes, the patterns of their phagocytosis are also different. Undischarged spermatozoa are phagocytosed by testicular nutritive phagocytes and become a part of their heterophagosomes, that is, undergo intracellular digestion [61–64]. Undischarged eggs are too large to undergo phagocytosis without being disintegrated. The large-scale fragmentation of undischarged eggs which we revealed in the ovaries of unspawned *S. intermedius* [59] corroborate the suggestion of Masuda and Dan [65] that sea urchin residual eggs are destructed into spherical fragments, probably through autophagy, which subsequently are phagocytosed by nutritive phagocytes and digested.

The resorption of relict gametes in the sea urchin gonad normally lasts for 2–3 months [64]. The much greater duration (over 7 months) of the cleaning process revealed in the ovaries of unspawned *S. intermedius* [59] is in all likelihood due to the much larger volume of the sex cells that were subject to resorption in seasons of 2008–2009. Development of a new generation of oocytes in *S. intermedius* took place from December to August (**Figure 5**). Thus, in the absence of spawning, the growth and differentiation of the new generation of oocytes occur simultaneously with the cleaning process.

3.2. Occurrence of spawning failure in *S. intermedius* populations along the coastline of the Primorye region and dynamics of environmental variables

To reveal the spatial (geographic) distribution of cases of spawning failure in *S. intermedius* populations in the northwestern sea of Japan, we carried out the following studies: (1) a one-time survey to examine the gonads of sea urchins sampled from Vostok Bay (42°53′ N, 132°43′ E) in November 2008 (in the post-spawning period of *S. intermedius* reproductive cycle); (2)

monthly analysis of sea urchin gonads during the period from May to November 2009 in Vostok Bay and Rudnaya Bay (44°20′ N, 145°49′ E); (3) a one-time analysis of the sea urchin gonads sampled in November 2009 in the Bays of Vrangel (42°45′ N, 133°3′ E), Shepalova (42°41′ N, 133°4′ E), Sokolovskaya (42°52′ N, 133°53′ E), and Kit (43°4′ N, 134°11′ E) (**Figure 6**).

Our interest to Vostok Bay is explained by the fact that (1) in this bay, the reproductive cycle of *S. intermedius* has been examined earlier for 5 years, in 1971–1975, and spawning failure has not been observed [37]; (2) according to satellite data (http://www.satellite.dvo.ru), the chlorophyll *a* (Chl *a*) concentration in Vostok Bay significantly exceeded that in Kievka Bay.

In sea urchin sample taken from Vostok Bay in November 2008, only 1 of 14 females (7%) was unspawned (**Figure 6**) and had a large gonad with degenerating eggs. Seasonal analysis of gonadal state showed that in 2009, sea urchins in Vostok Bay and Rudnaya Bay (extreme south and north sites) as well as in Kievka Bay (centrally positioned site) became mature almost simultaneously. In the first half of August, more than 90% of ripe sea urchins were found in all these populations, which allows to assume synchronous maturation in *S. intermedius* populations at other sites (the Bays of Vrangel, Shepalova, Sokolovskaya, and Kit). However, the percentage of unspawned females varied from 0% in Vostok and Sokolovskaya Bays to 68% in Kit Bay (**Figure 6**).

To reveal regional differences in the temperature of the water surface and phytoplankton production (determined as the Chl *a* concentration) in the surveyed area, satellite monitoring
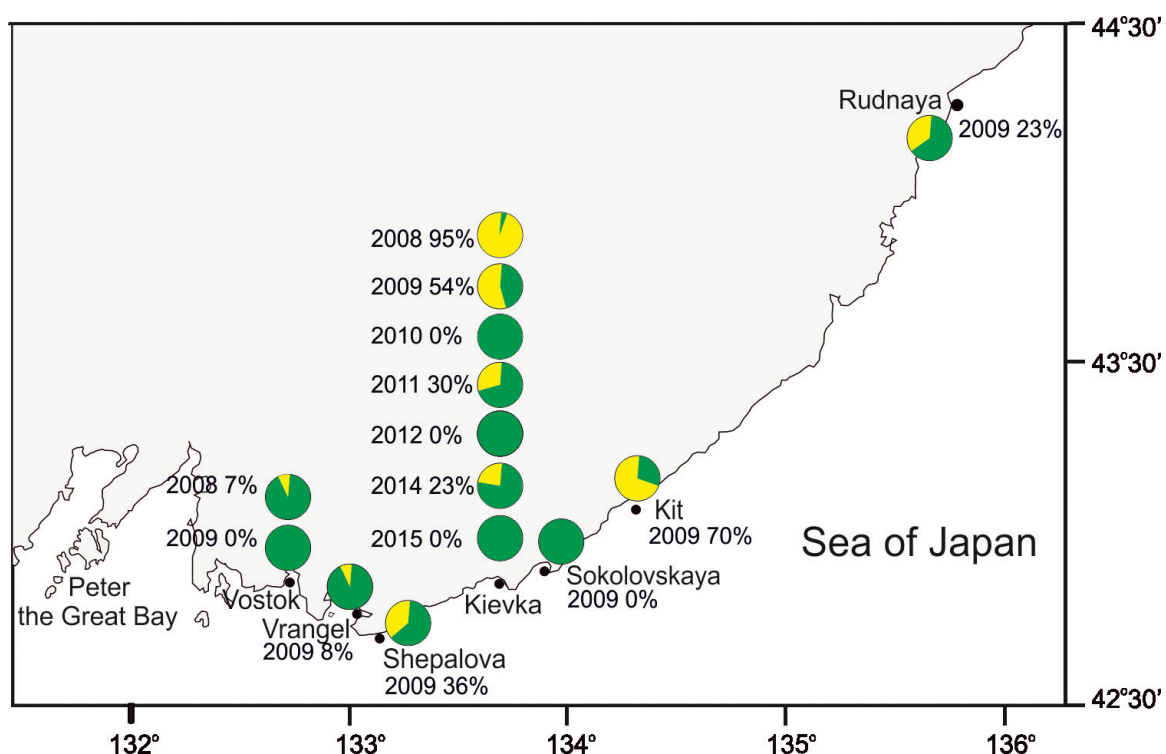

