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Sex differences in gamete release patterns during group spawning in Pacific herring

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Sex differences in gamete release patterns during group spawning in Pacific herring

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22 Abstract

23 Group spawning is common in fish, with individuals forming aggregations and
24 repeatedly releasing gametes. In such situations, the timing of gamete release is important
25 because it influences individual reproductive success. Pacific herring *Clupea pallasii* spawn
26 synchronously in large aggregations. Notably, herring sperm remain capable of fertilization
27 for up to 24 h, and pheromones in milt can induce spawning in other individuals. These
28 characteristics suggest that males and females may differ in their temporal patterns of
29 gamete release, particularly in its onset and temporal concentration. However, such sex-
30 specific patterns cannot be measured by direct observation. In this study, we used
31 biologging to detect sperm and egg release behaviors and examined sex differences in their
32 onset and temporal concentration during group spawning in experimental tanks. We
33 developed behavioral classification models using data from small-scale experiments and
34 applied them to acceleration data from 53 tagged individuals in a large-scale experiment
35 involving an aggregation of 911 fish. The number of detections varied among individuals,
36 ranging from 1 to 109 in males and from 1 to 328 in females. Although the onset of gamete

release did not differ between sexes, males repeatedly released sperm within a relatively short period of approximately 2 h, whereas some females repeatedly released eggs over a prolonged period of approximately 8 h. The prolonged fertilization capability of herring sperm may allow females to release eggs over an extended period. Resolving such individual contributions is important for understanding how group spawning events develop and persist in herring and potentially other fishes.

Keywords: Biologging, Reproductive Behavior, Group spawning, Spawning aggregation, Fish biology, Machine learning, Pacific herring

Introduction

Reproduction is a fundamental component of fish life history(McBride et al. 2015). Many fish species reproduce through group spawning, in which individuals form aggregations to spawn and males and females repeatedly release sperm and eggs, respectively(Domeier 2012). Even during group spawning, individuals are expected to behave in ways that maximize their reproductive success(Andersson and Iwasa 1996; Bekkevold et al. 2002; Molloy et al. 2012; Yamamoto et al. 2026). Because group spawning events emerge from the behaviors of participating individuals, understanding individual behavior, such as the timing of gamete release, is essential for explaining how these events unfold over time. Despite extensive observations of group spawning behavior(Takeuchi and Gushima 2006; Motohashi et al. 2010; Roff et al. 2017; Starr et al. 2018), little is known about the detailed temporal dynamics of gamete release at the individual level. This is largely because tracking individual behaviors within dense spawning aggregations is difficult through direct observation.

Pacific herring (*Clupea pallasii*) typically forms spawning aggregations of several hundred thousand to over a million individuals in coastal areas(Haegele and Schweigert 1985; Hay 1985; Hoshikawa et al. 2004). Herring are easy to obtain and maintain in captivity, and their behavior can be measured in experimental tanks(Batty et al. 1990;

65 Tomiyasu et al. 2019; Seki et al. 2025). During spawning, males and females release
66 gametes individually, without forming pairs, by rubbing their genital papillae against
67 seaweed.(Stacey and Hourston 1982; Rajasilta et al. 1997; Hoshikawa et al. 2004). Although
68 herring release sperm and eggs synchronously during group spawning, several reproductive
69 characteristics may influence the timing of gamete release. First, pheromones released with
70 sperm induce other individuals to release sperm and egg, causing gradual synchronization of
71 spawning among many individuals(Scott et al. 1991; Stacey and Hourston 1982; Carolsfeld
72 et al. 1997). Second, sperm can remain viable in seawater for approximately 24 – 48 hours
73 after release(Yanagimachi 1957b, Yanagimachi et al. 1992), whereas egg viability declines
74 substantially within 30 min and is maintained for no longer than 5 h(Yanagimachi 1957a).
75 These characteristics suggest that males may initiate sperm release earlier than females,
76 potentially inducing spawning in other individuals. Furthermore, the difference in the
77 duration of fertilization capability between sperm and eggs may allow males and females to
78 exhibit different temporal patterns of gamete release.
79 Biologging using acceleration loggers is useful for continuous measurement of fish
80 spawning behavior(Whitney et al. 2010; Yasuda et al. 2013; Seki et al. 2024). In our
81 previous study, we quantified temporal changes in spawning behaviors during a group
82 spawning event of Pacific herring using acceleration loggers in a large experimental

tank(Seki et al. 2025). However, the timing of sperm and egg release by individual fish remained unclear. By linking acceleration data with sperm and egg release behaviors under conditions in which these behaviors could be directly observed(Watanabe and Takahashi 2013; Nishizawa et al. 2013; Clarke et al. 2021), it would be possible to identify these behaviors from acceleration alone, allowing us to examine sex differences.

