Marine biology

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

Supplementary Information

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Development of RF model

For acceleration data processing, Static and dynamic acceleration components were separated for each axis. The absolute value of Roll (Roll_abs) was used to represent the magnitude of lateral body tilt regardless of direction. Eleven parameters, namely the raw data of each axis (AccX, AccY, AccZ), static acceleration (Pitch, Yaw, Roll_abs), dynamic acceleration (Surge, Heave, Sway), ODBA, and VeDBA were used to calculate statistical values for the time of behavior labeling. These statistics used the average, standard deviation, skewness, kurtosis, minimum and maximum values. Additionally, FFT analysis of Sway and its jerk was used to extract two dominant-frequency variables (Frequency and Frequency_DIF). Six statistical metrics were calculated for each of the eleven parameters, yielding 66 variables; together with the two frequency variables, 68 variables were used to construct the RF model.

RF hyperparameters were tuned using the *ranger* package in R. We tested mtry = 10, 15, and 20; min.node.size = 1, 5, and 10; and splitrule = "extratrees" or "gini". The optimal settings were mtry = 20, min.node.size = 1, and splitrule = "extratrees". We did not test mtry values >20 because this would use more than one-third of the 68 predictors at each split and could reduce model generalizability. Five-fold cross-validation was performed using the trainControl function in the *caret* package in R. In each fold, four subsets were used for training and one for testing, and performance metrics were averaged across the five folds.

Table S1

Definitions, notations, and formulas for each predictor variables used in the RF model. Static and dynamic acceleration were separated for each axis, and summary statistics [average, standard deviation, skewness, kurtosis, minimum and maximum] were computed within a 1-s window.

Parameter	**Notation**	**Calculation**	**Description**
Raw accelaration (G)	AccX, AccY, AccZ		The acceleration measured in each orthogonal axis (x, y and z)
Static acceleration (G)	Pitch, Yaw, Roll	A runnning 0.2 s mean of each raw acceleration	The posture of the fish in each axis
Dynamic accelation (G)	Surge, Heave, Sway	Raw acceleration - Static acceleration	The movement of the fish in each axis
Absolute roll acceleration (G)	Roll_abs	Absolute(Roll acceleration)	Absolute value of roll to evaluate the left and right rotation of the fish body equally

Overall Dynamic Body Acceleration (G)	ODBA	Absolute(Dynamic accX)＋ Absolute(Dynamic accY)＋ Absolute(Dynamic accZ)	The overall body movement of the fish
Vectoral Dynamic Body Acceleration (G)	VeDBA	√(Dynamic accX)²＋ (Dynamic accY)²＋ (Dynamic AccZ)²	The vectorial magnitude of the fish dynamic movement
Frequency (Hz)	Frequency		The dominant frequency obtained from the FFT analysis of sway acceleration in 1 sec
Frequency_DIF (Hz)	Frequency_ DIF		The dominant frequency obtained from the FFT analysis of the differentiated sway acceleration
Average	Ave		Mean value in 1 sec of the parameters
Standard deviation	SD		A measure of the variability of the parameters in 1 sec
Skewness	Skew		A measure of the symmetry of the parameters in 1 sec
Kurtosis	Kurt		A measure of the tail shape of the parameters in 1 sec
Minimam	Min		Minimam value 1 sec of the parameters
Maximam	Max		Maximam value 1 sec of the parameters

Generalizability to unseen individuals

To evaluate model generalizability to unseen individuals, leave-one-individual-out cross-validation was additionally performed. In each iteration, one individual was excluded from model training and used as independent test data, and model performance was evaluated using the confusion matrix. The F_1-Score for substrate spawning ranged from 0.36 to 0.99, and overall accuracy ranged from 0.62 to 0.97 among individuals. The low precision observed for ID 2_2 (0.22) reflected the small number of substrate-spawning observations in this individual, whereby a limited number of false-positive detections substantially reduced precision. The low accuracy for ID 2_5 (0.62) may reflect the reduced amount of substrate-spawning training data after excluding this individual, which contributed to the largest number of substrate spawning observations.