Figure 6. Occurrence of spawning failure in *Strongylocentrotus intermedius* populations along the coastline of the Primorye region (northwestern Sea of Japan). Yellow parts of the circles denote the percentages of unspawned females. The data shown are taken from Ref. [59], with addition of new data.

data over the period of 2008–2009 were used (see Ref. [59] for details). From our point of view, the comparison of temperature and Chl *a* dynamics in Kievka Bay and Vostok Bay is of most interest due to a large difference in the percentages of unspawned females in these bays.

The temperature profiles in Kievka and Vostok Bays during August and the beginning of October 2008 were well synchronized and strongly correlated (Pearson *r* = 0.81, *p* < 0.0001, *α* = 0.05) (**Figure 7A**). An abrupt temperature decrease caused by upwelling, which is typically observed in this season [59], occurred on September 28 at both stations simultaneously. At the same time, the concentrations of Chl *a* greatly differed among these two sites: the maximum and mean values in Vostok Bay were 5.5 and 3.5 times higher, respectively, than those in Kievka Bay (**Figure 7B**).
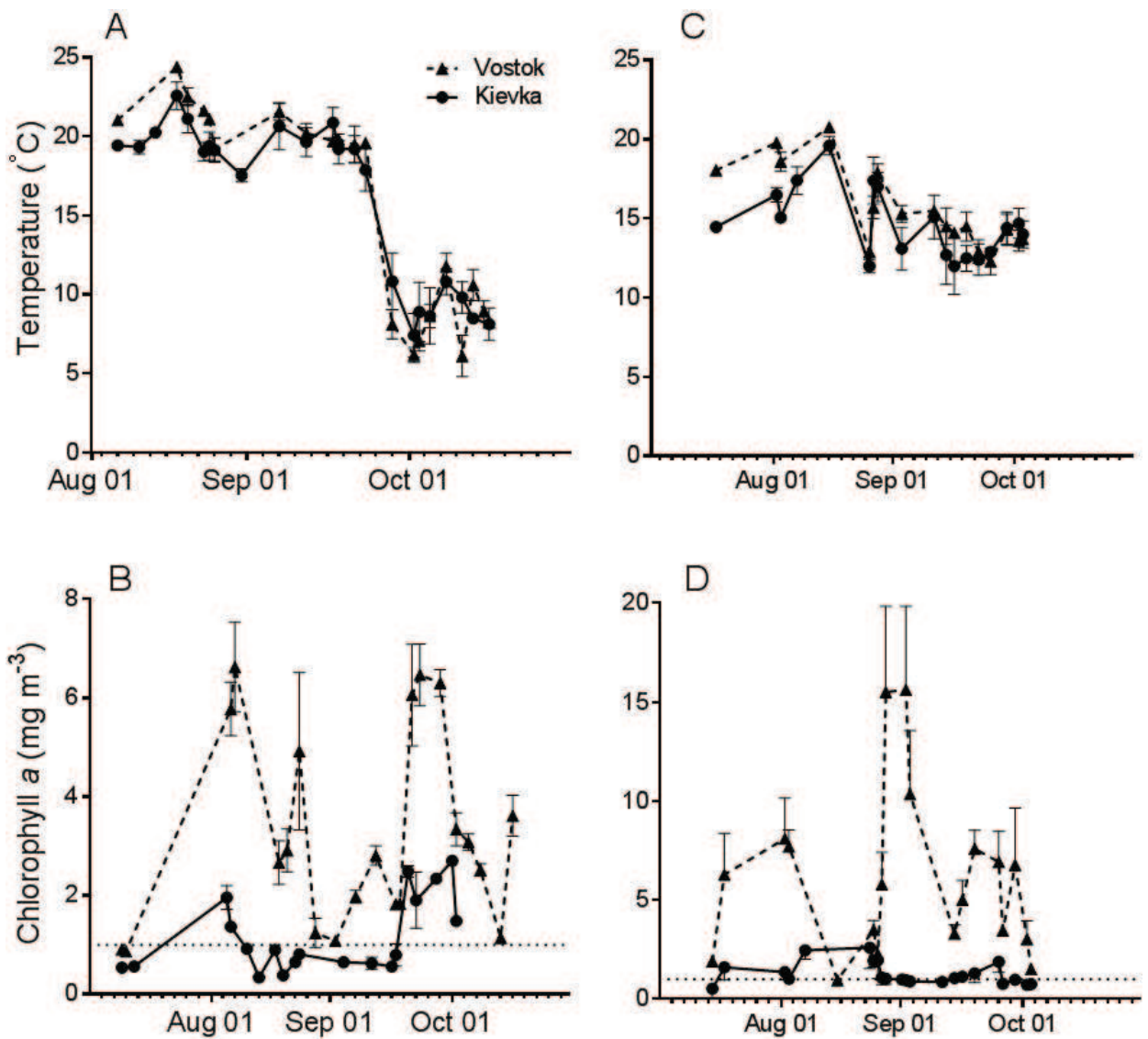
Figure 7. The satellite-based data (mean ± SD) showing temporal dynamics of the temperature (A, C) and Chl *a* concentration (B, D) in Vostok Bay and Kievka Bay (northwestern Sea of Japan) in summer–autumn seasons of 2008 and 2009. Horizontal dashed lines in (B, D) correspond to Chl *a* concentration of 1 mg m^{-3}. The data shown are extracted from **Figures 7–9** in Ref. [59].