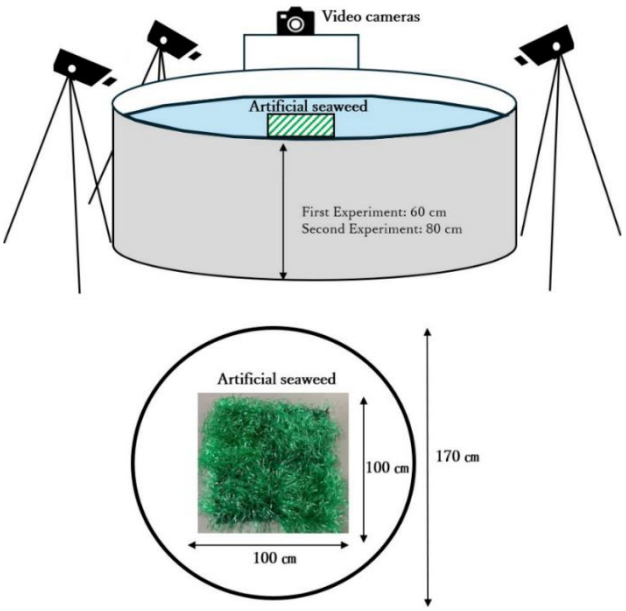
In this study, we used acceleration-based biologging to examine sex differences in the onset and temporal concentration of gamete release during group spawning in Pacific herring. To identify sperm and egg release behaviors, we developed behavioral classification models using acceleration data and direct observations from small-scale experiments and applied them to previously collected data from a large-scale experiment(Seki et al. 2025).

Methods

Development of behavioral classification models

Two experiments were conducted to collect training data for behavioral classification models. In the first experiment, 10 sexually mature herring (5 males and 5 females) collected in Hakodate, Hokkaido, Japan, were used, of which 9 individuals (5 males and 4 females) were equipped with tri-axial acceleration loggers (axy5 Depth, TechnoSmArt Co. Ltd.; 3.5 g in air). In the second experiment, 16 sexually mature herring (12 males and 4

females) collected using a coastal set net in Shibetsu, eastern Hokkaido, were used, of which 5 individuals (3 males and 2 females) were equipped with the same loggers. Loggers were externally attached to the fish following the procedures described in Seki et al. (2025). Cable ties and cushions of different colors were used to identify individual fish. Both experiments were conducted at the Hakodate Research Center for Fisheries and Oceans in circular tanks, with artificial seaweed provided as a spawning substrate (Fig. 1). After the experiments, we measured the sex, fork length, body mass, maturity stage, gonad weight, and gonadosomatic index (GSI) of all the individuals.



(Fig. 1: Experimental environment. The tank diameter was 170 cm and the depth was 60 or 80 cm. In both experiments, four video cameras were set up to observe fish behavior, and artificial seaweed as the spawning substrate was established in the center of the tank.)

Throughout both experiments, multiple cameras were positioned around the tanks to record whole-tank and close-up views of fish behavior (Tapo C200, TP-Link; HDR-CX900 and HDR-SR12, SONY; 1920 × 1080 pixels, 15.00 fps; Fig. 1). After the experiments, fish were observed for 2.5 h in the first experiment and 14 h in the second experiment. We classified herring behavior into five categories (swimming, dashing, turning, substrate testing, and substrate spawning) based on previous studies (Stacey and Hourston 1982) and our own observations (Movie. S1). Swimming was defined as typical swimming with slow tail movements and no noticeable change in body posture, dashing as rapid swimming with intense tail movements, turning as changing direction on the spot, substrate testing as inspecting the spawning substrate without proceeding to spawning, and substrate spawning as the release of sperm and egg with fine tail movements when the papilla and pelvic fins were in contact with the substrate. We recorded these behaviors, start time of the behavior, end time of the behavior, and the individual ID. Because it was difficult to classify behavior within an accuracy of 1 s, all behavioral labels were recorded at 1-s intervals. To increase the training data, when a behavior lasted for >1 s, it was divided into 1-s segments and labeled accordingly.

We used a random forest (RF) model to classify fish behaviors(Breiman 2001), a machine learning approach widely used for analyzing biologging data(Valletta et al. 2017;

133 Fehlmann et al. 2017; Dickinson et al. 2021; Clarke et al. 2021). In this study, tri-axis
134 acceleration was recorded at 25 Hz with a maximum value of ±4 G; depth and temperature
135 were recorded at 1 Hz. The X-, Y-, and Z-axis accelerations were recorded in the head-tail,
136 dorsoventral, and lateral directions, respectively. Acceleration along each axis was separated
137 into static and dynamic components using a 0.2-s running mean(Shepard et al. 2008). We
138 calculated 68 predictor variables from raw, static, and dynamic acceleration data, including
139 statistical and frequency-domain features, for each behavior-labeled period. Details of
140 variable calculations are provided in Supplementary Information (Table S1). These
141 parameters were calculated using IGOR Pro ver. 6.37 (WaveMetrics Inc.) and Ethographer
142 ver. 2.0(Sakamoto et al. 2009).
143 Data from all individuals were pooled and randomly split into training (70%) and
144 test (30%) datasets(Nathan et al. 2012; Sur et al. 2017). The RF model was developed using
145 R software version 4.3.1 and the "*ranger*" Package. RF hyperparameters were tuned using
146 the "ranger" package in R (Wright and Ziegler 2017), with the final model using mtry = 20,
147 min.node.size = 1, splitrule = "extratrees," and 500 trees based on previous biologging
148 studies (Fehlmann et al. 2017; Probst et al. 2019; Dickinson et al. 2021; Clarke et al. 2021).
149 Details of hyperparameter tuning and cross-validation, including leave-one-individual-out
150 validation to assess generalizability to unseen individuals, are provided in Supplementary

Information (Table S2).

