Quantitative study of the behavior of two broadcast spawners, the sea urchins *Strongylocentrotus intermedius* and *Mesocentrotus nudus*, during mass spawning events *in situ*

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Background. The spatial distribution of spawners and temporal parameters of spawning in motile invertebrates with external fertilization might influence reproductive success. However, to date, data on the prespawning and spawning behaviors of broadcast spawners in the field have been scarce and mostly qualitative. The present study was intended to clarify the behavioral adaptations of two sea urchin species, *Strongylocentrotus intermedius* and *Mesocentrotus nudus*, using quantitative analysis of their behavior during mass spawning events under natural conditions.

Methods: We analyzed *in situ* video recordings of sea urchin behavior obtained during 6 spawning seasons (2014–2019). The total number of specimens of each sea urchin species and the numbers of spawning males and females were counted. Quantitative parameters of sea urchin spawning (numbers of gamete batches, release duration of one gamete batch, time intervals between gamete batches and total duration of spawning) and movement (step length of spawners and nonspawners before and during spawning and changes in distances between males/nonspawners and females) were determined.

Results: For each species, 12 mass spawning events were recorded in which 10 or more individuals participated. The temporal dynamics of the numbers of males and females participating in mass spawning were well synchronized in both species; however, males began to spawn earlier and ended their spawning later than females. In both species, the most significant intersex difference was the longer spawning duration in males due to the longer pause between gamete batches. The total duration of gamete release did not differ significantly between sexes. The average duration of sperm release during mass spawning events was longer than solitary male spawning. Males and females showed significant increases in the locomotion rate 35 min before the start of spawning and continued to actively move during spawning. An increase in movement rate before spawning in males and females was induced by environmental factor(s). Nonspawners of both species showed increased locomotion activity but in the presence of spawning neighbors and less prominently than spawners. On a vertical surface, both echinoids moved strictly upward. On flat surfaces, males, females and nonspawners of both echinoids became closer during spawning.

Discussion: We showed that two sea urchin species with planktotrophic larvae display similar behavioral adaptations aimed at enhancing reproductive success. The high sensitivity of sea urchins, primarily males, to some environmental factors, most likely phytoplankton, may be considered a large-scale adaptation promoting the development of mass spawning events. The longer spawning duration in males and increased movement activity before and during spawning in both sexes may be considered small-

scale adaptations promoting approach of males and females and enhancing the chances of egg fertilization.

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4

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30 ## ABSTRACT

31 **Background.** The spatial distribution of spawners and temporal parameters of spawning in

32 motile invertebrates with external fertilization might influence reproductive success. However, to

33 date, data on the prespawning and spawning behaviors of broadcast spawners in the field have

34 been scarce and mostly qualitative. The present study was intended to clarify the behavioral

35 adaptations of two sea urchin species, *Strongylocentrotus intermedius* and *Mesocentrotus nudus*,

36 using quantitative analysis of their behavior during mass spawning events under natural

37 conditions.

38 **Methods:** We analyzed *in situ* video recordings of sea urchin behavior obtained during 6

39 spawning seasons (2014–2019). The total number of specimens of each sea urchin species and

40 the numbers of spawning males and females were counted. Quantitative parameters of sea urchin

41 spawning (numbers of gamete batches, release duration of one gamete batch, time intervals

42 between gamete batches and total duration of spawning) and movement (step length of spawners

43 and nonspawners before and during spawning and changes in distances between

44 males/nonspawners and females) were determined.

45 **Results:** For each species, 12 mass spawning events were recorded in which 10 or more

46 individuals participated. The temporal dynamics of the numbers of males and females

47 participating in mass spawning were well synchronized in both species; however, males began to

48   spawn earlier and ended their spawning later than females. In both species, the most significant

49   intersex difference was the longer spawning duration in males due to the longer pause between

50   gamete batches. The total duration of gamete release did not differ significantly between sexes.

51   The average duration of sperm release during mass spawning events was longer than solitary

52   male spawning. Males and females showed significant increases in the locomotion rate 35 min

53   before the start of spawning and continued to actively move during spawning. An increase in

54   movement rate before spawning in males and females was induced by environmental factor(s).

55   Nonspawners of both species showed increased locomotion activity but in the presence of

56   spawning neighbors and less prominently than spawners. On a vertical surface, both echinoids

57   moved strictly upward. On flat surfaces, males, females and nonspawners of both echinoids

58   became closer during spawning.

59   **Discussion:** We showed that two sea urchin species with planktotrophic larvae display similar

60   behavioral adaptations aimed at enhancing reproductive success. The high sensitivity of sea

61   urchins, primarily males, to some environmental factors, most likely phytoplankton, may be

62   considered a large-scale adaptation promoting the development of mass spawning events. The

63   longer spawning duration in males and increased movement activity before and during spawning

64   in both sexes may be considered small-scale adaptations promoting approach of males and

65   females and enhancing the chances of egg fertilization.

66

67   ## INTRODUCTION

68   During the course of evolution, marine invertebrates with external fertilization (broadcast

69   spawners) and feeding (planktotrophic) larvae have developed a number of reproductive

70   adaptations ensuring successful reproduction and thereby maintaining the continuity of species.

71   The broadcast spawning strategy involves the release of a large number of small eggs by females

72   and sperm by males during synchronous (often annual) spawning events (see *Olive, 1992*;

73   *Wangensteen, Turon & Palacín, 2017* for review). It is generally believed that the

74   synchronization of spawning of many individuals is provided by the ability of broadcast

75   spawners to perceive certain environmental cues to determine the conditions appropriate for

76   offspring development (see *Mercier & Hamel, 2009*; *Thorson, 1950* for review). For

77   echinoderms and sea urchins in particular, the external synchronizers are reported to be an

78   increased level of phytoplankton (*Bronstein et al., 2016*; *Egea et al., 2011*; *Gaudette, Wahle &*

79   *Himmelman, 2006*;*González-Irusta, De Cerio & Canteras,* 2010; *Himmelman, 1975*; *Starr,*
80   *Himmelman & Therriault, 1993*; *Zhadan et al., 2016*; *Zhadan, Vaschenko & Ryazanov, 2018*), an
81   increase or decrease in water temperature (*Byrne 1990; Egea et al., 2011*; *Guillou & Lumingas,*
82   *1998*; *Himmelman et al., 2008*; *King et al., 1994*; *Lamare & Stewart, 1998*; *Tsuji et al., 1989*),
83   photoperiod (*Byrne et al., 1998*), different phases of the moon cycle (*Coppard & Campbell,*
84   *2005*; *Gaudette, Wahle & Himmelman, 2006*; *Iliffe & Pearse, 1982*; *Lessios, 1991*; *Mercier &*
85   *Hamel, 2010*; *Zhadan et al., 2016*), time of day (*Zhadan, Vaschenko & Ryazanov, 2018*) and the
86   presence of released gametes in the environment (*Reuter & Levitan, 2010*; *Unger & Lott, 1994*).
87   Although sea urchin spawning may be influenced by multiple natural factors, phytoplankton
88   have been shown to be the most likely proximate cue for triggering spawning in some species
89   (*Gaudette, Wahle & Himmelman, 2006*; *Himmelman, 1975*; *Starr, Himmelman & Therriault,*
90   *1993*; *Zhadan, Vaschenko & Ryazanov, 2018*). Moreover, under conditions of low phytoplankton
91   abundance, spawning failure occurs in natural populations of the sea urchin *Strongylocentrotus*
92   *intermedius*, followed by prolonged resorption of the unspawned eggs and sperm(*Zhadan,*
93   *Vaschenko & Almyashova, 2015*; *Zhadan et al., 2016*). This phenomenon indicates that an
94   external stimulus is extremely important for the reproduction of some species and suggests a
95   high degree of impact of natural selection for the synchronization of spawning with
96   environmental factors.
97   In motile species, behaviors aimed at reducing nearest-neighbor distances or increasing
98   the likelihood of gamete survival and mixing may also favor fertilization success, especially at
99   low population densities (*Himmelman et al., 2008*; *Levitan, 2002*, *2005*; *McCarty & Young,*
100   *2004*). A classic example of spawning organisms being located in close proximity is the
101   pseudocopulation of ophiuroids and starfish (*Himmelman et al., 2008*; *Slattery & Bosch, 1993*;
102   *Tominaga, Nakamura & Komatsu, 2004*). The temporal parameters of pseudocopulation differ
103   among species. Males of dimorphic ophiuroids such as *Ophiodaphne formata*are constantly
104   attached to females (*Tominaga, Nakamura & Komatsu, 2004*). Males and females of the sand sea
105   star *Archaster typicus* form pairs two months before spawning, and males spawn only when
106   females release eggs (*Run et al., 1988*). In other sea stars and ophiuroids, approaching of males
107   and females occurs just before or during spawning. During echinoderm mass spawning events
108   off the Mingan Islands (the northern Gulf of St. Lawrence, Canada), many instances of
109   pseudocopulation of the ophiuroids *Ophiopholis aculeata* and *Ophiura robusta* and sea star

110   *Asterias vulgaris* as well as of approaching individuals have been observed (*Himmelman et al.,*
111   *2008*).

112   Climbing onto elevated surfaces before spawning is a typical behavior for many
113   echinoderms (see *Levitan, 1998*; *Mercier & Hamel, 2009* for review). In cases when both sexes
114   move upward, such displacement evidently promotes approach of males and females. Spawning
115   of starfish, ophiuroids and sea urchins *in situ* was observed on the tops of corals, stones, and
116   even algae (*Babcock et al., 1992*; *Gladstone, 1992*; *Hagman & Vize, 2003*; *Himmelman et al.,*
117   *2008*; *Johnson & Ranelletti, 2017*; *McEuen, 1988*; *Minchin, 1992*). The ophiuroids *O. aculeata,*
118   *O. robusta*, *Ophioderma rubicundum* and *Ophioderma squamosissimum* leave their shelters in
119   crevices before spawning and move to higher elevations (*Hagman & Vize, 2003*; *Himmelman et*
120   *al., 2008*). Highly accelerated upward movement on a vertical surface immediately before and
121   during spawning was also recorded in the sea urchin *Strongylocentrotus intermedius* (*Zhadan,*
122   *Vaschenko & Ryazanov, 2018*). Some starfish and ophiuroids take a characteristic pose before
123   spawning, raising the aboral disc above the bottom surface (*Gladstone, 1992*; *Hagman & Vize,*
124   *2003*; *Hendler & Meyer, 1982*; *Himmelman et al., 2008*). Many holothuroid species spawn with
125   their anterior ends lifted off the substratum (*Hendler & Meyer, 1982*; *McEuen, 1988*). The
126   behavior aimed at maximal elevation of the body in the water column seems to promote gamete
127   dispersion and mixing and to increase gamete survival, mitigating their falling to the bottom and
128   being wasted (*Hendler & Meyer, 1982*; *Himmelman et al., 2008*).

129   The above observations indicate that motile echinoderms are able to change their
130   behavior before spawning events. Moreover, some species, including sea urchins, display an
131   increase in locomotion rate before spawning (*Himmelman et al., 2008*; *Zhadan, Vaschenko &*
132   *Ryazanov, 2018*), prompting the suggestion that this may be the first response of mature
133   individuals to some exogenous and/or endogenous factors stimulating spawning. However, to
134   date, only a few laboratory and field studies have attempted to quantitatively assess sea urchin
135   movement in response to presumable spawning stimuli. During natural spawning events of the
136   sea urchin *Mesocentrotus (=Strongylocentrotus) franciscanus,* which coincided with
137   phytoplankton blooms, *Levitan (2002)* found no changes in the nearest-neighbor distances
138   between males and females or between nonspawners. In laboratory experiments, phytoplankton,
139   sperm and a combination of sperm and phytoplankton increased the rate of movement of
140   sexually mature *Lytechinus variegates* males and females up the wall of the experimental beaker,

141 whereas only sperm and sperm+phytoplankton treatments induced vertical movement of sexually

142 immature individuals (*Reuter & Levitan, 2010*). In the field experiments of *McCarthy & Young*

143 *(2004)*, sperm added to sea water did not influence the distance traveled per 1 h or the nearest-

144 neighbor distance in the sea urchin *L. variegates* with mature and immature gonads. Recent field

145 studies have shown that an increased phytoplankton concentration stimulates both prespawning

146 movement activity and spawning in males of the sea urchin *S. intermedius* (*Zhadan, Vaschenko*

147 *& Ryazanov, 2018*). These findings indicate that a detailed quantitative analysis of the

148 prespawning and spawning behaviors of sea urchins during mass spawning events is needed to

149 understand whether the changes in movement activity may contribute to the enhancement of

150 fertilization success. However, to date, there have been only rare and mostly descriptive

151 observations of sea urchins' mass spawning events under natural conditions.