In August–September 2009, temperature dynamics in Kievka and Vostok Bays (**Figure 7C**) as well as at other five sites (the Bays of Rudnaya, Vrangel, Shepalova, Sokolovskaya, and Kit, see **Figure 5** in Ref. [59]), was very similar. A sharp decrease in temperature due to upwelling was observed at all stations between August 28 and 31 followed by the second (lower) temperature oscillation. The proportions of females that failed to spawn, however, were different between different localities (**Figure 6**) and positively correlated with mean Chl *a* concentrations at these sites [59]. The highest Chl *a* concentration was observed in Vostok Bay (**Figure 7C**) where no unspawned females were found in 2009 (**Figure 6**).

All these data indicate that a link appeared to be between spawning success in *S. intermedius* populations and the phytoplankton level in the study area. In summer 2010, from July to October, we had the opportunity to measure the Chl *a* concentration, water temperature and salinity in Kievka Bay directly at the site of sea urchin sampling near the bottom. Analysis of gonadal state of the sea urchins was carried out at intervals of 8–14 days. Three subsequent spawning events were revealed between August 6 and September 8 based on significant decrease in the GI and a sharp increase in completely spawned sea urchins (**Figure 8A**). In 2010, sea urchin spawning was the most successful for over three years (2008–2010): in the sample collected on September 22, only one unspawned female of the 22 examined was found (**Figure 8A**).
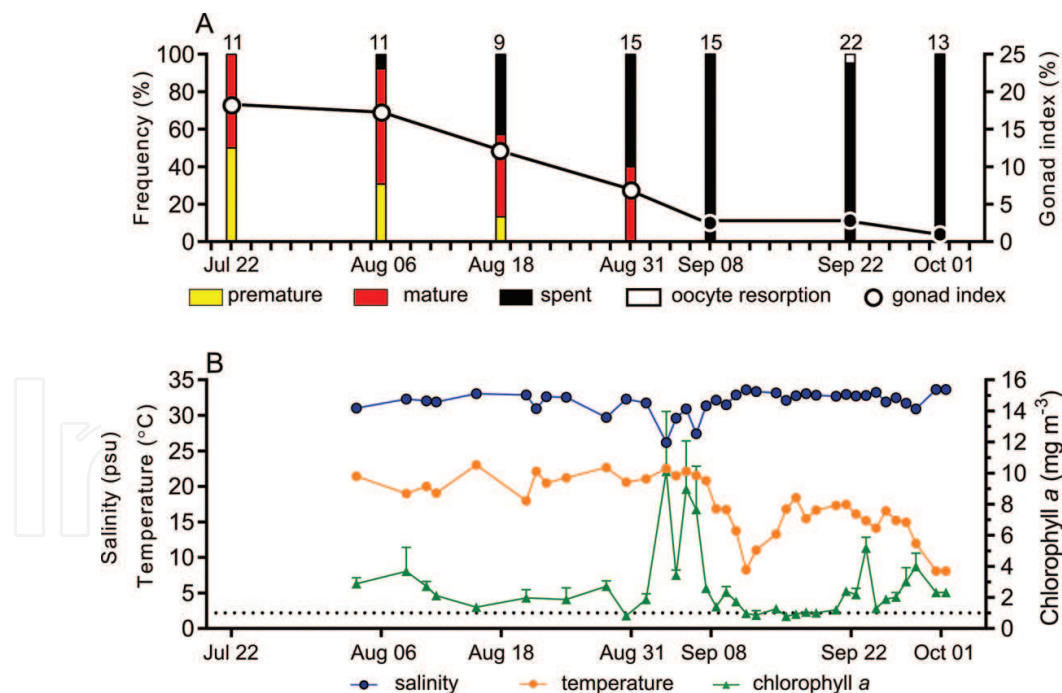
Figure 8. Temporal dynamics of gonadal development in *Strongylocentrotus intermedius* females (A) and environmental variables (B) in summer–autumn season of 2010 in Kievka Bay (northwestern Sea of Japan). (A) The percentages of the reproductive cycle stages (columns) and the GI values (circles and line; the figures above the columns show the number of sea urchins from which the frequency was calculated). (B) Temporal variation of the temperature, Chl *a* concentration and salinity (data of direct measurements in the bottom boundary layer). Horizontal dashed line corresponds to the Chl *a* concentration of 1 mg m⁻³; solid vertical lines denote standard deviation. The data shown are compiled from **Figure 10B** and D in Ref. [59].