Model performance was evaluated using confusion matrices generated with the "*confusionMatrix*" function in the "*caret*" package in R. Four classifications were set for each behavior: true positive (TP; the behavior was observed and correctly predicted by the model), true negative (TN; the behavior was not observed and was not predicted), false positive (FP; the behavior was not observed, but was incorrectly predicted by the model), and false negative (FN; the behavior was observed, but was not predicted by the model). From these, the model accuracy, precision, and recall F_1 score were calculated. Accuracy evaluates the overall performance of the model across all behavior classes. The precision and recall were calculated for each behavior, and the F_1 score was used to assess the balance between them.

$$\text{Precision} = \frac{\text{TP}}{\text{TP} + \text{FP}}$$

$$\text{Recall} = \frac{\text{TP}}{\text{TP} + \text{FN}}$$

$$\text{F}_1\ \text{score} = 2 \times \frac{\text{Precision} \times \text{Recall}}{\text{Precision} + \text{Recall}}$$

Application to the model to a group spawning event in a large tank

The experiment was conducted from 21 to 22 April 2023 using a large experimental tank (10 m wide × 5 m long × 3.4 m deep). A total of 911 individuals were

used, from which acceleration data were obtained from 15 males and 38 females. The same type of acceleration logger as in the small-scale experiments was used. After tagging, fish were allowed to acclimate for about 2 h. The tank was then set to dark conditions to induce a spawning event. The start of the experiment was defined as 19:00 on 21 April 2023, after fish behavior had stabilized following the lights-off. Detailed experimental procedures and conditions are described in our previous study(Seki et al. 2025).

Tri-acceleration, depth, and temperature were recorded using the same sampling settings as in the small-scale experiments. We analyzed data for 10 h (from 19:00 on 21 April 2023 to 5:00 on 22 April 2023). The same 68 variables which were used for model development were calculated from acceleration data within a 1-s moving time window, shifting every 0.4 s. These time-series features were then inputted into the trained RF models to extract the predicted timing of substrate spawning for each individual. Overlapping substrate spawning predictions in the time series were merged and treated as a single substrate spawning event. Detected substrate spawning events were treated as sperm-release behaviors in males and egg-release behaviors in females, based on sex determined by anatomical examination. Then, the number of detected events was summarized every 10 min and visualized as a time series. The time-series dynamics of detections were compared with previously reported patterns of acceleration changes during spawning events.

187 To investigate relationships between substrate spawning and sex, individuals with
188 no detected behaviors were excluded from the analysis, and 50 individuals (14 males and 36
189 females) were used. To examine whether males release sperm earlier than females, the first
190 detection time was calculated for each individual as the elapsed time (minutes) from 19:00
191 on 21 April 2023. We also calculated the interquartile range (IQR) of detection timing to
192 evaluate the distribution of behaviors. IQR was used instead of the duration between the
193 first and last detections because the latter was highly variable. Individuals with fewer than
194 two detections were excluded, resulting in 46 individuals (12 males and 34 females) for the
195 IQR analysis. To examine whether sex affect the first detection time and IQR, generalized
196 Linear Model (GLM) with a Gamma distribution was used. First detection time and IQR
197 were treated as response variables, and sex was included as an explanatory variable. Model
198 fitting was carried out using the software R v. 4.0.2 with the *glm*() function. Likelihood ratio
199 tests were used to compare the fitted models with the null models. Statistical significance
200 was set at $p < 0.05$.
201

202 # Results

203 ## Development of behavioral classification models

204 A total of 1190 substrate spawning behaviors (625 sperm release and 565 egg
205 release behaviors; Table 1) were obtained from 6 males (mean fork length 25.83 ± 9.29 cm;

206 mean mass 172.55 ± 24.78 g) and 3 females (mean fork length 28.17 ± 1.61 cm; mean mass
207 210.17 ± 23.24 g). In addition, 180 swimming, 39 dashing, 951 turning and 149 substrate
208 testing were obtained (Table 1). Most sperm and egg release behaviors lasted for 1–2 s;
209 however, some reached 8 s. The duration of other behaviors (dashing, turning, and substrate
210 testing) ranged from approximately 1 to 2 s.
211 (Table 1: Number of observed behaviors per individual used as training data for model
212 classification. Behavioral events recorded from video observations are shown for each
213 individual. IDs 1_1 to 1_9 were used in the first experiment, and the others were used in the
214 second experiment. Further information on these individuals and those without loggers is
215 available in Supplementary Information, Table S4)