152   The present study was intended to obtain quantitative data on the behavior of two

153 cohabiting sea urchin species, *Strongylocentrotus intermedius* (A. Agassiz, 1864) and

154 *Mesocentrotus nudus* (A. Agassiz, 1864) (= *Strongylocentrotus nudus*), during mass spawning

155 events and to determine whether this behavior might contribute to the reproductive success of the

156 studied species. To achieve this goal, we analyzed *in situ* video recording data of sea urchin

157 behavior obtained during 3 spawning seasons (2017–2019). To increase the dataset, together

158 with the data for the seasons 2017–2019, we additionally reanalyzed the data for the 2014–2016

159 seasons. We addressed the following: (1) how, and for how long before spawning, the behavior

160 in two sea urchin species changes; (2) whether the distances between males and females change

161 as a result of spawning behavior; (3) whether there is a link between spawned material and the

162 changes in the prespawning and spawning behaviors of sea urchins; and (4) whether the behavior

163 of nonspawning sea urchins changes during the spawning of conspecifics. We also compared the

164 temporal characteristics of solitary and mass spawning and the dynamics of the numbers of

165 spawners during mass spawning events in the two sea urchin species.

166
167 **MATERIALS AND METHODS**

168 **Study areas, sea urchins and video recordings**

169 The field studies were conducted in two bays in the northwestern Sea of Japan, Kievka Bay

170 (42.830°N, 133.691°E) and Alekseev Bay (42.981°N, 131.730°E). Six separate sets of

171 observations were performed from 2014–2019, two in Kievka Bay (August–September of 2014

172   and 2015) and four in Alekseev Bay (May–June of 2016, July– September of 2017, June–

173   September of 2018 and 2019). The objects of our studies were two sea urchin species, *S.*

174   *intermedius* and *M. nudus*, which are common inhabitants of coastal communities in the study

175   areas. The timing of the studies was chosen to coincide with the spawning seasons of these

176   echinoids. For *S. intermedius,* it was May–September in Alekseev Bay and July–September in

177   Kievka Bay (*Zhadan, Vaschenko & Almyashova, 2017*; *Zhadan, Vaschenko & Ryazanov, 2018*).

178   The spawning season of *M. nudus* in both bays was July–August (*P. Zhadan, 2020, pers. comm.;*

179   *this study*).

180   In Kievka Bay, the studies were performed at a depth of 6 m on relatively flat bedrock

181   surrounded by large stones. In Alekseev Bay, the studies were performed at a depth of 2 m on a

182   flat bottom covered with medium-sized gravel. To register the spawning behavior of sea urchins,

183   continuous time-lapse video recording was performed with TLC200 Pro (Brinno Incorporated,

184   Taipei City, Taiwan) video cameras, which were mounted on stanchions approximately 1 m

185   above the bottom. The videos were taken in 1-min intervals at a resolution of 1280 × 720 pixels.

186   During the night, the cameras' fields of view (approximately 1.0 × 1.5 m) were illuminated by

187   LED lamps (1 W) synchronized with the cameras by a flash LED indicator, with impulse

188   duration of 1 s.

189   To attract sea urchins to the cameras' fields of view, flat mesh containers filled with the

190   kelp *Saccharina japonica* (Laminariales, Phaeophyta), which is known to stimulate foraging

191   activity in *M. nudus* and *S. intermedius* (*Zhadan & Vaschenko, 2019*), were used. Each container

192   (hereafter the feeder) was composed of 2 steel frames with mesh stretched across them, 1.1 ×

193   0.75 m in size, and contained 30–40 kg of kelp. In both study areas, the density of each sea

194   urchin species was 1 to 2 ind. m^{-2} (*Zhadan, Vaschenko & Ryazanov, 2018*). In addition, 200–300

195   specimens of *S. intermedius* in Kievka Bay (2014, 2015) and 200–300 specimens of each species

196   in Alekseev Bay (2016–2019) were collected within a 100- to 200-m radius of the cameras and

197   carefully transplanted within an approximately 10-m radius. Sea urchins of both species

198   themselves found the feeders, and within approximately 2 days, 30–70 individuals of each

199   species populated the feeders. The feeders were replaced every 2–3 weeks when approximately

200   80% of laminaria was consumed by sea urchins. Sea urchins were carefully transferred from the

201   old feeders to the bottom, and then they found themselves the feeders with fresh laminaria and

202   populated them within several hours. It took approximately 5 min to change one feeder.

203   In both bays, four video cameras were used. In Kievka Bay, each camera was directed at
204   the feeder. The entire area of the feeder and an additional 5–10 cm around its edges were in the
205   cameras' fields of view. In Alekseev Bay, where two feeders were located close to the vertical
206   wall of the concrete pier (2016) or to a pyramid built of stones (2017–2019), two cameras were
207   directed at the feeders, and two others were directed at the pier wall or the surface of the stone
208   pyramid.
209

210   **Video recording and statistical analyses**
211   Just after the changes the feeders, isolated spawning males of *M. nudus* but not *S. intermedius*
212   were noted on them. Therefore, to exclude the possibility that our manipulations could affect the
213   results, video recordings taken for 8 h after the feeders were changed were not analyzed. During
214   spawning, the sexes of both sea urchin species are easily distinguishable due to different colors
215   of gametes: white in males and orange and light-yellow in females of *S. intermedius* and *M.*
216   *nudus*, respectively. The recorded videos were viewed frame by frame, and the total number of
217   specimens of each sea urchin species in the cameras' fields of view and the numbers of spawning
218   males and females were counted. In cases when at least 10 individuals took part in spawning for
219   at least 1 h, we used the term "mass spawning".
220   By means of the free software "Tracker" for video analysis (www. open
221   sourcephysics.org/items/detail.cfm?ID=7365) we traced the spawning males and females with an
222   interval of 1 min. The distance between two successive positions of the sea urchin (1 min apart)
223   was defined as the step length (*Lauzon-Guay, Scheibling & Barbeau, 2006*; *Zhadan &*
224   *Vaschenko, 2019*). The cell size (2 × 2 cm) of the feeder mesh was used as a scale. For males and
225   females participating in mass spawning events, the tracing was started 60 min before the
226   beginning of spawning of the first individual and finished when the last individual ceased to
227   spawn (see Table S1 for raw data). Each track lasted approximately 180 min. For nonspawning
228   sea urchins that were present on the feeders during mass spawning events, the duration of tracing
229   was the same as that for spawners. In cases when sea urchins spawned alone or the spawners'
230   numbers were less than 10, the tracing began 60 min before the start of spawning of each
231   spawner and lasted approximately 180 min.
232   Only full sea urchin tracks were included in the statistical analysis (see Table S1 for raw
233   data). Exceptions included the tracks of sea urchins that spawned on the vertical surfaces of the

234   pier wall or stone pyramid. In these cases, sea urchins before and during spawning moved strictly

235   upward with a high speed and left the camera's field of view before it was safe to conclude that

236   they had finished spawning.

237   The beginning and end of the release of each gamete batch and duration of the intervals

238   between the gamete batches were determined with an accuracy of 1 min. Total spawning

239   duration was determined as a sum of total duration of gamete release and total duration of the

240   intervals between the gamete batches (see Table S1 for raw data). In the cases when spawning of

241   the specimen was recorded on only one frame, the individual spawning duration was considered

242   to be 1 min. To analyze the sex-specific, species-specific and habitat-related differences in

243   quantitative parameters of spawning (the release duration of one gamete batch, total duration of

244   gamete release, time intervals between gamete batches, total duration of the intervals between

245   the gamete batches and total duration of spawning) and to compare the time to reach the

246   maximum numbers of spawning males and females participating in mass spawning events, the

247   corresponding datasets were created and tested for adherence to a normal distribution

248   (D'Agostino and Pearson omnibus normality test, $P < 0.05$). Since most of the datasets were not

249   normally distributed, the nonparametric Mann–Whitney test, Wilcoxon matched-pairs signed-

250   rank test and Spearman's rank-order correlation were used. The same procedure was performed

251   to compare temporal parameters of solitary and mass spawning in males of both sea urchin

252   species.

253   To determine the moment of change (inflection point) in the locomotion rate of sea

254   urchins before spawning, the 180-min time series of step lengths of spawning individuals were

255   combined into 4 datasets, separately for each species and each sex, and aligned so that the

256   beginnings of spawning of all individuals coincided. Within each dataset, the 60-min interval

257   preceding the start of spawning was segmented by 11 possible inflection points (5 min apart).

258   Each such point segmented the time-series dataset into two samples, one of which was assumed

259   as the expected period before the beginning of change in sea urchin locomotion rate ("before

260   start") and the other as the expected period after the beginning of locomotion change ("after

261   start"). Thus, 11 datasets were obtained, which were then compared using a nonparametric two-

262   sample permutation test (*Efron & Tibshirani, 1993*). The test statistic Θ was estimated as the

263   absolute difference between the medians, i.e., $\Theta = |Me_{before\ start} - Me_{after\ start}|$. The accumulated

264   significance level (*ASL*, analogous to "*P*" of parametric tests) was determined as the proportion

265   of cases when the absolute difference between two medians after data permutation (Θ_{Perm}) was

266   higher than our observed result (Θ_{Exp}). The first inflection point with $ASL \leq 0.01$ was determined

267   as a moment of statistically significant change in the locomotion rate of the sea urchins. The time

268   interval preceding this point was defined as the "control interval", and the following interval was

269   defined as the "prespawning interval", which ended with the onset of spawning. The 35-min

270   interval after the beginning of spawning was defined as the "spawning interval".

271   To analyze the sex-specific and species-specific differences in quantitative parameters of

272   movement as well as to determine whether the locomotion rate in nonspawners changes during

273   mass spawning events, the datasets on the step length of spawners and nonspawners of each sea

274   urchin species measured during the control, prespawning and spawning intervals were pooled

275   into corresponding datasets, checked for adherence to a normal distribution and tested with the

276   Kruskal–Wallis test followed by Dunn's multiple comparison test.

277   To determine whether sperm released by the males that began to spawn first during mass

278   spawning events (hereafter "leading males") influenced the locomotion rate of males that began

279   to spawn 40 min later when there were already approximately 50% spawning males (hereafter

280   "outsider males"), the step lengths of these two groups of males measured during the

281   prespawning period were compared. The datasets on step lengths for leading males were

282   supplemented with data on step lengths of males that spawned first in the cases that were not

283   referred to as mass spawning events. After checking for adherence to a normal distribution, the

284   data on step lengths of leading and outsider males of each sea urchin species were compared by

285   the Mann–Whitney test.

286   To determine whether spawning males, females and nonspawning individuals come

287   closer together during mass spawning, 2 analyses were performed. First, the distances from

288   males, females and nonspawners to their common center of mass were determined. When

289   calculating the center of the sea urchin mass, the conditional mass of males or females was taken

290   as a multiple of the time of gamete release, and the conditional mass of nonspawners was taken

291   as a value of 1. Second, the distances from each of the males and nonspawners to the center of

292   mass of females were measured. Then, the differences between the distances measured 1 min

293   apart during the 25-min control interval and during the spawning period, from the beginning of

294   spawning to its completion in 95% of males, were determined, and the medians of these

295   differences for the control and spawning periods were compared using the Mann–Whitney test.

296   For graphical representation, the values of the displacement of each individual relative to its

297   location at the beginning of the control interval were calculated 1 min apart. As these values

298   were normally distributed, they are presented as the mean and 95% confidence interval.

299

300   **RESULTS**

301   **General characteristics of sea urchin spawning**

302   In Kievka Bay in 2014–2015, spawning was recorded in 86 males and 6 females of *S.*

303   *intermedius* and in 21 males of *M. nudus*. In Alekseev Bay in 2016–2019, spawning was

304   recorded in 510 males and 19 females of *S. intermedius* and in 824 males and 39 females of *M.*

305   *nudus*. Spawning occurred both in the presence and absence of spawning females. In the absence

306   of females, the maximum numbers of *S. intermedius* and *M. nudus* spawning males were 11 and

307   20, respectively. For the entire duration of the observations, only a few females that spawned in

308   the absence of males were recorded: 2 females of *S. intermedius*, which spawned on different

309   days, and 2 females of *M. nudus*, which began to spawn with an interval of 4 min. The ratios of

310   females to males for all spawning individuals were 1:24 for *S. intermedius* and 1:22 for *M.*

311   *nudus*.