Between August 6 and August 18, 2010, when the first spawning event took place, the water salinity was relatively stable, whereas during August 18–31 and August 31–September 8, when the second and third spawning events occurred, a short-term decrease in salinity by 2–5‰ was recorded (**Figure 8B**). During the first and second spawning events, the temperature variations were from 17 to 23°C, whereas the third spawning event occurred under conditions of fairly stable temperature (**Figure 8B**). A sharp decline in temperature caused by upwelling occurred on September 11, when the sea urchin spawning was virtually completed. The Chl *a* concentration varied from 0.83 to 2.7 mg m^{-3} during the first and second spawning events and rose up to 10.1 mg m^{-3} during the third spawning event (**Figure 8B**).

Taken together, the results of our study indicate that a certain environmental stimulus or combination of stimuli is necessary for triggering the spawning in *S. intermedius* natural population. A sufficiently high concentration of phytoplankton, which serves as food for larvae, and water temperature suitable for larval development are the factors ensuring survival of sea urchin offspring; therefore, *S. intermedius* spawning may be triggered by these environmental stimuli. Our findings are not consistent with the studies presuming the changes in temperature may serve as proximal cues triggering the spawning of sea urchins (see for review, Ref. [46]). At the same time, the results of our analysis support a hypothesis that an increase in the phytoplankton concentration may be the most appropriate candidate for inducing sea urchin spawning.

Primary production in nearshore waters substantially depends on the influx of biogenic elements from the terrigenous runoff, upwelling and anthropogenic sources (see for review, Refs. [66–68]). The study area is characterized by spatially different level of anthropogenic pressure and significant year-to-year variations in the magnitude of river runoff and the upwelling schedule [56–59]. We hypothesized that the phenomenon of spawning failure in natural populations of *S. intermedius* seemed to be attributable to combination of environmental factors responsible for low primary productivity in water column during the sea urchin spawning season. The results of our subsequent studies showed that the phenomenon of spawning failure seems to be common in *S. intermedius* populations in Kievka Bay, so the sea urchin spawning season can last for more than 2 months [58], and spawning failure was observed during 4 reproductive seasons between 2008 and 2015 (**Figure 6**).

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 Chl *a*, temperature, salinity, moon phases, and tide level) which were monitored directly in the animal habitats, along with examination of gonadal state of sea urchins sampled at a fine temporal scale (3–12 days, on average 4.2 days) [58].

4. Environmental variables influencing *S. intermedius* spawning

The studies were carried out in Kievka Bay at Sites 1–3 in 2011 and at Sites 1–4 in 2012 (**Figure 9**). An analysis of sea urchin gonads sampled at intervals of 3–12 days (on average, 4.2 days) was performed during the period from the beginning of August to the end of September (in the pre-spawning, spawning, and post-spawning periods of the reproductive cycle [59]).
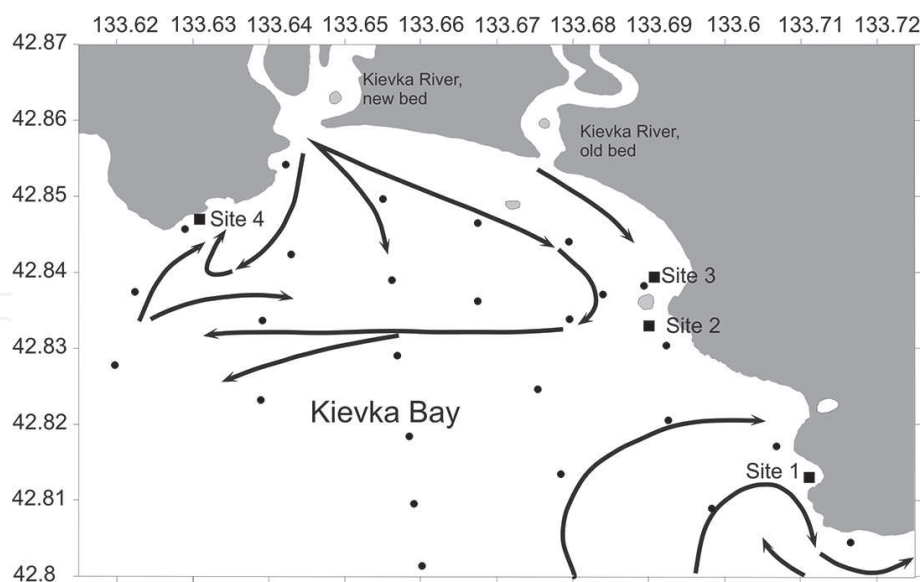
Figure 9. Map of Kievka Bay located in the northwestern Sea of Japan. The sites where sea urchins were collected are denoted by the squares, and 26 sites where the sounding of the water column was performed are denoted by small circles. Solid lines with arrowheads show surface currents in Kievka Bay in August (according to: Ref. [69]).