		Variation of behavior					
						Substrate Spawning	
ID	**Sex**	**Swimming**	**Dashing**	**Turning**	**Substrate Testing**	**Sperm Release**	**Egg Release**
1_1	M	20	0	20	3	91	−
1_4	M	20	0	19	0	75	−
1_5	F	20	0	26	9	−	128
1_7	M	20	0	19	0	179	−
1_8	F	20	1	16	6	−	96
1_9	M	20	1	7	6	34	−
2_2	M	20	9	344	12	10	−
2_3	M	20	14	223	33	236	−
2_5	F	20	14	277	80	−	341
Total		180	39	951	149	625	565

216

RF model performance was evaluated using accuracy, precision, recall, and F_1 score calculated from the confusion matrix of predicted and reference behaviors. Under this five-category framework, the overall accuracy of the RF model was 0.88. Among the five classified behaviors, substrate spawning had the best performance with a precision of 0.90, recall of 0.96, and F_1 score of 0.93 (Table 2). Turning with an F_1 score of 0.90 and swimming with an F_1 score of 0.89 performed well. However, dash and substrate testing had low precision, recall, and F_1 score values (Table 2). Additionally, leave-one-individual-out cross-validation showed that model performance was generally maintained when evaluated on individuals that were completely excluded from model training (Table S2).

(Table 2: Matrix of predicted behavior, reference behavior, and model performance metrics for behavior classification using the RF model. TP, TN, FP, and FN were obtained for each behavior, using 30% of the data for testing. Based on those values, precision, recall, and F_1 value were calculated for each behavior.)

	Predicted behavior					**Performance Metrics**		
Reference behavior	**Swimming**	**Dashing**	**Turning**	**Substrate Testing**	**Substrate Spawning**	**Precision**	**Recall**	**F1-Score**
Swimming	45	0	5	0	0	0.88	0.90	0.89
Dashing	0	1	11	0	1	1.00	0.08	0.14
Turning	6	0	269	2	17	0.88	0.91	0.90

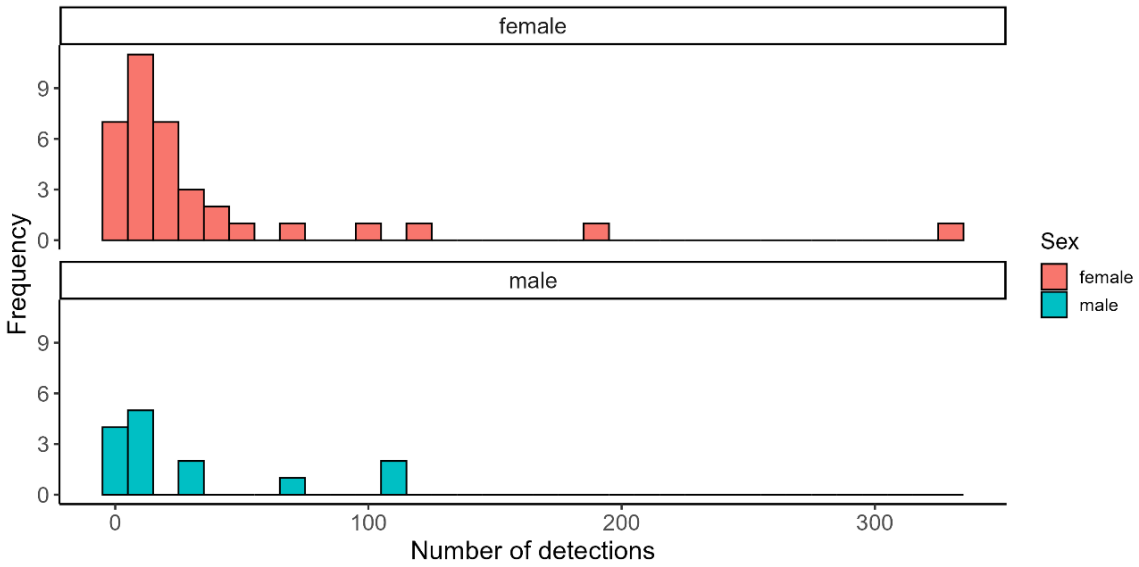
Substrate Testing	0	0	12	4	20	0.44	0.11	0.18
Substrate Spawning	0	0	10	3	347	0.90	0.96	0.93