312   Calculations made from the total sea urchin number in the camera field of view, number

313   of spawners and the female/male ratio show that males seem to be able to spawn several times

314   during the spawning season. This is clearly demonstrated by a video recording obtained by one

315   of the cameras, which in 2018 (June 11–17) recorded 3 mass spawning events with the

316   participation of 41 males of *M. nudus*. Another 27 males spawned during the intervals between

317   mass spawning events. Taking into account that the total sea urchin number in the camera field

318   of view during this week decreased from 41 to 34 individuals and that the female/male ratio was

319   1:1, one may calculate that each of the males spawned approximately 3 times during this period.

320   The total duration of sperm release during this week decreased by 3 times, but this change was

321   not significant (Mann–Whitney test, $U = 15$, $P = 0.08$).

322   For each species, 12 mass spawning events were recorded, in which 10 and more

323   individuals participated (Table 1). The percentage of spawning individuals ranged from 18% to

324   78% of the total number of sea urchins in the cameras' fields of view. A total of 142 males and

325   15 females of *S. intermedius* and 183 males and 34 females of *M. nudus* participated in mass

326   spawning. Thus, the numbers of *S. intermedius* and *M. nudus* males participating in mass

327   spawning were 4 and 5 times lower and the numbers of females were 2.1 and 1.7 times lower,

328   respectively, than the corresponding total numbers of spawning individuals. Both sexes

329   participated in 8 of 12 spawning events for *S. intermedius* and in 10 of 12 spawning events for

330   *M. nudus*. The ratios of females to males were 1:9 and 1:5 for *S. intermedius* and *M. nudus*,

331   respectively.

332   Spawning occurred at dusk or at night in 19 of 24 mass spawning events recorded for

333   both species (see Table S1). Twelve of 24 mass spawning events took place within the range

334   from 0 to 4 days near the new moon, 2 events were recorded the day before the full moon, 3

335   events occurred when the moon was in waxing phase (first quarter), and 7 events were observed

336   when the moon was in waning phase (third quarter) (see Table S1).

337   Despite the spawning seasons of *S. intermedius* and *M. nudus* overlap in the areas studied

338   and the simultaneous spawning of several individuals of both sea urchins was sometimes

339   observed, no one case of synchronous mass spawning with participation of 10 and more

340   individuals of each species was recorded. However, on July 13, 2018, a case of simultaneous

341   spawning of 29 individuals (23 males and 6 females) of *M. nudus* and 5 individuals (4 males and

342   1 female, i.e., < 10 individuals) of *S. intermedius* was recorded in Alekseev Bay (see Sheet 18 in

343   Table S1). One can see that spawning of both sea urchin species is synchronous.

344

345   **Temporal parameters of spawning**

346   Males and females of both sea urchin species released gametes in batches. The number of

347   batches in males varied from 1 to 10 in *S. intermedius* (median = 3) and from 1 to 9 in *M. nudus*

348   (median = 2) and was significantly higher in *S. intermedius* (Mann–Whitney test, $U = 4534$, $P =$

349   0.0183). The numbers of batches in females of the two species did not differ significantly (range

350   from 1 to 6, median = 2 for *S. intermedius* and range from 1 to 6, median = 1 for *M. nudus*,

351   Mann–Whitney test, $U = 138.5$, $P = 0.205$). The release duration of one gamete batch (the time

352   in which a gamete clot was present in the area near the gonopores), time intervals between

353   gamete batches and total duration of spawning ranged widely (Table 2). Analysis of intersex

354   differences showed that in both sea urchin species, the spawning duration of males was longer

355   than that of females ($P = 0.039$ and $P = 0.009$ for *S. intermedius* and *M. nudus*, respectively)

356   because although the total duration of gamete release did not differ significantly between sexes

357   ($P = 0.66$ and $P = 0.58$), the total duration of intervals between gamete batches was significantly

358   higher in males ($P = 0.001$ and $P < 0.0001$) (Table 2). Analysis of interspecific differences

359   showed that in males, the release duration of one gamete batch, total duration of gamete release,

360   total duration of intervals between gamete batches and total duration of spawning were

361   significantly higher in *S. intermedius* than in *M. nudus* (Table 2). In females of the two sea

362   urchin species, there were no significant differences in any of the spawning parameters studied.

363   Comparison of spawning parameters in *S. intermedius* sea urchins living in different bays

364   showed that intersex differences were slightly more pronounced in sea urchins from Alekseev

365   Bay (Table 3). In *S. intermedius* males from Alekseev Bay, all temporal parameters of spawning

366   were significantly higher than those in Kievka Bay (Table 3). In *S. intermedius* females from

367   different bays, there were no significant differences in any of the spawning parameters.

368   A comparison of the temporal parameters of solitary and mass spawning in males of *S.*

369   *intermedius* and *M. nudus* from Alekseev Bay showed that in both species, the duration of

370   release of one gamete batch and the total duration of gamete release were significantly higher

371   during mass spawning events (Table 4). In *S. intermedius* males, the total duration of intervals

372   between gamete batches and total spawning duration were also higher during mass spawning.

373

374   **Dynamics of mass spawning**

375   The spawning dynamics of males and females of both sea urchin species were generally similar:

376   the number of spawners gradually increased, reaching a maximum, and then decreased to zero

377   (Figs. 1A, 1B). The spawning males demonstrated similar dynamics both in the presence and

378   absence of females (Figs. 1C, 1D). Figure 2 shows the dynamics of the total numbers of males

379   and females of *S. intermedius* (Fig. 2A) and *M. nudus* (Fig. 2B) for all events of mass spawning.

380   In most cases, the spawning duration of both sea urchin species was approximately 100 min

381   (Figs. 2A, 2B). However, in 2 of 12 mass spawning events of *S. intermedius*, a second spawning

382   wave took place, in which approximately 55% and 80% of females and males of the first

383   spawning wave participated, respectively (Fig. 2A).

384   In most mass spawning events of *S. intermedius*, the males started to spawn first (Fig.

385   2A). In 2 of 12 mass spawning events, *S. intermedius* females began to spawn first, but one of

386   these cases was doubtful because when the female shifted 4 min after the start of spawning, the

387   spawning male was found underneath. *M. nudus* males began to spawn first in all 12 mass

388   spawning events (Fig. 2B). In all cases, males of both species finished to spawn after females

389   (with exception of one case when the female finished to spawn simultaneously with males, see

390   Sheet 24 in Table S1).

391   On average, *S. intermedius* and *M. nudus* females began to spawn 26.5 (–4–43) min and

392   24 (1–67) min (the data are presented as median and range) after the start of the first male

393   spawning, respectively, when a great number of males had already spawned, and finished to

394   spawn 12.0 (1–214) and 15.5 (0–70) min before the last male spawning (Figs. 1A, 1B, 2A, 2B).

395   Although the females began to spawn later than the males, the times to reach the maximum

396   numbers of spawning males and females did not differ significantly (Wilcoxon matched-pairs

397   signed-rank test: $n = 8$, $W = –18$, $P = 0.16$ for *S. intermedius* and $n = 10$, $W = –2$, $P = 0.95$ for *M.*

398   *nudus*). Spearman's correlation analysis revealed significant positive relationships between the

399   numbers of simultaneously spawning males and females for both species (Spearman's $r = 0.722$

400   and 0.845 for *S. intermedius* and *M. nudus*, respectively, $P<0.0001$).

401

402   **Movement activity of sea urchins**

403   Our data show that sea urchins of both species increased their movement activity shortly before

404   and during spawning. A significant increase in step length in both sexes of both sea urchin

405   species occurred from 30 to 35 min before the start of spawning (identified by the intersection of

406   the threshold value of $ASL = 0.01$) (Figs. 3A – 3D).

407   During the prespawning interval, the average step length in males and females of both

408   species was significantly higher than that in the control (Table 5). During the spawning interval,

409   both sexes of *M. nudus* and males of *S. intermedius* accelerated even more, while *S. intermedius*

410   females slowed down (Table 5). As the end of mass spawning approached, the movement rate in

411   both sexes of both species gradually decreased (Figs. 3A – 3D).

412   Leading males that began to spawn first during mass spawning events showed a

413   significant increase in the average step length in both species during the prespawning period

414   compared with the control, followed by further acceleration during the spawning interval (Fig. 4;

415   Table 5). This finding indicates that accelerated locomotion of sea urchins before spawning

416   resulted from external cues.

417   Comparison of the time series of step length of two groups of spawning males, leading

418   males and outsider males, during the prespawning period showed no significant differences

419   (Table 6). This result indicated that sperm of leading males did not influence the locomotion rate
420   of outsider males during the prespawning period.

421   The number of females that spawned in the absence of other spawners was too small for
422   statistical analysis (two *S. intermedius* females and two *M. nudus* females). However, it should
423   be noted that in the only *S. intermedius* female, which undoubtedly began to spawn first during
424   mass spawning, the average step length during the prespawning period was 3.9 times longer than
425   that during the control period, and the average prespawning step length in two *M. nudus* females,
426   which spawned in the absence of spawning males, was 2.1 times longer.

427   Analysis of the temporal dynamics of step length in nonspawning individuals showed that
428   in both species, there was no significant difference in locomotion rate between the control and
429   prespawning intervals, while a small but significant increase in this parameter occurred during
430   the spawning interval (Fig. 5; Table 5).

431   Despite the absence of complete synchronization of the changes in locomotion rate, a
432   significant positive correlation between the time series of step length of males and nonspawning
433   individuals was found in 8 out of 12 mass spawning events for *S. intermedius* and in all 12 mass
434   spawning events for *M. nudus* (ranges of Spearman's r from 0.55 to 0.11 and P from < 0.0001 to
435   0.31 for *S. intermedius*, Spearman's r from 0.58 to 0.27 and P from < 0.0001 to 0.004 for *M.*
436   *nudus*).

437

438   **Spatial distribution of sea urchin males and females during spawning**
439   Measurement of the distances from males, females and nonspawners to their common center of
440   mass revealed significant approach of sea urchins of both species during the spawning period
441   (Fig. 6; Table 7). Measurement of the distances from males and nonspawners to the center of
442   mass of females revealed significant approach during spawning in both sea urchin species (Fig.
443   7; Table 7). Fig. 7 demonstrates the dynamics of the mean difference between the initial and
444   measured 1-min interval distances from males and nonspawners to females. Since only one
445   female took part in 5 of 8 mass spawning events of *S. intermedius* with the participation of both
446   sexes, and in one case, two females were close to each other, the data in Fig. 7A mainly reflect a
447   decrease in the distance from males and nonspawners to females. Since a larger number of
448   females participated in the mass spawning events of *M. nudus* (Table 1), the data in Fig. 7B
449   reflect a decrease in the distance from males and nonspawners to the area where females moved

450   during spawning. For both sea urchin species, the displacement of males towards females was

451   approximately two times higher than that of nonspawning individuals.

452   It should be noted that there was variability between mass spawning events in the median

453   distances of males and nonspawners to the center of mass of females: the distances could

454   decrease (in most cases), increase or remain almost unchanged (see Table S1 for raw data).

455   The spatial distribution of sea urchins during spawning on the vertical surface (pier wall)

456   or inclined surface of stone pyramids was significantly different from that on flat food substrates.

457   Males and females of both species moved strictly upward both before and during spawning.

458   Spawning males, reaching the top of a large stone, continued to actively move along its surface

459   in the absence of females. In cases when there was a spawning female nearby, the males slowed

460   down or stopped. In contrast, females usually stopped active movement during spawning after

461   reaching the top of the stone. *S. intermedius* females also displayed similar behaviors on food

462   substrates when they were on top of other individuals. In *M. nudus*, the formation of such groups

463   was not recorded in any of the mass spawning events.

464

465   **DISCUSSION**

466   Despite the high density of sea urchins on food substrates, only 24 mass spawning events (each

467   of 10 and more spawners) were recorded for both species over 6 spawning seasons (2014–2019),

468   in which approximately 4 times fewer sea urchins were involved compared with the total number

469   of spawners. This finding indicates that in *S. intermedius* and *M. nudus,* mass spawning is a

470   relatively rare phenomenon that apparently occurs due to the complex interaction of both

471   intrinsic (i.e., gonad maturity) and extrinsic (some environmental cue(s), primarily

472   phytoplankton), factors. The application of continuous around-the-clock time-lapse video

473   recordings and feeders with kelp attractive to sea urchins allowed us to perform a high-temporal-

474   resolution (at 1-min intervals) quantitative analysis of the behaviors of *S. intermedius* and *M.*

475   *nudus* before and during mass spawning events under natural conditions. To the best of our

476   knowledge, this is the first such analysis to examine the representatives of mobile broadcast

477   invertebrates with planktotrophic larvae. The high temporal resolution of the video recording

478   method provides an opportunity for the mutual interpretation of results from laboratory and field

479   studies, and long-term around-the-clock observations allowed us to replenish the data on the

480   behavior of broadcast invertebrates during mass spawning obtained *in situ* by diving.