Fresh smears were used to determine the state of maturation of *S. intermedius* females. Six stages of ovarian maturation were determined: (1) immature, (2) premature, (3) partially mature, (4) mature, (5) spent, and (6) over-mature [58].

The measurement of depth, water temperature, salinity, photosynthetically active radiation, and concentrations of dissolved oxygen and Chl *a* was performed with a multi-parameter sonde directly at the sites of sea urchin sampling. At site 3, environmental variables were continuously (every 15 min) recorded by a YSI 6920V2 data logger which was installed 50 cm above the bottom. Additionally, these parameters were measured at 26 sites (**Figure 9**) in the bay by the CTD-sounding of the water column up to depth of 60 m. Details of the methods and statistical analysis are given in Ref. [58].

4.1. Gonadal maturity and spawning events

Examination of gonadal development (GI dynamics, ovarian maturity, and dynamics in the proportion of sea urchins ready for spawning) in *S. intermedius* at different sites in Kievka Bay throughout two reproductive seasons (August–October of 2011 and 2012) showed that sea urchins become mature by the August, so they have the potential to spawn at any time in August and most parts of September. However, mass spawning may be both synchronous and asynchronous between sea urchin populations within a bay; moreover, it was shown that not all populations spawned completely even within a comparatively small area. The differences in the dynamics of sea urchin spawning between the sites and the years are most evident from a comparison of the parameters of gonadal development in sea urchins from site 1 and those from other sites (**Figure 9**). In 2011, 30% of females and approximately 60% of males from site 1 failed to spawn which was evident from the analysis of the final sample taken on October 21 (**Figure 10A**). The percentages of females and males ready for spawning and mean GI value
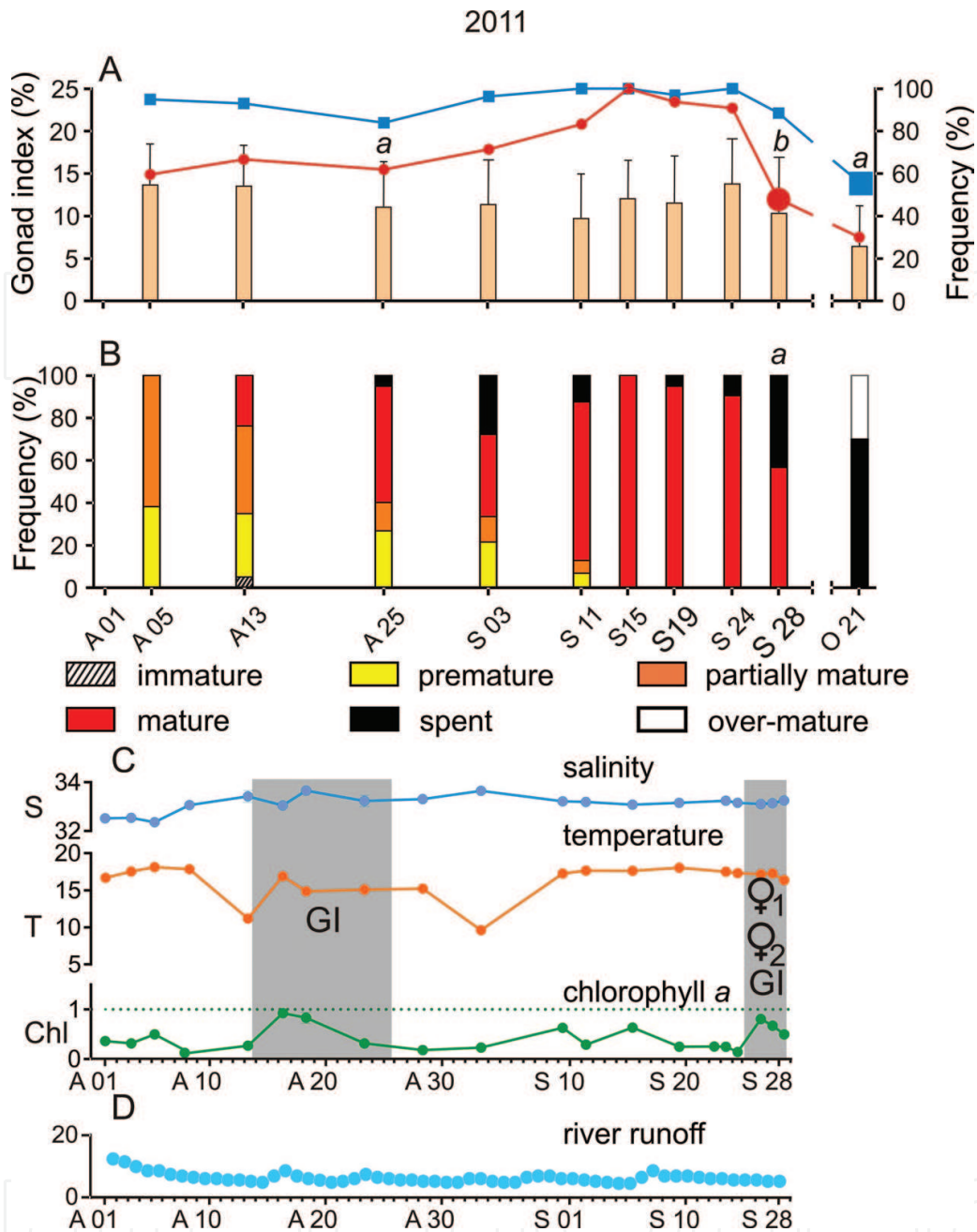
Figure 10. The timing of the spawning of the sea urchin *Strongylocentrotus intermedius* and the associated environmental conditions in August–September of 2011 at site 1 in Kievka Bay. (A) Temporal dynamics of the gonad index (columns: mean ± SD) and the percentages of the the the males (lines with squares) and females (lines with circles) ready for spawning. Letters above the columns denote significant mean difference compared to previous month (*a*: $p < 0.05$, *b*: $p < 0.01$, one-way ANOVA, Kruskal-Wallis statistics, Dunn's multiple comparison test). Large squares and circles indicate significant differences compared to previous month ($p < 0.05$, binomial statistics). (B) Temporal dynamics of the percentages of the ovarian maturity stages in *S. intermedius* females. Letter "*a*" above the column denotes significant increase in the portion of spent females ($p < 0.05$, binomial statistics). (C) Spawning events and the dynamics of environmental variables: Chl—chlorophyll *a* (mg m⁻³), S—salinity (psu), T—temperature (°C). Horizontal dashed line corresponds to the Chl *a* concentration of 1 mg m⁻³. 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; ♀$_2$: significant increase in the portion of spent females. (D) Daily runoff of the Lazovka River, the main tributary of the Kievka River that flows into Kievka Bay (m³ s⁻¹). *x*-axis: month and date. The data shown are extracted from **Figures 2, 5,** and **7** in Ref. [58].