231

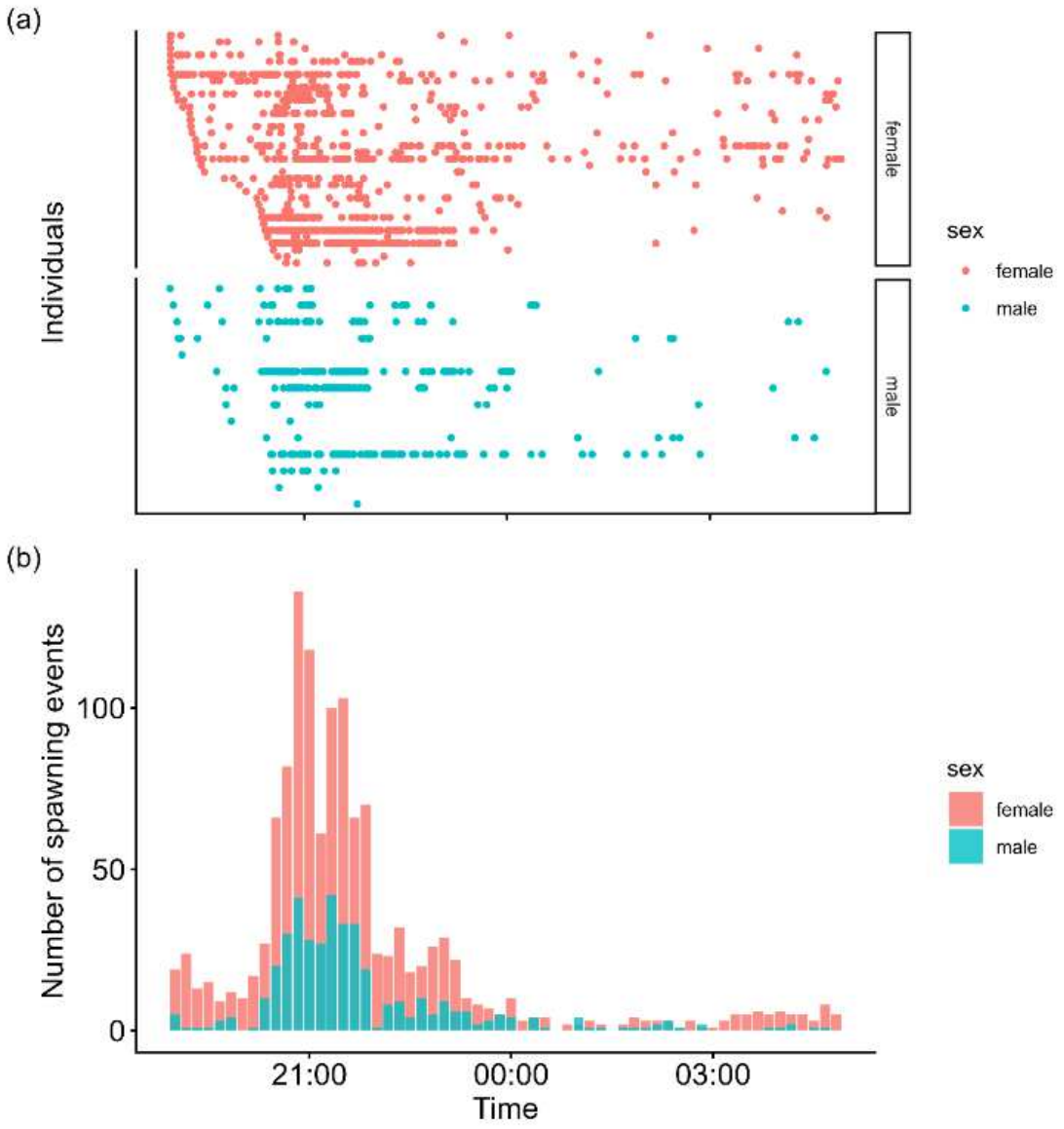
232 Application to the model to a group spawning event in a large tank

233 The trained RF model was applied to a large-scale group spawning event which was
234 difficult to observe directly. In the experiment, a total of 911 herring were used in the large
235 tank, from which acceleration data were obtained from 15 males (mean fork length 28.44 ±
236 1.47 cm; mean mass 275.50 ± 55.00 g) and 38 females (mean fork length 28.86 ± 2.03 cm;
237 mean mass 317.06 ± 80.18 g). A detailed description of the experiment has been reported
238 previously(Seki et al. 2025). The group spawning event occurred at night; however, direct
239 observation of sperm and eggs release behaviors was difficult under these conditions.
240 Sperm and egg release behaviors were detected in 50 of 53 individuals (14 males and
241 36 females) using the trained RF model, and no sperm or egg release behaviors were
242 detected in 2 females and 1 male. In males, the number of detections ranged from 1 to 109
243 per individual (Fig. 2). Time-series visualization at the individual level showed that sperm
244 release behaviors were detected in 5 males between 19:00 and 19:10, immediately after the
245 start of the experiment (Fig. 3a; first five individuals in the male panel). Subsequently,
246 sperm release behaviors were detected in additional males by 30 min after the start of the

247 experiment (19:30). By 1 h 30 min after the start of the experiment (20:30), nearly all males
248 had initiated sperm release, except for one male. In females, the number of detections
249 ranged from 1 to 328 per individual (Fig. 2). In females, egg release behaviors were detected
250 in 11 females between 19:00 and 19:10 (Fig. 3 (a); first eleven individuals in the female
251 panel). Subsequently, egg release behaviors were detected in additional females, and all
252 females started egg release by 1 hour and 40 min after the start of experiment (20:40). In
253 both sexes, behavioral patterns varied among individuals: some individuals exhibited
254 concentrated release around 21:00, whereas others showed prolonged or infrequent release
255 events. Combined sperm and egg release behaviors by sex revealed temporal changes during
256 the group spawning event (Fig. 3 (b)). Detections remained relatively low until 1 h 20 min
257 after the start of the experiment (20:20), but increased between 20:20 and 20:30. The
258 number of detections peaked around 2 h after the start of the experiment (21:10).
259 Thereafter, the number of detections repeated increases and decreases, but gradually
260 declined over time. After 00:00, few sperm and eggs release behaviors were detected. These
261 temporal patterns closely resembled the time-series dynamics reported in our previous study
262 (Seki et al. 2025).
263
264



(Fig. 2: The histograms of the number of detections for males and females. The vertical axis represents the number of individuals in each bin, and the horizontal axis represents the number of detections. The number of detections ranged from 1 to 328 in females, and from 1 to 109 in males.)