481

482 **Temporal characteristics and quantitative dynamics of mass spawning are similar in _S._**

483 **_intermedius_ and _M. nudus_**

484 Males and females of both sea urchin species exhibited an intermittent ("pulse") pattern of

485 spawning, as shown earlier for _S. intermedius_ (_Zhadan, Vaschenko & Ryazanov, 2018_). The most

486 significant difference in temporal parameters of spawning was the longer duration of male

487 spawning in terms of both intersex comparison (in both species, males spawned longer than

488 females) and interspecies comparison (_S. intermedius_ males spawned longer than _M. nudus_

489 males). This result is consistent with the conclusion of _Lotterhos & Levitan (2010)_ based on an

490 analysis of data reported in studies on spawning duration in males and females of 13 taxonomic

491 groups of broadcast invertebrates, including echinoderms. However, in this review, the spawning

492 process of 3 sea urchin species (_Strongylocentrotus franciscanus_, _S. droebachiensis_ and

493 _Diadema antillarum_) was characterized as continuous ("plume") based on occasional diving

494 observations. In our studies, along with the intermittent ("pulse") pattern of spawning, we

495 showed that in _S. intermedius_ and _M. nudus_, the longer spawning duration of males was due to

496 the longer pause between sperm batches, while there were no sex differences in the total duration

497 of gamete release.

498   Taking into consideration that the duration of gamete release in our study was assessed by

499 the presence of gamete clot on the aboral surface of sea urchin test, a question may arise whether

500 different spawning duration of males and females may be due to different physical properties of

501 sperm and eggs and the influence of water advection on gametes' dispersion. To date, only a few

502 studies addressed these issues in sea urchins (_Thomas, 1994_; _Yund and Meidel, 2003_; see also

503 _Crimaldi and Zimmer, 2014_ for review). Based on the comparison of the retention times of eggs

504 and sperm on the tests of three morphologically different sea urchin species (_Tripneustes gratilla_,

505 _Echinometra mathaei_ and _Colobocentrotus atratus_) under different water velocity (_Thomas,_

506 _1994_) and our results on temporal parameters of _S. intermedius_ and _M. nudus_ spawning, we may

507 suggest that the earlier cessation of spawning in females of _S. intermedius_ and _M. nudus_

508 compared to males during mass spawning events is due to longer spawning duration of males but

509 not different physical properties of female and male gametes.

510   In both _S. intermedius_ and _M. nudus_, the temporal dynamics of the numbers of males and

511 females participating in mass spawning were well synchronized, so that the maximum numbers

512 of simultaneously spawning sexes coincided. Currently, to the best of our knowledge, there is

513 surprisingly little information on the temporal patterns of spawning *in situ* both for sea urchins

514 and other groups of broadcast spawners. In terms of quantification of the dynamics of mass

515 spawning, the studies of *in situ* spawning of the sea cucumber *Cucumaria frondosa*, the

516 echinoderm species with long-lived planktonic lecitothrophic larva, seem to be the most

517 comprehensive (*Hamel & Mercier, 1995, 1996*). Mass spawning event started from the spawning

518 of isolated males. The number of males reached maximum (83%) after 10 h, whereas maximum

519 number (87%) of females was recorded after 12 h when the males' number was less than 32%.

520 Thus, the maxima of spawning activity in males and females of *C. frondosa* did not coincide.

521 However, this delay between the peaks of male and female spawnings could have a benefit for

522 fertilization success because it allows to attain a maximum concentration of sperm in the water

523 column prior to female spawning (*Hamel & Mercier, 1996*). We believe that the differences in

524 the temporal dynamics of the numbers of spawners of *C. frondosa* (*Hamel & Mercer, 1995*) and

525 *S. intermedius* and *M. nudus* (our study) during mass spawning can be explained by interspecific

526 variations in reproductive physiological adaptations and behavior associated with different life-

527 history models and aimed at optimization of gamete dispersion and fertilization success in each

528 species.

529   Generally, males of *S. intermedius* and *M. nudus* exhibited much more active spawning

530 behavior than females. First, they began to spawn earlier and ended spawning later than females

531 in all cases except for one mass spawning event when one *S. intermedius* female undoubtedly

532 began to spawn before males. Moreover, males of both sea urchin species were capable of

533 spawning in the absence of females, demonstrating temporal dynamics of spawner numbers

534 similar to those during mass spawning with the participation of both sexes. In contrast, females

535 began to spawn after several males had already spawned, and over 6 spawning seasons, for each

536 species, only two cases were recorded as not belonging to mass spawning when females spawned

537 in the absence of males. Our observations support the currently accepted view that in broadcast

538 spawners, males predominantly spawn before females (see *Levitan, 1998*; *Mercier & Hamel,*

539 *2009*; *Thorson, 1950* for review). For sea urchins, rare exceptions have been encountered in field

540 studies, for example, spawning of *S. droebachiensis* females in the absence of males (*Pearse et*

541 *al., 1988*).

542   Second, the numbers of males participating in mass spawning were significantly higher

543   than those of females (the female/male ratios were 1:9 and 1:5 for *S. intermedius* and *M. nudus*,

544   respectively). This is significantly different from the sex ratio in the natural populations of these

545   species, which is close to 1:1 (*Zhadan, Vaschenko & Ryazanov, 2018*; *P. Zhadan, 2020, pers.*

546   *comm.*). Similar female/male ratios during mass spawning in the field were found for other sea

547   urchin species, such as *Strongylocentrotus purpuratus* (1:4, *Levitan, 2002*), *M. franciscanus* (1:8,

548   *Levitan, 2002*) and *S. droebachiensis* (1:4, *Himmelman et al., 2008*).

549   The significant excess of spawning males over the number of spawning females can be

550   explained in several ways. First, males develop the ability to release gametes earlier than females

551   and retain this ability after the completion of female spawning, as was shown for *S. intermedius*

552   (*Zhadan, Vaschenko & Almyashova, 2015*; *Zhadan, Vaschenko & Ryazanov, 2018*). The same

553   sexual maturity pattern was reported for males of the sea urchin *Lytechinus variegates* (*Reuter &*

554   *Levitan, 2010*). Second, males seem to be able to spawn several times during the spawning

555   season (*Zhadan, Vaschenko & Ryazanov, 2018*; *this study*). Third, males seem to be more

556   sensitive to external triggers of spawning than females. Consistent with this suggestion,

557   laboratory experiments demonstrated the higher sensitivity of males to external stimuli such as

558   phytoplankton and sperm for the sea urchins *S. droebachiensis* and *L. variegatus*, mussel *Mytilus*

559   *californianus* and crown-of-thorns starfish *Acanthaster* cf. *solaris* (*Caballes & Pratchett, 2017*;

560   *Reuter & Levitan, 2010*; *Starr, Himmelman & Therriault, 1990*).

561   As proposed previously by several researchers, the dynamics of the numbers of spawners

562   during mass spawning of sea urchins are likely to form due to the presence of positive feedback

563   (*Reuter & Levitan, 2010*; *Starr, Himmelman & Therriault, 1990*). Thus, spawning products of

564   the individuals most sensitive to environmental factors stimulate the spawning of neighbors, as

565   shown in laboratory experiments in which sperm alone induced spawning in *L. variegates*

566   (*Reuter & Levitan, 2010*) and a combination of phytoplankton and sperm had a synergistic effect

567   on spawning induction in *S. droebachiensis* females (*Starr, Himmelman & Therriault, 1992*). It

568   should be noted, however, that in the other laboratory and field experiments, no effect of water-

569   borne gametes of *L. variegatus* on spawning induction in sexually mature conspecifics of both

570   sexes was found (*McCarthy & Young, 2004*). Our results showed that in both *S. intermedius* and

571   *M. nudus*, the duration of sperm release during mass spawning events was longer than that of

572   solitary spawning. These findings indicate, on the one hand, that during mass spawning,

573   fertilization success may be enhanced by not only the larger number of spawners but also the

574   higher intensity of gamete release. On the other hand, our results suggest that (1) water-borne

575   gametes cannot be considered a primary cue for the induction of mass spawning and (2) one of

576   the reasons for the development of mass spawning events in broadcast spawners may be

577   favorable environmental conditions for the stimulation of spawning, for example, phytoplankton

578   concentrations higher than those during solitary spawning. Our previous field studies showed

579   that an increase in phytoplankton concentration triggers spawning in natural *S. intermedius*

580   populations (*Zhadan, Vaschenko & Almyashova, 2015*; *Zhadan et al., 2016*; *Zhadan, Vaschenko*

581   *& Ryazanov, 2018*). The timing, duration and completeness of spawning at the population level

582   clearly depended on phytoplankton abundance during the spawning season. In the present study,

583   we showed that the temporal parameters of spawning in *S. intermedius* males were different in

584   bays with different levels of phytoplankton. In the bay with the higher phytoplankton level, *S.*

585   *intermedius* males exhibited significantly longer durations of gamete release, intervals between

586   gamete batches and total spawning processes. Considering that most mass spawning events in *S.*

587   *intermedius* and *M. nudus* occurred at night and close to new or full moon phases, the nighttime

588   and lunar phases may be additional environmental factors increasing the probability of mass

589   spawning.

590   In the present work, we did not include data on the role of phytoplankton in the

591   stimulation of spawning in *M. nudus*. Our preliminary data provide evidence that an increased

592   concentration of phytoplankton triggers spawning in the *M. nudus* population (*P. Zhadan, 2020,*

593   *pers. comm.*). However, the relationships between spawning and environmental factors for this

594   species are more complex than those for *S. intermedius*. At a low concentration of phytoplankton

595   in the environment, mature gametes accumulated in *M. nudus* gonads and were released during

596   storm events. These data will be the subject of a future article.

597

598   **Sea urchins increase movement activity before and during mass spawning**

599   In a previous study, we reported that *S. intermedius* males began to move actively just before

600   spawning and retained this activity during the spawning process (*Zhadan, Vaschenko &*

601   *Ryazanov, 2018*). In the present work, a precise time was determined when an increase in the

602   locomotion rate of sea urchins happened before spawning. In both sexes of *S. intermedius* and *M.*

603   *nudus,* this phenomenon occurred 35 min before the start of spawning. *M. nudus* males and

604 females and *S. intermedius* males gradually increased the locomotion rate right up to the time of

605 spawning, while *S. intermedius* females reached a plateau in the average step length

606 approximately 15 min before the beginning of spawning and exhibited a lower locomotion rate

607 during spawning than males (Fig. 3; Table 6). Many *S. intermedius* females stopped moving after

608 climbing to the top of a stone or to another individual. These results are consistent with our

609 previous conclusion about the increased movement activity of *S. intermedius* males during

610 spawning (*Zhadan, Vaschenko & Ryazanov, 2018*).

611   Another important finding of this study was that sea urchins that started to spawn first

612 during mass spawning (leading males) as well as males that spawned alone increased their

613 movement rate before spawning in the absence of other spawning individuals. This observation

614 clearly indicates that the increase in locomotion activity in *S. intermedius* and *M. nudus* before

615 spawning is due to environmental factor(s). Moreover, we found no differences in the

616 prespawning locomotion rate between leading males and outsider males, which started to spawn

617 when the sperm of leading males was already present in the environment. Nevertheless, this

618 finding cannot exclude the influence of sperm as a factor contributing to the stimulation of

619 spawning in sea urchins. Our results showing that nonspawning individuals of both species

620 increased locomotion activity in the presence of spawning males and females suggest a possible

621 effect of released gametes on the movement activity of nonspawners during mass spawning

622 events. This suggestion is congruent with the results of laboratory experiments in which

623 phytoplankton and/or sperm treatments stimulated spawning behavior defined as climbing up the

624 sides of the beaker in *L. variegatus* males and females, whereas only sperm and the combination

625 of phytoplankton and sperm stimulated such a behavior in sexually immature individuals (*Reuter*

626 *& Levitan, 2010*).