were rather a high due to a presence of unspawned individuals in the sample. Unspawned females had disintegrating egg in the ovaries, which corresponded to the over-mature stage (6) of the ovarian maturation (**Figure 10B**). Two sea urchin spawning events were revealed by a significant increase in the number of spent females and significant decrease in the GI and/or the percentage of females and males ready for spawning (**Figure 10C**, shaded areas). At the same time, at sites 2 and 3, the dynamics of the GIs, readiness for spawning, and level of ovarian maturity were significantly different from those at site 1 [58]. Three and two subsequent spawning events occurred during August–September, respectively, and all the females were spent on October 21. The GIs and percentages of females and males ready for spawning at sites 2 and 3 sharply decreased by September 28 and were close to zero on October 21.

In 2012, seasonal dynamics of the GIs, proportion of sea urchins ready for spawning and level of ovarian maturity at sites 1–4 were synchronous [58]. At site 1, more than a 50% decrease in the GI occurred between August 14 and August 18, and then, the GIs gradually decreased until October 17 up to values less than 5% (**Figure 11A**). At other three sites, the GIs exhibited very similar dynamics [58]. At all four sites, the seasonal dynamics of the proportions of females and males ready for spawning showed drops, mostly coincident with significant decreases in the GIs (see **Figure 11A**, for example). In September, the percentages of spent females at different sites constituted from 25 to 55% of the total female number (see **Figure 11B**, for example). From two to three sea urchin spawning events were revealed at different sites, and the analysis of the final sample taken on October 17 showed that the reproductive cycle of *S. intermedius* in all four populations studied ended in virtually complete spawning (see **Figure 11B** and **C**, for example).

In sum, in 2011 and 2012, 16 sea urchin spawning events (spawning windows) were revealed by a significant increase in the number of spawned females and significant decreases in the GI and/or the percentage of females ready for spawning.

4.2. Relationship between environmental variables and spawning events

The temporal patterns of *S. intermedius* spawning events throughout two reproductive seasons indicate that the exact timing of spawning during the spawning period depends on the local properties of the environment, that is, a certain external stimulus or a favorable combination of environmental conditions is necessary for spawning. Analysis of data logger measurements showed that the summer–autumn seasons of 2011 and 2012 differed in weather and hydrological conditions: (1) the quantity of precipitation and associated changes in the terrigenous input into Kievka Bay and bottom layer salinity were substantially higher in 2012 than in 2011; (2) 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 in the water column in 2011 (see **Figures 10C** and **D** and **11C** and **D** for comparison). The oxygen content in the waters of Kievka Bay was close to saturation in both seasons.