271

272 (Fig. 3 (a): Temporal distribution of detected sperm and egg release events for individual

273 females and males. Each dot represents a sperm- or egg-release event detected by the

274 random forest model. Individuals are arranged along the vertical axis, and variation in the

275 timing and duration of sperm release events can be observed among individuals.

276 (b): Temporal changes in detections during the group spawning event. Red bars represent

277 detections by females (36 individuals), and blue bars represent detections by males (14

278 individuals). The height of each bar indicates the number of detections per 10-min interval
279 for each sex. The figure shows a pronounced peak in spawning activity at the group level,
280 despite substantial variation among individuals in the timing and duration of spawning
281 activity.)

282 For the first detection time, the GLM showed that the null model and the model
283 including sex had similar support (ΔAIC < 2; Table 3), providing no strong support for
284 either model. In contrast, for the IQR, the model including sex was selected as the best
285 model (Table 3) and sex had a significant effect on IQR ($p < 0.05$). Males had significantly
286 smaller IQRs than females, indicating that sperm release was concentrated within a
287 relatively short period (approximately 1–2 h), whereas females showed greater variability,
288 with some individuals releasing eggs over periods exceeding 8 h (Fig. 4).

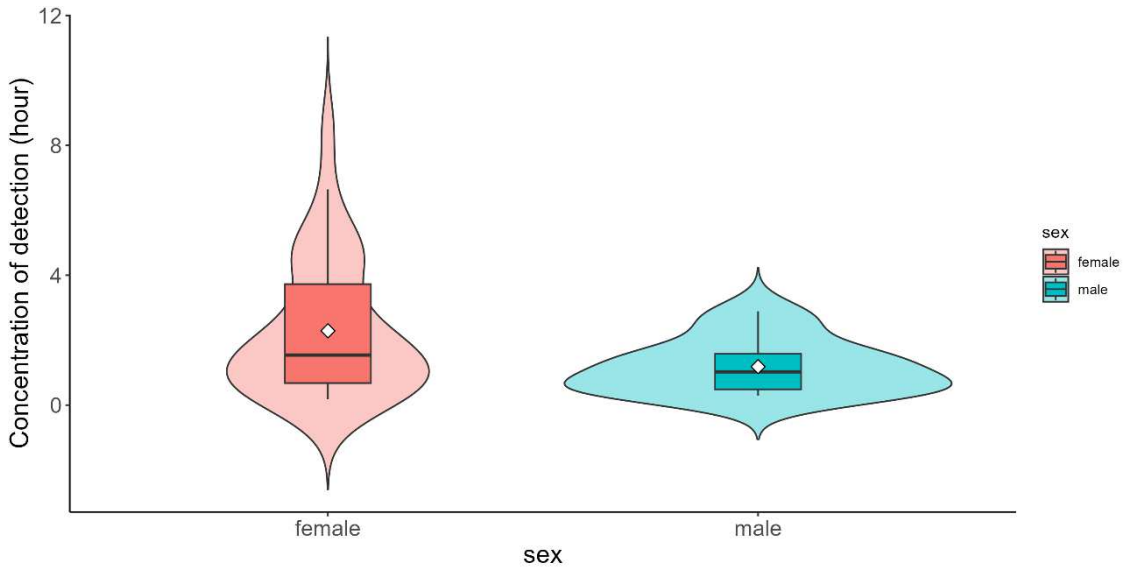
289

290 (Table 3: Generalized linear models testing the effects of sex on the first detection time and
291 distribution of detection (IQR). The significance of each fixed effect was evaluated using
292 likelihood ratio tests comparing nested models. Asterisks indicate significant values. The
293 first detection time was defined as the elapsed time from the start of the experiment.
294 Abbreviations: AIC, Akaike's information criteria; ΔAIC, difference for model relative to
295 smallest AIC in the model set; SE, standard error of the coefficient.)

First detection time						
Models	AIC	ΔAIC	Log-likelihood	Coefficient	S.E.	*p*-value
Null	512.14	0.00	-255.07	—	—	—
Sex	512.64	0.50	-250.32	0.03	0.02	0.23
IQR						
Models	AIC	ΔAIC	Log-likelihood	Coefficient	S.E.	*p*-value
Null	158.19	2.72	-77.01	—	—	—
Sex	155.47	0.00	-74.74	-0.66	0.30	0.03*

296

297



298

299

300 (Fig. 4: The relationship between IQR of detection and sex. Blue and red indicate males and

females, respectively. Violin plots represent kernel density estimates, and boxplots indicate the median and interquartile range (with whiskers showing the data range). The IQR of females was significantly larger than that of males.)