627   Simultaneously, we observed that in some cases, nonspawners began to actively move

628 before their neighbors started to spawn. Sheet 34 in Table S1 demonstrates that on the nonfood

629 substrate where there were 4 *M. nudus* individuals, 2 females and 2 nonspawners, the

630 nonspawners began to actively move before the females began to spawn. Moreover, we also

631 observed that in September, after all *M. nudus* individuals had already spawned, with an increase

632 in the concentration of phytoplankton up to 5–10 µg l^{-1}, sea urchins moved from the feeders to a

633 stone pyramid, and with a decrease in phytoplankton concentration, they returned again to the

634 food substrate. These observations suggest that immature sea urchins have the ability to perceive

635   some environmental signal(s), in the case of their high intensity, which stimulates spawning

636   behavior.

637

638   **Sea urchin males and females become closer during mass spawning**

639   One of the most interesting questions concerning the reproductive behavior of sea urchins, to

640   which there is still no answer, is the question of whether sea urchins approach each other during

641   spawning. On flat food substrates, the movement of spawning males of *S. intermedius* and *M.*

642   *nudus* appeared multidirectional (*Zhadan, Vaschenko & Ryazanov, 2018*; *this study*). However,

643   comparison of the distances between males and females measured during the control and

644   spawning periods revealed that during spawning, males and females of both sea urchin species

645   became significantly closer. Moreover, nonspawners also approached females, although this

646   approach was less pronounced than that between males and females.

647   To date, due to rare direct field observations of sea urchin spawning, information on the

648   spatial distribution of sea urchin species before and during spawning has been scarce. The sea

649   urchin *S. droebachiensis* formed no spawning aggregations during echinoderm mass spawning

650   events off the Mingan Islands in the northern Gulf of St. Lawrence in eastern Canada

651   (*Himmelman et al., 2008*), whereas sea urchins *T. gratilla* off the island of Maui (Hawaii)

652   spawned within small groups of 2–5 (*Johnson & Ranelletti, 2017*). One case of *L. variegates*

653   spawning in an aggregation of several hundred sea urchins was observed in St. Joseph Bay,

654   Florida (*Reuter & Levitan, 2010*). To the best of our knowledge, the study reported by *Levitan*

655   *(2002)* is the only work where nearest-neighbor distances between spawning and nonspawning *S.*

656   *franciscanus* sea urchins during mass spawning events in the field were determined. Based on

657   the data on the distribution of sea urchins that were mapped at 30-min intervals for 2 h, it was

658   concluded that spawners and nonspawners did not become more aggregated during spawning. In

659   our study, sea urchin trajectories were traced at 1-min intervals for 3 h, and the changes in

660   distances from the mass centers of females to males and nonspawners were determined. This

661   approach allowed us to show that sea urchins of *S. intermedius* and *M. nudus* did not form true

662   aggregations before and during mass spawning events, but they undoubtedly approached each

663   other. The most significant changes in the distances between spawning and nonspawning *S.*

664   *intermedius* and *M. nudus* sea urchins occurred during the first 50–60 min after the beginning of

665   mass spawning (Fig. 7), and the ranges of these changes varied widely, as males/nonspawners

666 both approached the females and moved away (Table 7). Therefore, the time resolution of 30

667 min (*Levitan, 2002*) did not seem to be sufficient to detect the changes in nearest-neighbor

668 distances between sea urchins during spawning.

669   Since fertilization success in sea urchins depends on the distance between the male and

670 female (*Levitan, 2002, 2005*), their approach during mass spawning is important for reproductive

671 success. The meaning of the approach between nonspawning individuals and females is not very

672 obvious. In this regard, a question arises concerning whether spawning males and nonspawning

673 individuals exhibit similar behaviors due to their responses to some chemical cues (pheromones)

674 that are released together with gametes shed by conspecifics, most likely females. The last

675 supposition is based on our observations that despite females moving more actively than males

676 immediately before spawning, they usually stopped after climbing the elevated surface, followed

677 by the start of spawning. Simultaneously, after the beginning of spawning, males accelerated in

678 the absence of females but stopped near females.

679   Both experimental and field studies show that sea urchins use chemosensation to avoid

680 predators as well as to find food (*Campbell et al., 2001*; *Mann et al., 1984*; *Spyksma, Taylor &*

681 *Shears, 2017*; *Zhadan & Vaschenko, 2019*). Genomic analysis suggests that an elaborate

682 chemosensory system involving several hundred putative chemoreceptor genes, in particular

683 those encoding olfactory receptors, operates in sea urchins (*Burke et al., 2006*; *Raible et al.,*

684 *2006*). However, to date, there is no information on the presence and chemical nature of

685 pheromones in sea urchins. Recently, mass spectrometry, genomic and proteomic analyses were

686 performed to identify the protein composition of water-borne plumes released from aggregating

687 spawning crown-of-thorn starfish (*Acanthaster planci*) (*Hall et al., 2017*). The proteins secreted

688 by *A. planci* have been shown to consist largely of signaling factors and hydrolytic enzymes that

689 may be detected by putative olfactory receptors expressed in the external tissues of starfish. We

690 believe that the new data on the patterns of sea urchin locomotion activity during mass spawning

691 obtained in the present study will serve as the physiological basis for the search for biomolecules

692 that may play a role in pheromones in this group of echinoderms.

693

694 **CONCLUSION**

695 Our results show that two sea urchin species with planktotrophic larvae, *S. intermedius* and *M.*

696 *nudus*, which were monitored in their natural environment, display similar behaviors during mass

697   spawning events. Males and females of both species responded to some environmental cue(s),

698   most likely phytoplankton, by increasing their locomotion rate 35 min before the start of

699   spawning. Subsequently, they accelerated until the beginning of and during spawning. Males

700   appeared to be more sensitive to external trigger(s) of spawning than females; therefore, during

701   mass spawning events they began to actively move earlier, in the absence of other spawners.

702   Nonspawners of both species also increased their locomotion activity, but at a later time, in the

703   presence of spawning males and females, and less prominently than spawners. On a vertical

704   surface, both echinoids moved strictly upward, whereas on a flat food substrate, their movement

705   was multidirectional. Spatial distribution analysis showed that although neither echinoid formed

706   spawning aggregations on flat surfaces, the males and, to a much lesser extent, nonspawners

707   approached females during mass spawning.

708   The temporal dynamics of the numbers of males and females participating in mass

709   spawning were well synchronized in both echinoids so that the maximum numbers of

710   simultaneously spawned sexes coincided. However, males of both sea urchin species exhibited

711   much more active spawning behavior than females: 1) males began to spawn earlier and ended

712   spawning later than females; (2) the spawning duration of males was longer due to the longer

713   pause between sperm batches; and (3) males seemed to be able to spawn several times during the

714   spawning season.

715   Temporal and quantitative patterns of behavior of the sea urchins *S. intermedius* and *M.*

716   *nudus* before and during mass spawning may be considered a set of behavioral adaptations aimed

717   at increasing fertilization success. The high sensitivity of males to environmental factor(s),

718   primarily phytoplankton, appears to be a large-scale adaptation characteristic for many broadcast

719   spawners with planktotrophic larvae and is apparently one of the prerequisites for the

720   development of mass spawning events. The nighttime and new and full moon phases apparently

721   to be modulating factors increasing the probability of mass spawning. The longer spawning

722   duration in males compared with females, longer duration of sperm release during mass

723   spawning events compared with that during solitary male spawning, longer durations of sperm

724   release and total time of spawning in males inhabiting the bay with higher levels of

725   phytoplankton and approach of males and females during mass spawning may be considered

726   small-scale adaptations that promote the likelihood of fertilization.

727

728 **ACKNOWLEDGEMENTS**

731

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Table 1(on next page)

Locations and dates of mass spawning events in the sea urchins *Strongylocentrotus intermedius* and *Mesocentrotus nudus*.

1 **Table 1** Locations and dates of mass spawning events in the sea urchins *Strongylocentrotus intermedius* and *Mesocentrotus*

2 *nudus*.

Location	Species	Total number of sea urchins	Percentage of spawning sea urchins	Date	Number of full tracks of males / total number of males	Number of full tracks of females / total number of females	Sheet number in Table S1
Kievka Bay	*S. intermedius*	24	43	16.08.2014	3/7	3/3	1
Kievka Bay	*S. intermedius*	43	23	03.08.2015	9/10	0/0	2
Kievka Bay	*S. intermedius*	29	39	03.08.2015	7/11	0/0	3
Kievka Bay	*S. intermedius*	38	45	14.09.2015	12/16	1/1	4
Kievka Bay	*S. intermedius*	56	18	16.09.2015	3/9	1/1	5
Alekseev Bay	*S. intermedius*	45	22	17.05.2016	6/10	0/0	6
Alekseev Bay	*S. intermedius*	65	26	19.05.2016	13/15	2/2	7
Alekseev Bay	*S. intermedius*	72	28	20.05.2016	14/19	1/1	8
Alekseev Bay	*S. intermedius*	66	33	22.05.2016	12/17	5/5	9
AlekseevBay	S. intermedius	39	27	01.06.2016	5/9	1/1	10
Alekseev Bay	*S. intermedius*	38	26	02.06.2016	6/10	0/0	11
Alekseev Bay	*S. intermedius*	39	26	14.07.2018	5/9	1/2	12
Alekseev Bay	*M. nudus*	56	52	13.07.2017	8/21	5/8	13

Alekseev Bay	*M. nudus*	40	25	19.07.2017	6/10	0/0	14
Alekseev Bay	*M. nudus*	38	53	21.07.2017	13/20	0/0	15
Alekseev Bay	*M. nudus*	35	40	11.07.2018	7/11	2/3	16
Alekseev Bay	*M. nudus*	41	27	11.07.2018	8/9	1/2	17
Alekseev Bay	*M. nudus*	37	78	13.07.2018	18/23	5/6	18
Alekseev Bay	*M. nudus*	63	41	13.07.2018	13/21	3/5	19
AlekseevBay	*M. nudus*	57	18	17.07.2018	4/9	1/1	20
Alekseev Bay	*M. nudus*	34	32	17.07.2018	8/9	2/2	21
Alekseev Bay	*M. nudus*	37	49	16.08.2018	14/16	2/2	22
Alekseev Bay	*M. nudus*	50	42	21.07.2019	10/18	2/3	23
Alekseev Bay	*M. nudus*	47	38	26.07.2019	11/16	1/2	24

3

Table 2(on next page)

Temporal parameters of spawning of the sea urchins *Strongylocentrotus intermedius* and *Mesocentrotus nudus* participating in mass spawning events.

The data for 15 females (f) and 96 males (m) of *S. intermedius* and 24 females and 119 males of *M. nudus* are presented as the median and range (in parentheses). "*n*" is the number of measurements for each parameter. The Mann–Whitney test was used to reveal intersex and interspecies differences.

1

2

Parameter	*S. intermedius*		*M. nudus*		Intersex comparison		Interspecies comparison	
	Male	Female	Male	Female	*S. intermedius*	*M. nudus*	Males	Females
Release duration of one gamete batch (min)	3 (1–90) $n = 390$	4 (1–34) $n = 38$	2 (1–34) $n = 379$	6 (1–21) $n = 50$	m ≈ f $U = 6238$; $p = 0.1040$	m < f $U = 6148$; $p = 0.0001$	$m_{int} > m_{nud}$ $U = 66350$; $p = 0.0049$	$f_{int} ≈ f_{nud}$ $U = 869$; $p = 0.4966$
Total duration of gamete release (min)	13.5 (1–92) $n = 96$	14 (2–61) $n = 15$	9 (1–62) $n = 119$	14 (3–37) $n = 24$	m ≈ f $U = 683.5$; $p = 0.6567$	m ≈ f $U = 1611$; $p = 0.5833$	$m_{int} > m_{nud}$ $U = 4847$; $p < 0.0067$	$f_{int} ≈ f_{nud}$ $U = 181$; $p = 0.5804$
Time interval between gamete batches (min)	4 (0–162) $n = 292$	1.5 (0–35) $n = 28$	4 (0–79) $n = 282$	1 (0–11) $n = 37$	m > f $U = 2862$; $p = 0.0072$	m > f $U = 2657$; $p < 0.0001$	$m_{int} ≈ m_{nud}$ $U = 40895$; $p = 0.8645$	$f_{int} ≥ f_{nud}$ $U = 401.5$; $p = 0.1148$
Total duration of intervals between gamete batches (min)	15 (0–162) $n = 96$	1 (0–50) $n = 15$	9 (0–113) $n = 119$	0 (0–11) $n = 24$	m > f $U = 348.5$; $p = 0.001$	m > f $U = 871.5$; $p < 0.0001$	$m_{int} > m_{nud}$ $U = 4760$; $p = 0.0037$	$f_{int} ≥ f_{nud}$ $U = 162.5$; $p = 0.062$
Spawning duration (min)	36 (1–188) $n = 96$	24 (2–111) $n = 15$	24 (1–140) $n = 119$	15 (1–48) $n = 24$	m > f $U = 482$; $p = 0.0398$	m > f $U = 1178$; $p = 0.0089$	$m_{int} > m_{nud}$ $U = 4423$; $p = 0.0003$	$f_{int} ≥ f_{nud}$ $U = 162.5$; $p = 0.1826$

3

Table 3(on next page)

Temporal parameters of spawning of the sea urchin *Strongylocentrotus intermedius* inhabiting different bays.