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, that is, a value of 1 was assigned to each inter-sample time interval when spawning
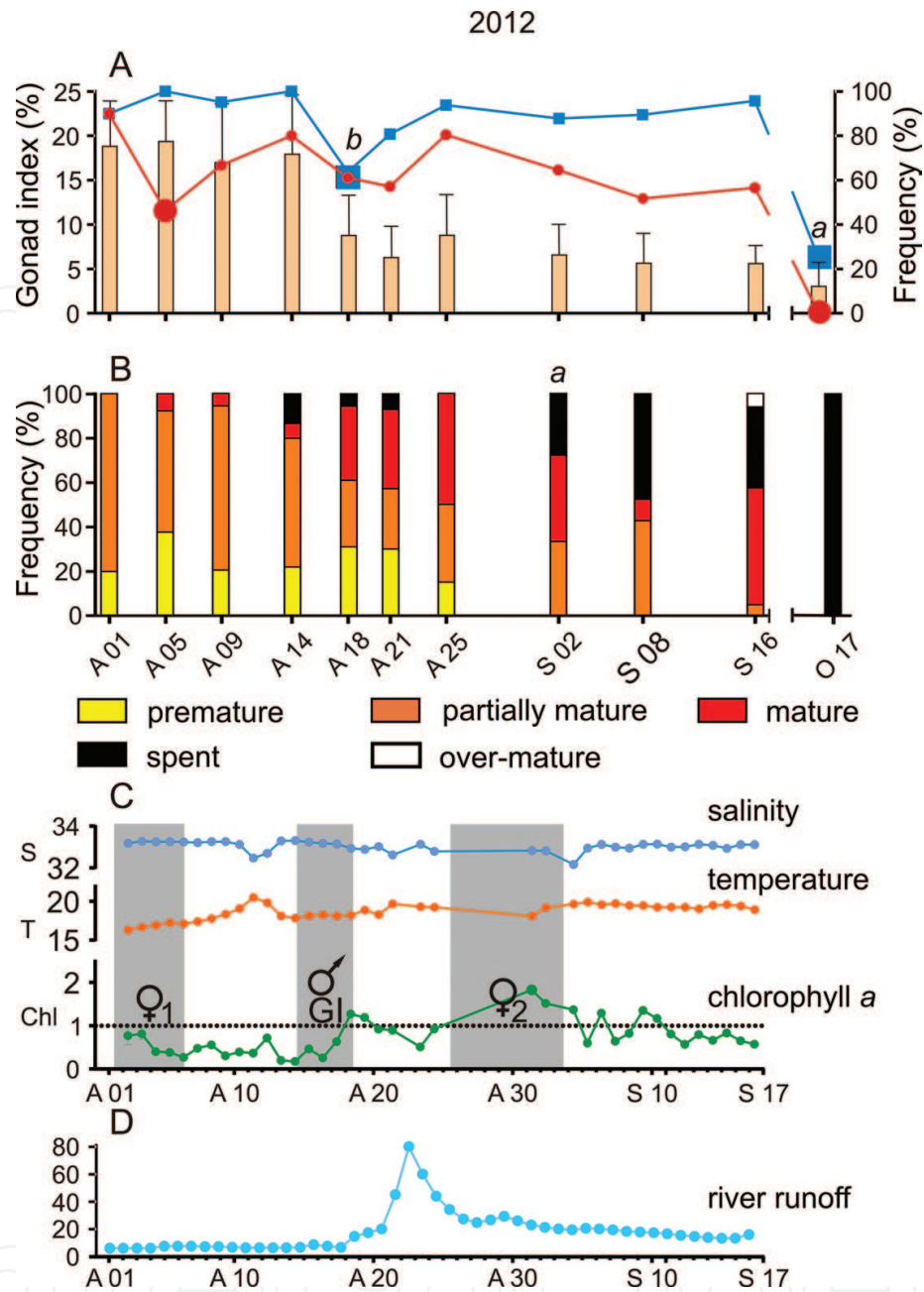
Figure 11. The timing of the spawning of the sea urchin *Strongylocentrotus intermedius* and the associated environmental conditions in August–September of 2012 at site 1 in Kievka Bay. (A) Temporal dynamics of the gonad index (columns: mean ± SD) and the percentages of the males (lines with squares) and females (lines with circles) ready for spawning. Letters above the columns denote significant mean difference compared to previous month (*a*: $p < 0.05$, *b*: $p < 0.01$, one-way ANOVA, Kruskal-Wallis statistics, Dunn's multiple comparison test). Large squares and circles indicate significant differences compared to previous month ($p < 0.05$, binomial statistics). (B) Temporal dynamics of the percentages of the ovarian maturity stages in *S. intermedius* females. Letter "*a*" above the column denotes significant increase in the portion of spent females ($p < 0.05$, binomial statistics). (C) Spawning events and the dynamics of environmental variables: Chl—chlorophyll *a* (mg m^{-3}), S—salinity (psu), T—temperature (°C). Horizontal dashed line corresponds to the Chl *a* concentration of 1 mg m^{-3}. 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; ♀$_2$: significant increase in the portion of spent females. (D) Daily runoff of the Lazovka River, the main tributary of the Kievka River that flows into Kievka Bay (m^3 s^{-1}). *x*-axis: month and date. The data shown are extracted from **Figures 4, 6,** and **8** in Ref. [58].

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). Different combinations of environmental variables 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 using free and open-source R software [70].