Discussion

In this study, we developed a behavioral classification model to detect sperm and egg release behaviors from acceleration data and applied it to a previously collected dataset to examine sex differences in gamete release patterns. This approach enabled us to examine individual variation in gamete release during group spawning.

Reliability of behavioral classification

Random forest models have been used to classify animal behaviors in various species(Yu et al. 2021; Dunford et al. 2024; Schoombie et al. 2024), including fishes such as yellowtail kingfish *Seriola lalandi* and lemon sharks *Negaprion brevirostris*(Brewster et al. 2018; Clarke et al. 2021). In these previous studies, classification performance varied among behaviors, with relatively high F_1 scores (approximately 0.8–0.9) reported for behaviors with distinctive movement patterns, such as swimming and chafing(Brewster et al. 2018, Clarke et al. 2021). Similarly, in our results, substrate spawning showed high F_1 score (0.93; Table 2). In contrast, classification performance was relatively low for dashing and substrate

319 testing. In particular, substrate testing was often misclassified as substrate spawning,
320 possibly due to limited training data and similarities in body movements between these
321 behaviors. Increasing the amount of labeled data may improve the discrimination of these
322 behaviors. Nevertheless, substrate spawning was detected with high accuracy, which was
323 sufficient for our subsequent analyses. The training data were unevenly distributed among
324 individuals, with some individuals, such as ID2_3 and ID2_5, contributing more data than
325 others. However, leave-one-individual-out cross-validation showed some variation indicated
326 that the model generally retained its ability to detect substrate spawning in individuals
327 excluded from training, although classification performance varied among individuals (Table
328 S2).

329 When applying the classification model developed from the small-scale experiments
330 to the large-tank experiment, differences in experimental conditions should be considered,
331 particularly differences in substrate configuration. However, in both experiments, herring
332 rubbed their bodies against the substrate while adopting various body angles and postures
333 (Fig. S3 (b),(c); Seki et al. 2025). Thus, the variation in body postures observed during the
334 small-scale experiments may have helped the RF model accommodate differences in
335 spawning posture between the two experimental settings. In addition, the characteristic
336 whole-body vibration associated with rubbing against the substrate was consistently

observed in both experiments, as reported in previous study(Stacey and Hourston 1982). Although differences in substrate configuration may have influenced body posture and associated acceleration signals, these shared behavioral characteristics support the application of the model to the large-scale dataset. Additionally, individual spawning patterns were evaluated using summary statistics of the temporal distribution of detected events, including the interquartile range (IQR), rather than relying on individual detections alone. Although the RF model may not generalize equally well across all experimental conditions, the evidence supports its use for characterizing broad temporal patterns of spawning behavior at the individual level.

Temporal patterns of gamete release and sex difference

Sperm and egg release behaviors were detected in 50 individuals (14 males, 36 females) out of 53 individuals. The number of detections ranged from 1 to 109 in males, and from 1 to 328 times in female (Fig.2), indicating marked individual variation in release frequency. Although the overall temporal pattern of detected sperm and egg release was broadly consistent with those reported in our previous study(Seki et al. 2025), the onset of substrate spawning detected in this study did not fully correspond to that identified by direct video observation that previous study. The difference suggests that some substrate

355 spawning may have been missed by direct observation because of limitations in camera
356 angle and visibility within the aggregation. Therefore, resolving sperm and egg release
357 timing at the individual level is important for revealing the temporal dynamics of group
358 spawning, particularly when some release events are not captured by direct observation.
359 With respect to the onset of spawning events, many studies have reported that
360 pheromones contained in milt induce sperm and egg release behaviors in other
361 individuals(Scott et al. 1991; Stacey and Hourston 1982; Carolsfeld et al. 1997; Sherwood et
362 al. 1991). During the reproductive season, steroid hormones in the blood of both sexes are
363 excreted into the urine as conjugated cortisol(Scott et al. 1991; Carolsfeld et al. 1997),
364 which can function as a pheromone(Stacey and Hourston 1982). These effects have been
365 demonstrated in milt mixed with urine, whereas seawater washes of dissected, ovulated
366 herring ovaries did not induce such responses(Stacey and Hourston 1982). These findings
367 strongly suggest that milt during sperm release behaviors play an important role in
368 triggering spawning events. However, in this study, no clear differences between sexes were
369 obtained. Time-series plot of sperm and egg release behaviors show that both of sperm and
370 egg release behaviors were detected between 19:00 and 19:10 (4 males and 11 females; Fig 3
371 (a)). In addition, GLM showed no relationship between the first time of detection and sexes
372 (Table 3). These results suggested the sperm release behaviors by only a few males may be