The data are presented as the median and range (in parentheses). "*n*" is the number of measurements for each parameter. The Mann–Whitney test was used to reveal the differences.

1

Parameter	Alekseev Bay		Kievka Bay		Intersex comparison		Interhabitat comparison	
	Male	Female	Male	Female	Alekseev Bay	Kievka Bay	Males	Females
Release duration of one gamete batch (min)	4 (1–90) $n = 257$	5.5 (1–34) $n = 22$	2 (1–13) $n = 133$	3 (1–24) $n = 9$	m < f $U = 2106$; $p = 0.0449$	m ≤ f $U = 2106$; $p = 0.2847$	$m_{Alek} > m_{Kiev}$ $U = 11580$; $p < 0.0001$	$f_{Alek} \geq f_{Kiev}$ $U = 54.5$; $p = 0.0517$
Total duration of gamete release (min)	25 (1–92) $n = 61$	17 (5–61) $n = 10$	6.5 (1–45) $n = 35$	7 (2–24) $n = 5$	m ≈ f $U = 278$; $p = 0.6101$	m ≈ f $U = 85$; $p > 0.9999$	$m_{Alek} > m_{Kiev}$ $U = 433$; $p < 0.0001$	$f_{Alek} \geq f_{Kiev}$ $U = 15$; $p = 0.0856$
Time interval between gamete batches (min)	4 (0–162) $n = 201$	2 (0–35) $n = 21$	2 (0–35) $n = 91$	1 (0–6) $n = 7$	m > f $U = 1463$; $p = 0.0192$	m ≥ f $U = 157.5$; $p = 0.0905$	$m_{Alek} > m_{Kiev}$ $U = 6575$; $p < 0.0001$	$f_{Alek} \geq f_{Kiev}$ $U = 401.5$; $p = 0.2148$
Total duration of intervals between gamete batches (min)	23 (0–162) $n = 61$	4.5 (0–50) $n = 10$	7.5 (0–47) $n = 35$	0 (0–11) $n = 5$	m > f $U = 141.5$; $p = 0.0048$	m > f $U = 36.5$; $p = 0.0337$	$m_{Alek} > m_{Kiev}$ $U = 598.5$; $p = 0.0004$	$f_{Alek} \geq f_{Kiev}$ $U = 50$; $p = 0.2547$
Spawning duration (min)	59 (8–188) $n = 61$	29 (5–111) $n = 10$	20 (1–59) $n = 35$	8 (2–24) $n = 5$	m > f $U = 186$; $p = 0.04354$	m ≥ f $U = 55$; $p = 0.2183$	$m_{Alek} > m_{Kiev}$ $U = 395$; $p < 0.0001$	$f_{Alek} \geq f_{Kiev}$ $U = 10.5$; $p = 0.0799$

2

Table 4(on next page)

Temporal parameters of solitary and mass spawning in males of the sea urchins *Strongylocentrotus intermedius* and *Mesocentrotus nudus*.

The data are presented as the median and range (in parentheses). "*n*" is the number of measurements for each parameter. The Mann–Whitney test was used to reveal differences between solitary and mass spawning.

1

Parameter	Strongylocentrotus intermedius			Mesocentrotus nudus		
	Solitary spawning	Mass spawning	Statistics	Solitary spawning	Mass spawning	Statistics
Release duration of one gamete batch (min)	2 (1–16) $n = 72$	4 (1–90) $n = 257$	$U = 7024$; $p = 0.0015$	2 (1–12) $n = 52$	3 (1–34) $n = 379$	$U = 8160$; $p = 0.0396$
Total duration of gamete release (min)	13 (1–26) $n = 20$	25 (4–92) $n = 61$	$U = 372.5$; $p = 0.0069$	6 (1–23) $n = 20$	9 (1–62) $n = 119$	$U = 741$; $p = 0.0021$
Time interval between gamete batches (min)	4 (0–48) $n = 36$	4 (0–162) $n = 201$	$U = 3188$; $p = 0.2551$	4 (0–48) $n = 36$	4 (0–79) $n = 282$	$U = 4916$; $p = 0.7574$
Total duration of intervals between gamete batches (min)	6.5 (0–62) $n = 20$	23 (0–162) $n = 61$	$U = 277$; $p = 0.0001$	7 (0–76) $n = 20$	9 (0–113) $n = 119$	$U = 1186$; $p = 0.5993$
Spawning duration (min)	20.5 (1–71) $n = 20$	59 (8–188) $n = 61$	$U = 218$; $p < 0.0001$	15 (1–99) $n = 20$	24 (1–140) $n = 119$	$U = 943.5$; $p = 0.0588$

2

Table 5(on next page)

Changes in the step length (cm) of the sea urchins *Strongylocentrotus intermedius* and *Mesocentrotus nudus* during different periods of mass spawning events.

The medians of step length were compared with the Kruskal–Wallis test followed by Dunn's multiple comparison test. "*n*" is the number of full tracks of sea urchin movement; "ns" – not significant.

1

Interval	Statistical parameter	S. intermedius		M. nudus		Leading males		Nonspawning individuals	
		Male	Female	Male	Female	S. intermedius	M. nudus	S. intermedius	M. nudus
	n	96	15	119	24	21	33	85	50
Control	Median	0	0.14	0.035	0	0	0	0.1	0.071
	Range	0–9.9	0–7.8	0–13.1	0–11.5	0–4.5	0–11.4	0–7.1	0–4.6
Prespawning	Median	0.14	0.36	0.1	0.14	0.1	0.1	0	0
	Range	0–8.0	0–7.6	0–13.2	0–13.6	0–6.4	0–8.6	0–2.1	0–13.0
Spawning	Median	0.32	0.27	0.47	0.42	0.45	0.42	0.1	0.1
	Range	0–14.0	0–7.0	0–12.2	0–14.2	0–8.0	0–13.3	0–10.78	0–9.5
Prespawning / Control comparison	Mean rank diff.	519.1	262.7	443.6	207.6	133.4	237.1	−84.28	−4.1
	p	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	ns	ns
Spawning / Control comparison	Mean rank diff.	1869	131.8	243	505.7	520.3	670.7	336.3	161.9
	p	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.001
Spawning / Prespawning comparison	Mean rank diff.	1350	−130.9	1995	298	386.9	433.6	420.6	166
	p	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

2

Table 6(on next page)

Comparison of the step length (cm) of leading and outsider sea urchin males during the prespawning period.

The data are presented as the median and range (in parentheses) for 35-min intervals before the start of spawning. The Mann–Whitney test was used for comparison. "*n*" is the number of full tracks of sea urchin movement.

1

Species	Leading males	Outsider males	Statistics
Strongylocentrotus intermedius	0.1 (0–6.4) *n* = 21	0.14 (0–6.3) *n* = 29	$U = 355145, p = 0.0756$
Mesocentrotus nudus	0.1 (0–8.6) *n* = 33	0.14 (0–8.4) *n* = 30	$U = 583245, p = 0.2407$

2

Table 7(on next page)

The distances (cm) from spawners and nonspawners to their common center of mass (CCM) and the center of mass of females (FCM) during sea urchin mass spawning.

The data are presented as the median and range (in parentheses). "*n*" is the number of full tracks of sea urchin movement.

1

Interval	Statistical parameter	Strongylocentrotus intermedius			Mesocentrotus nudus		
		From all individuals to CCM	From males to FCM	From nonspawners to FCM	From all individuals to CCM	From males to FCM	From nonspawners to FCM
	n	131	67	49	165	101	34
Control	Median	17.16	20.50	23.74	19.15	25.06	30.18
	Range	1.15–36.91	4.96–46.89	5.92–50.50	0.74–58.80	3.07–68.16	2.58–72.04
Spawning	Median	13.53	15.75	21.62	16.02	19.35	28.43
	Range	0.19–39.16	2.93–55.13	4.80–54.88	0.15–46.71	1.82–46.69	1.29–101.0
Control / Spawning comparison	Mann–Whitney U	12400000	1920000	2222000	21010000	4757000	775386
	p	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	= 0.0007
	Number of values	12746	4962	5220	11106	7699	3058

2

Figure 1

Examples of temporal dynamics of the numbers of simultaneously spawning sea urchins during mass spawning events.

(A, C) *Strongylocentrotus intermedius*. (B, D) *Mesocentrotus nudus*. (A, B) Spawning of sea urchins of both sexes. (C, D) Spawning of males in the absence of females. Blue and red lines indicate the numbers of males and females, respectively.
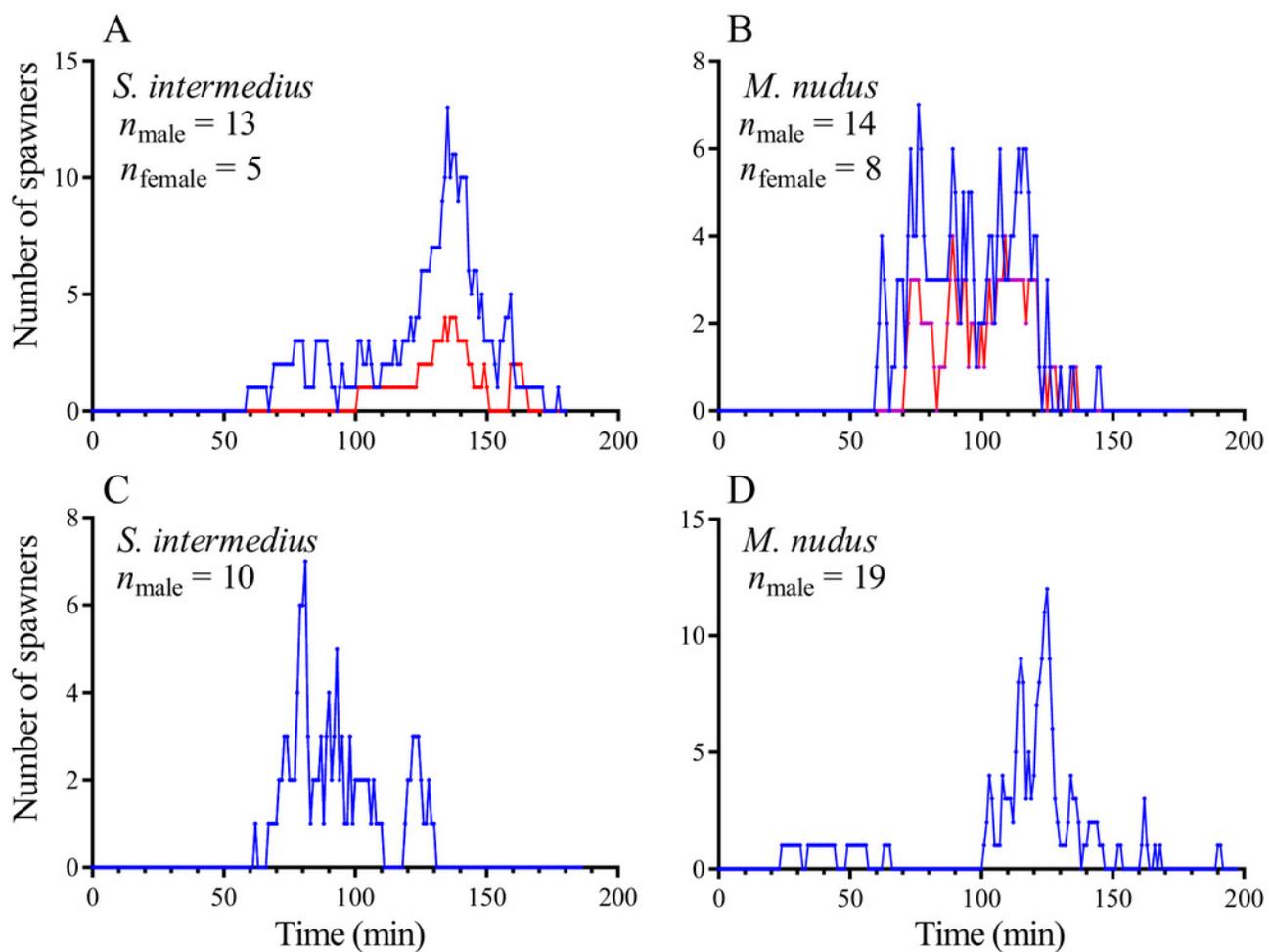
Figure 2

Temporal dynamics of the numbers of simultaneously spawning males and females of the sea urchins during mass spawning.