No apparent relationship was revealed between temperature or salinity and the timing of spawning in *S. intermedius* populations. At the same time, our statistical analysis showed a significant positive relationship between Chl *a* concentration and spawning events in *S. intermedius* 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 [13, 15–17, 71]. Based on the results of the laboratory experiments, 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, phytoplankton alone did not induce spawning in experiments with the sea urchin *Lytechinus variegatus*, though the introduction of phytoplankton 75 min before sperm greatly accelerated the initiation of male spawning followed by female spawning [21]. In the field experiments, however, no consistent reaction of *L. variegatus* to sperm was observed [72]. The phenomenon of spawning failure repeatedly registered in *S. intermedius* populations from Kievka Bay during four reproductive seasons between 2008 and 2015 (**Figure 6**) indicates that *S. intermedius* males with mature gonads are not able to induce spawning in the absence of external stimuli. From a fine-scale temporal analysis of the dynamics of sea urchin gonadal development and environmental parameters, we can draw a conclusion that phytoplankton concentration is the main factor driving the initiation of *S. intermedius* spawning [58].

Our study showed that an increase in phytoplankton concentration in the bottom water layer of Kievka Bay, as judged from measured Chl *a*, occurred under different temperature conditions, that is, increasing, decreasing, or almost constant temperatures [58]. 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. The coincidence of increasing phytoplankton concentration and a stable warm or rising temperature, it seems, 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 [58]. 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 [73]. We suggest a mechanism, involving a rapid response of *S. intermedius* parental individuals to increasing phytoplankton density, which 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 next interesting finding of our study is a coincidence between the majority of *S. intermedius* spawning events and new and full moons [58]. This is in consistence with field observations

of spawning events in a number of echinoids, both tropical (two species of the genus *Diadema* and two species of the genus *Echinothrix* [74]) and temperate (*S. droebachiensis* [16]), as well as with laboratory experiments on *L. variegatus* [21] which provide evidence of lunar periodicity in some species. However, the differently pronounced influence of the moon cycle on the spawning of *S. intermedius* in different years, as judged from different levels of both spawning success and synchronization between spawning events and lunar phases, forced us to suggest that this factor seems 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* [21].

5. Conclusion

In the northwestern Sea of Japan along 400 km of the coast of the Primorye region of Russia, three types of populations of the sea urchin *S. intermedius* were found that differ from each other in the proportions of individuals with early spawning (the end of May–June) and late spawning (September–early October). All *S. intermedius* populations studied live at the same latitude and experienced the same photoperiodic conditions, but the timing and implementation of spawning are different both between and within localities. The individuals with early spawning breed in spring and spawn in late spring–early summer under conditions of rising photoperiod (the duration of daytime) and temperature. However, the temperature in this period in the study area is much lower as compared to the warmest summer season when gonad maturation in the individuals with late spawning takes place. These individuals spawn under conditions of decreasing photoperiod and relatively stable warm temperature. Sharp temperature fluctuations in the study area caused by upwelling/downwelling do not drive *S. intermedius* spawning. Moreover, we found that, in some years, sea urchins from several localities failed to end their reproductive cycle with complete spawning despite similar temperature and photoperiod changes. Hence, neither photoperiod nor temperature may be considered as the primary external factors that determine *S. intermedius* temporal patterns of gonad maturation and the timing of spawning. We also found no apparent relationships between salinity, dissolved oxygen or tidal activity, and the spawning events.

Our analysis showed that since 1970–1980s, the shift in spawning season from autumn to early summer occurred in *S. intermedius* populations inhabiting anthropogenically polluted areas of Peter the Great Bay whereas sea urchins inhabiting relatively clean areas retained an autumn pattern of spawning. Based on these observations and on the results of the studies of population genetic structure of several *S. intermedius* populations with different spawning schedule which revealed no significant differences between the individuals with early and late spawning, we hypothesized that the shift of spawning seasonality from autumn to early summer would be a phenotypic switch related to the changes in environmental conditions caused by chronic anthropogenic pollution. Considering that (1) there is statistically significant relationship between *S. intermedius* spawning events and Chl *a* (phytoplankton) concentration; (2) spawning failure occurs in *S. intermedius* populations under conditions

of low phytoplankton concentration throughout summer–autumn season; and (3) human activity is associated with the eutrophication of the seawater and phytoplankton increase, we propose that phytoplankton may be the main environmental factor driving reproductive cycle and spawning in *S. intermedius* populations. We hypothesize that permanent increased levels of phytoplankton and its metabolites in the eutrophic seawater would be environmental cues that are used by the sea urchins for assessing the appropriateness of environmental conditions for reproductive success in terms of benefits to offspring survival. We believe that *S. intermedius* reproductive cycle in the area not subjected to anthropogenic impact (Kievka Bay) may be considered as a "baseline" (reference) reproductive cycle for this species in the northwestern Sea of Japan. Analysis of the temporal patterns of *S. intermedius* gonadal maturation in Kievka Bay showed that a vast majority of individuals become mature by the end of July; however, approximately 5% of mature females and more than 50% of mature males were found in June [59]. Moreover, in June 2009, approximately 5% of spent individuals of both sexes were found. This indicates that *S. intermedius* possesses high reproductive plasticity which promotes the possibility of both early and late spawning depending on the local properties of the environment. Considering mentioned above, 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.

Acknowledgements

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