373 sufficient to trigger a group spawning event in herring. Alternatively, some individuals of
374 both sexes may release sperm and eggs at the onset of spawning event, and once pheromone
375 concentrations reach a certain threshold, many individuals are induced, resulting in a large-
376 scale event. Hay (1985) suggested that males release sperm earlier than females release eggs
377 because the decrease in gonad wight in males is more gradual than in females in the wild.
378 However, our results are more consist with the hypothesis of Stacey and Hourston, in which
379 milt released by a few males induce spawning events(Stacey and Hourston 1982).
380 Nevertheless, the motivation or selective advantage of the males, which take a risk and
381 release their sperm first remains unclear, as also discussed by Hay (1985). Future work
382 should aim to clarify these mechanisms, by integrating behavioral measurements obtained
383 using loggers with information on physiological condition.
384 GLM results showed that temporal distribution of sperm and egg release behaviors
385 differed between sexes (Table 3). Many males released sperm within a relatively short
386 period (approximately 2 h), whereas females included both short-duration egg release and
387 prolonged release lasting in some cases over 8 h (Fig. 4). These sex-specific patterns may
388 have different implications for fertilization success. Previous experimental studies have
389 shown that fertilization success in herring is influenced by sperm concentration(Hourston
390 and Rosenthal 1976; Rosenthal et al. 1988). Although sperm concentrations in natural

391 spawning grounds are expected to be considerably lower than those observed under
392 experimental conditions, concentrated sperm release may increase local sperm
393 concentration and thereby contribute to fertilization success. Female may also increase
394 fertilization success by releasing eggs in synchrony with sperm release by males. However,
395 prolonged egg release may also provide opportunities for successful fertilization because
396 herring sperm possess unique characteristics that allow them to remain fertile for extended
397 periods. Unlike sperm of many other fishes, Pacific herring sperm remain largely immotile
398 after release into seawater and initiate motility in response to egg-derived factors
399 (Yanagimachi 1957b, Yanagimachi et al. 1992). These characteristics may allow females to
400 obtain sufficient opportunities for successful fertilization even when releasing eggs over
401 extended periods, provided that sufficient sperm remain available. These findings suggest
402 that different temporal patterns of egg release may be compatible with successful
403 fertilization in Pacific herring. Further studies are needed to examine the relationship
404 between individual egg-release patterns and actual fertilization success.
405 In our analysis, to characterize the temporal distribution of detections while
406 reducing the influence of occasional false-positive detections, we used the interquartile
407 range (IQR) rather than the full range of detection times. In addition, when analyzing the
408 onset of sperm and egg release, we did not apply thresholds based on detection frequency or

409 exclude individuals, because some detections may not have represented genuine gamete
410 release and there was no objective basis for excluding individuals according to the number of
411 detections. Despite these considerations, the observed sex-specific patterns were consistent
412 with plausible ecological interpretations. However, the imbalance in sample size between
413 sexes should also be considered. The number of males was smaller than that of females (15
414 males and 38 females), and the larger female sample may have captured a broader range of
415 behavioral variation. This sampling imbalance may therefore have influenced the apparent
416 differences in the onset and temporal spread of sperm and egg release. Accordingly,
417 although the present results suggest sex-related differences in gamete-release timing,
418 further experiments with more balanced sample sizes and additional individuals are needed
419 to evaluate the generality of these patterns.

420 In this study, behavioral classification of acceleration data enabled the timing of
421 sperm and egg release to be detected for individuals during a group spawning event. This
422 revealed the temporal patterns of sperm and egg release that would be difficult to identify
423 from observations of the aggregation as a whole. In this species, although sperm and egg
424 release behavior appear synchronized at the group level based on direct observation,
425 individuals differed in when and for how long they release sperm and egg. The number of
426 detections also varied among individuals, suggesting that participation in spawning may

differ among individuals even within the same aggregation. Such variation may partly reflect differences in individual condition, because variation in maturation state and age can occur even within herring spawning groups(Rajasilta et al. 1997; MacCall et al. 2019; Slotte et al. 2025). These individual differences in the timing, duration, and frequency of gamete release may contribute to how spawning events begin, become concentrated, persist, and eventually cease. Therefore, resolving how individual fish contribute to a spawning event is important for understanding its temporal dynamics of group spawning. More broadly, although many fish species spawn in aggregations, such individual-level temporal dynamics remain poorly understood (Colin 2012). Resolving them will therefore be important for understanding the processes underlying group spawning across fish species.

Declarations

Ethics approval

This study was conducted in accordance with the animal experimental protocol approved by the Hokkaido University (approval number: 4-3). All the researchers who participated in this study completed an ethics course on animal experiments at Hokkaido University. All experiments were performed in compliance with the Institutional Guidelines for Animal Experiments at Hokkaido University.

Consent for publication

Not applicable.

Availability of data and materials

The datasets are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

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Authors contributions declaration

K. S., M. T., M. K., and K. M. designed the experiment. K. S. and M. I. performed the fieldwork. K. S., Z. Y., and K. M. conducted the experiment. K. S., M. T., and N. S. analyzed the data. K. S. wrote the manuscript. All authors contributed to the writing of the manuscript.

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