(A) *Strongylocentrotus intermedius*. (B) *Mesocentrotus nudus*. All data on the numbers of males and females participating in mass spawning events were combined into corresponding pools and aligned on the X-axis at the time point coinciding with the start of the first spawning in each mass spawning event (denoted by a vertical dotted line). Blue and red lines indicate the numbers of males and females, respectively.
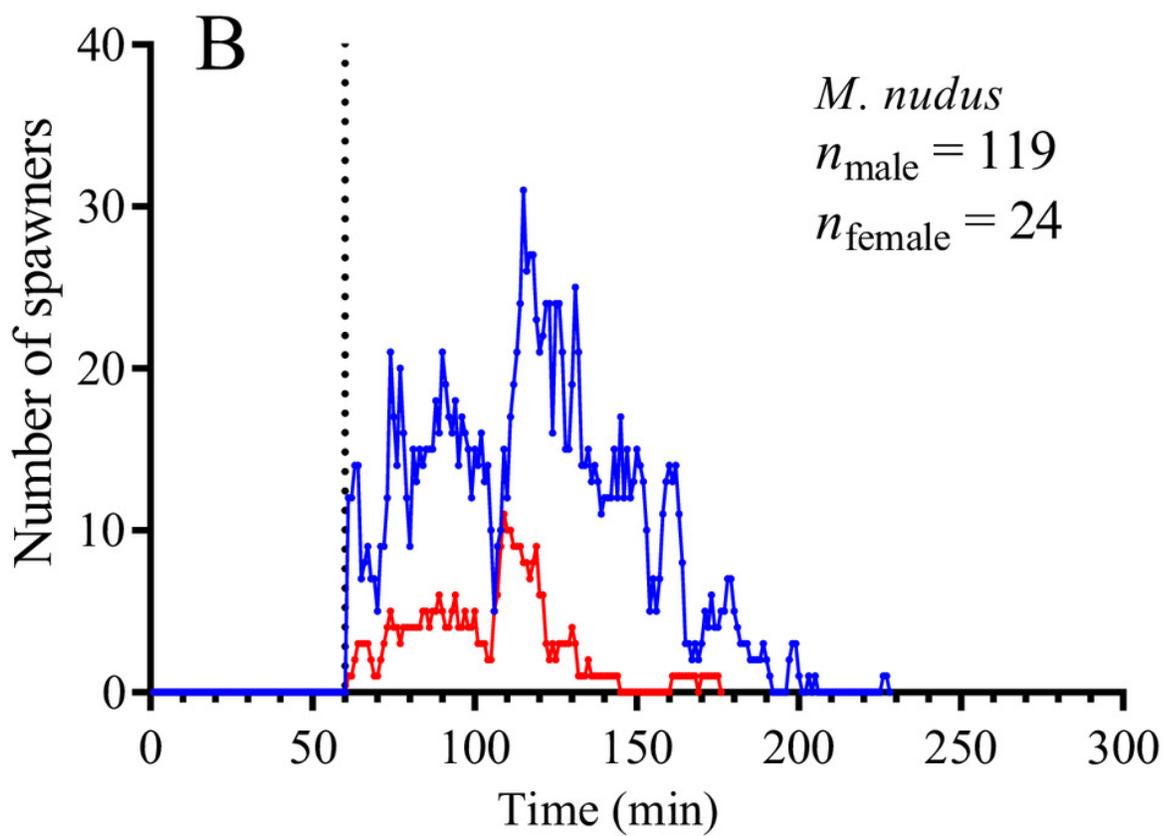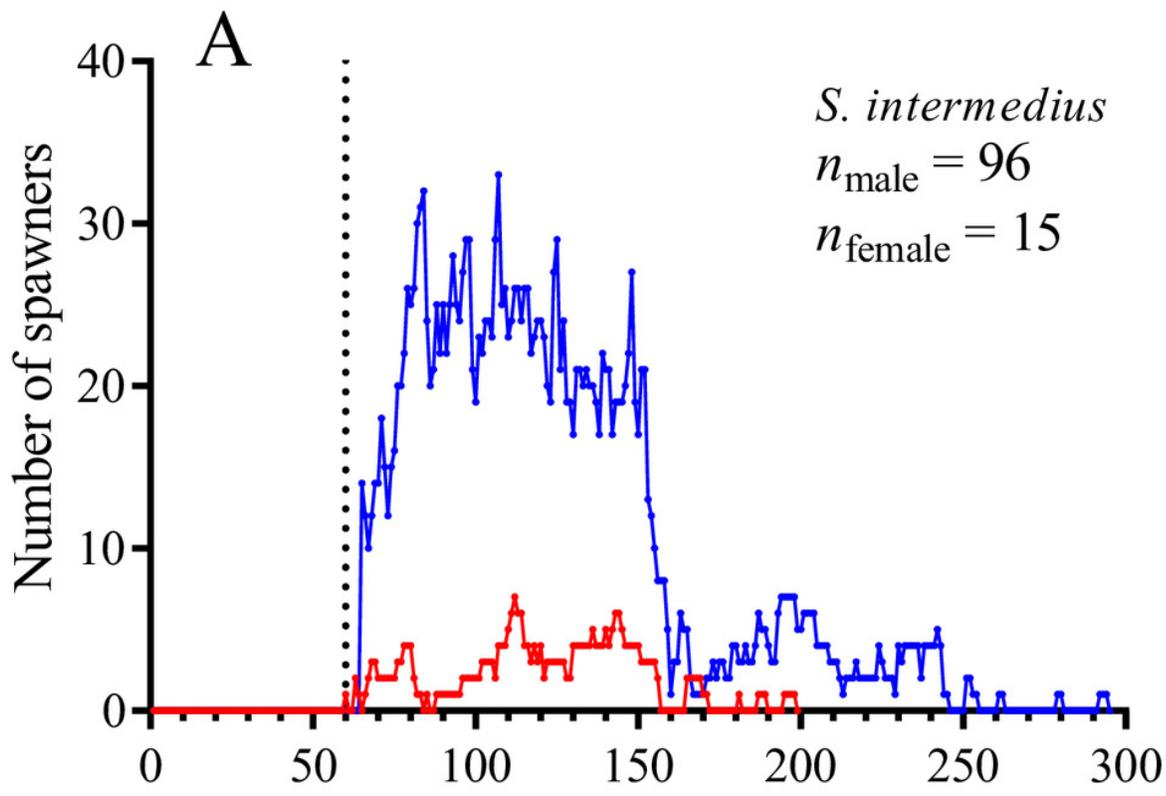
Figure 3

Temporal dynamics of the locomotion activity of males and females of the sea urchins before and during mass spawning.

(A, B) *Strongylocentrotus intermedius*. (C, D) *Mesocentrotus nudus*. The data on the length of sea urchins' steps and the numbers of spawners were combined into corresponding pools and aligned on the X-axis at the time point coinciding with the beginning of spawning of each individual. Vertical dashed lines denote the boundaries of the control, prespawning and spawning intervals. The black solid line denotes the median step length of sea urchins. Vertical lines indicate the interquartile range (IQR). Blue and red lines indicate the numbers of spawning males and females, respectively (*n*).
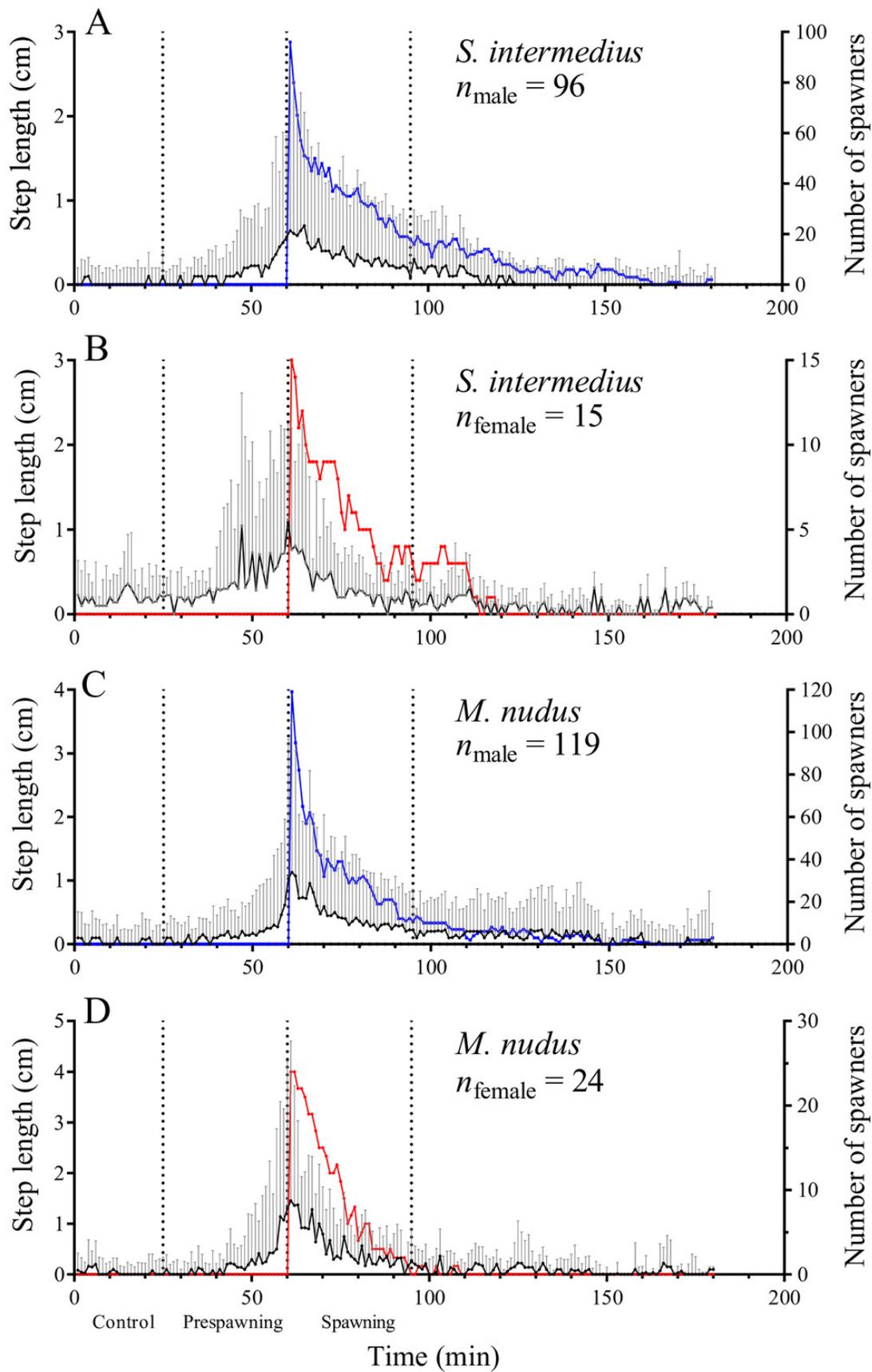
Figure 4

Temporal dynamics of the locomotion activity of males that started to spawn first during mass spawning (leading males).

(A) *Strongylocentrotus intermedius*. (B) *Mesocentrotus nudus*. The data on the step lengths of leading males and the numbers of spawners were combined into corresponding pools and aligned on the X-axis at the time point coinciding with the beginning of spawning of each male. Vertical dashed lines denote the boundaries of the control, prespawning and spawning intervals. The black solid line denotes the median step length of sea urchins. Vertical lines indicate the interquartile range (IQR). The blue line indicates the number of spawning males (*n*).
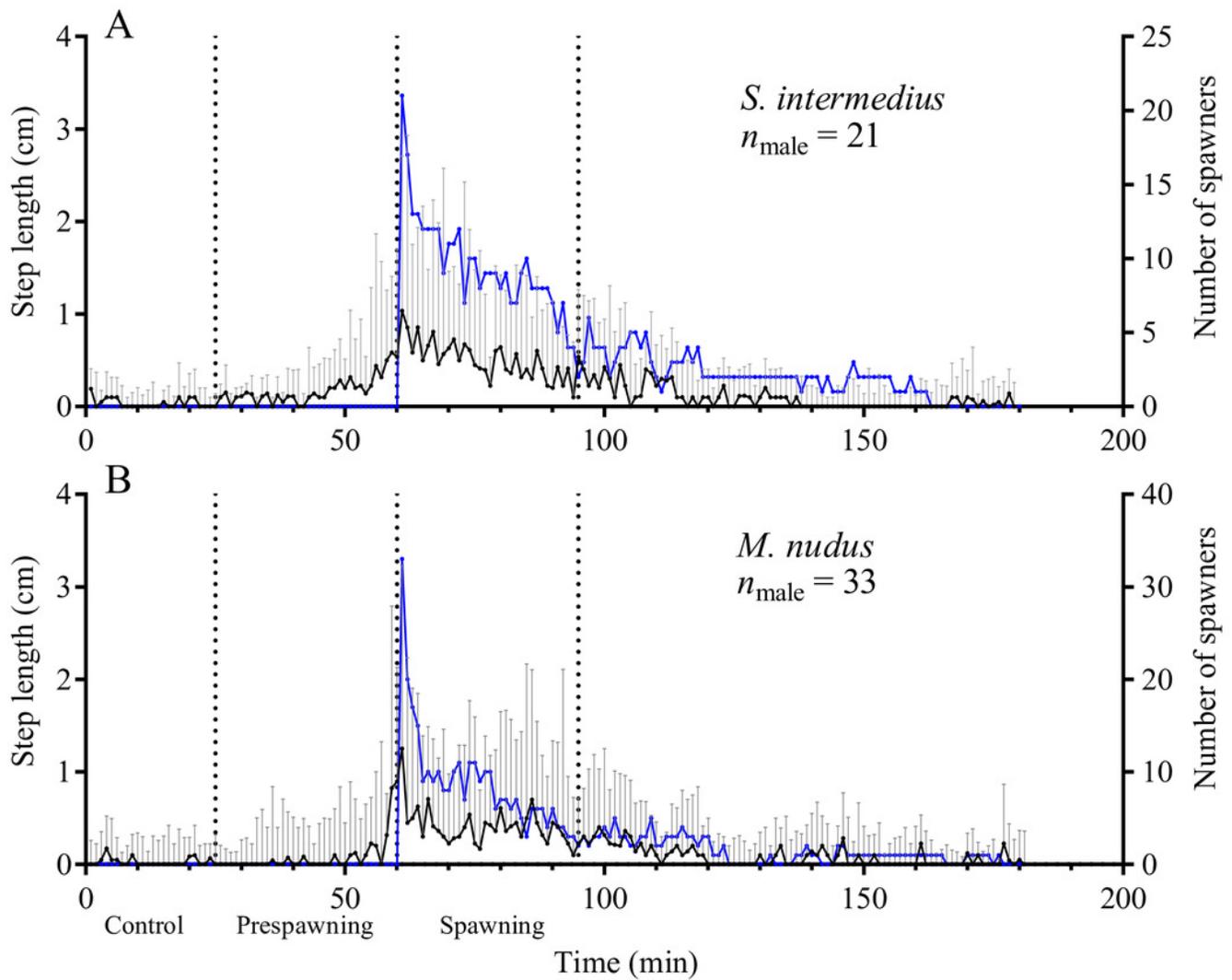
Figure 5

Temporal dynamics of the locomotion activity of males (blue solid line) and nonspawners (green solid line) during mass spawning events.

(A) *Strongylocentrotus intermedius*. (B) *Mesocentrotus nudus*. The data on the step lengths of males and nonspawners were combined into corresponding time series and aligned on the X-axis at the time point coinciding with the beginning of spawning of the first individual in each mass spawning event. Vertical dashed lines denote the boundaries of the control, prespawning and spawning intervals. Vertical solid lines indicate the interquartile range (IQR).
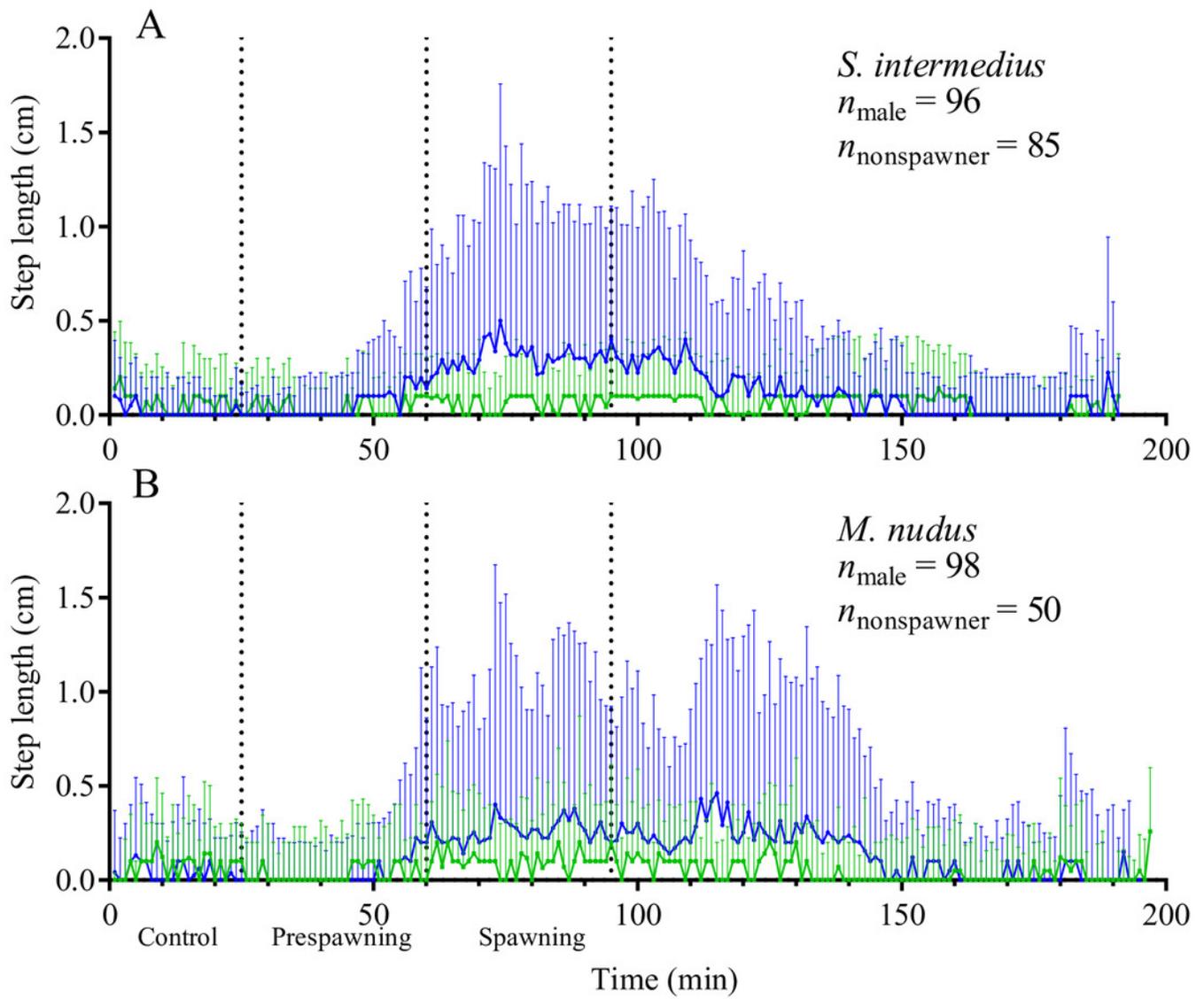
Figure 6

The changes in distances from males, females and nonspawners to their common center of mass during mass spawning of sea urchins.

When calculating the common center of mass, the conditional mass of spawning individuals was taken as a multiple of the time of gamete release. For nonspawners (denoted by a green color), the conditional mass was taken as a value of 1. The data for *Strongylocentrotus intermedius* and *Mesocentrotus nudus* are denoted by brown and black colors, respectively. X-axis: time of the sea urchin movement track (min). Left Y-axis: changes in the distances presented as the mean of differences between the initial and measured 1-min interval distances (cm) and 95% confidence intervals. Right Y-axis: data on the percentage of spawning males. Vertical dashed lines, from left to right, denote the boundary of the control interval, the time point when the first male in each mass spawning event began to spawn, and the time point when 95% of males spawned.
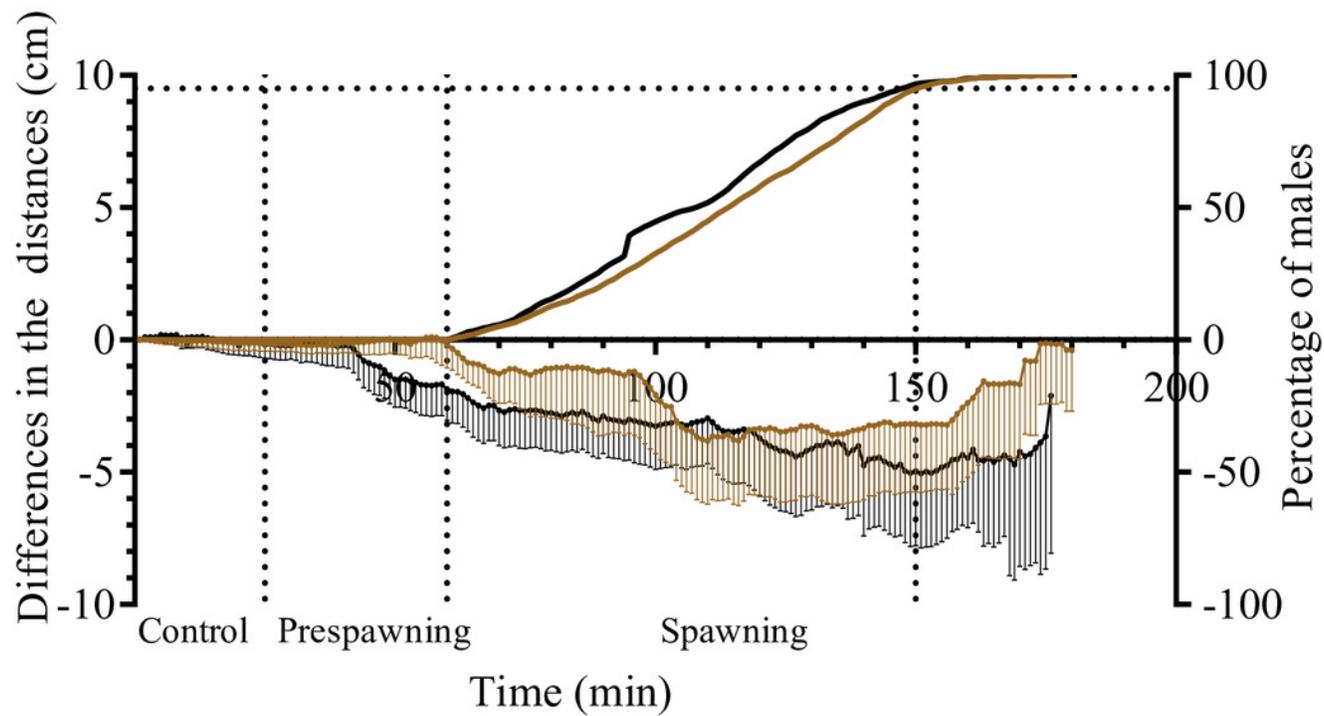
Figure 7

The changes in distances from males and nonspawners to the center of mass of females during mass spawning of the sea urchins.

(A) *Strongylocentrotus intermedius*. (B) *Mesocentrotus nudus*. When calculating the center of mass of females, female conditional mass was taken as a multiple of the time of gamete release. For nonspawners (denoted by a green color), the conditional mass was taken as a value of 1. X-axis: time of the sea urchin movement track (min). Left Y-axes: changes in distances presented as the mean of differences between the initial and measured 1-min interval distances (cm) and 95% confidence intervals. Right Y-axes: data on the percentage of spawning males. Vertical dashed lines, from left to right, denote the boundary of the control interval, the time point when the first male in each mass spawning event began to spawn, and the time point when 95% of males spawned.