Table S2

Classification performance evaluated using leave-one-individual-out cross-validation, including overall accuracy and class-specific performance metrics for substrate spawning. Performance metrics were calculated as described in the main text.

ID	Substrate spawning	Other behaviors	TP	FP	FN	Precision	Recall	F_1-Score	Accuracy
1_1	91	43	91	7	0	0.93	1.00	0.96	0.92
1_4	75	39	74	0	1	1.00	0.99	0.99	0.97
1_5	128	55	123	9	5	0.93	0.96	0.95	0.91
1_7	179	45	178	9	1	0.95	0.99	0.97	0.93
1_8	96	43	94	11	2	0.90	0.98	0.94	0.86
1_9	34	28	33	3	1	0.91	0.97	0.94	0.84
2_2	10	385	10	35	0	0.22	1.00	0.36	0.88
2_3	236	290	225	22	11	0.91	0.95	0.93	0.87
2_5	341	391	191	18	150	0.91	0.56	0.70	0.62

Classification performance when sperm and egg release were treated separately

When sperm and egg release behaviors were classified separately, overall model accuracy decreased slightly to 0.85. Classification performance was lower for both sperm release (precision = 0.81, recall = 0.95, F_1 = 0.88) and egg release (precision = 0.86, recall = 0.72, F_1 = 0.79) than for the combined substrate spawning category (precision = 0.90, recall = 0.96, F_1 = 0.93; Table 2). The relatively low recall for egg release (0.72) may reflect the limited number of female individuals in the training dataset and differences in body size between sexes. In our experiments, females were generally larger than males, which may have influenced acceleration features associated with gamete release. Although the model demonstrated some ability to distinguish sperm release and egg release behaviors, these differences in sample size and body size may have limited its classification performance and generalizability. Therefore, we concluded that treating sperm and egg release as a single category, substrate spawning, was the most suitable approach for the present analysis.

Table S3

Classification performance of the six-category RF model. Overall accuracy was 0.85, lower than that of the five-category model. The sperm release (precision = 0.81, recall = 0.95, F_1 = 0.88) and egg release classes (precision = 0.86, recall = 0.72, F_1 = 0.79) showed lower F_1 scores than the combined substrate spawning class (precision = 0.90, recall = 0.96, F_1 = 0.93) in the five-category model.

	Predicted behavior						**Performance Metrics**		
Reference behavior	Swimming	Dashing	Turning	Substrate Testing	Sperm Release	Egg Release	Precision	Recall	F1-Score
Swimming	45	0	5	0	0	0	0.88	0.90	0.89
Dashing	0	1	10	0	2	0	1.00	0.08	0.14
Turning	6	0	279	2	3	4	0.86	0.95	0.90
Substrate Testing	0	0	14	8	5	9	0.67	0.22	0.33
Sperm Release	0	0	3	0	183	7	0.81	0.95	0.88
Egg Release	0	0	12	2	32	121	0.86	0.72	0.79

Variable importance based on Gini impurity

Variable importance was evaluated based on the decrease in Gini impurity associated with splits on each predictor variable. The standard deviation of heave (heave_sdev) was the most important of all factors. This was followed by the minimum heave acceleration (heave_min), the mean and minimum absolute roll acceleration (roll_abs_avg and roll_abs_min), and the mean ODBA and VeDBA (ODBA_avg and VeDBA_avg). Variables related to Z-axis acceleration and pitch also showed relatively high importance.

Although rapid tail-fin movements were characteristic of substrate spawning during direct observations, sway acceleration showed relatively low importance in the model. This suggests that tail-fin movements alone may not be sufficient to distinguish substrate spawning from other behaviors, such as turning or dashing. During spawning, herring rolled their bodies and rubbed their genital papillae against the substrate (Supplementary Movie S1), which may explain the high importance of heave- and roll-related variables. In addition, the relatively high importance of ODBA and VeDBA suggests that whole-body movements, rather than tail-fin movements alone, contributed to the classification of substrate spawning.

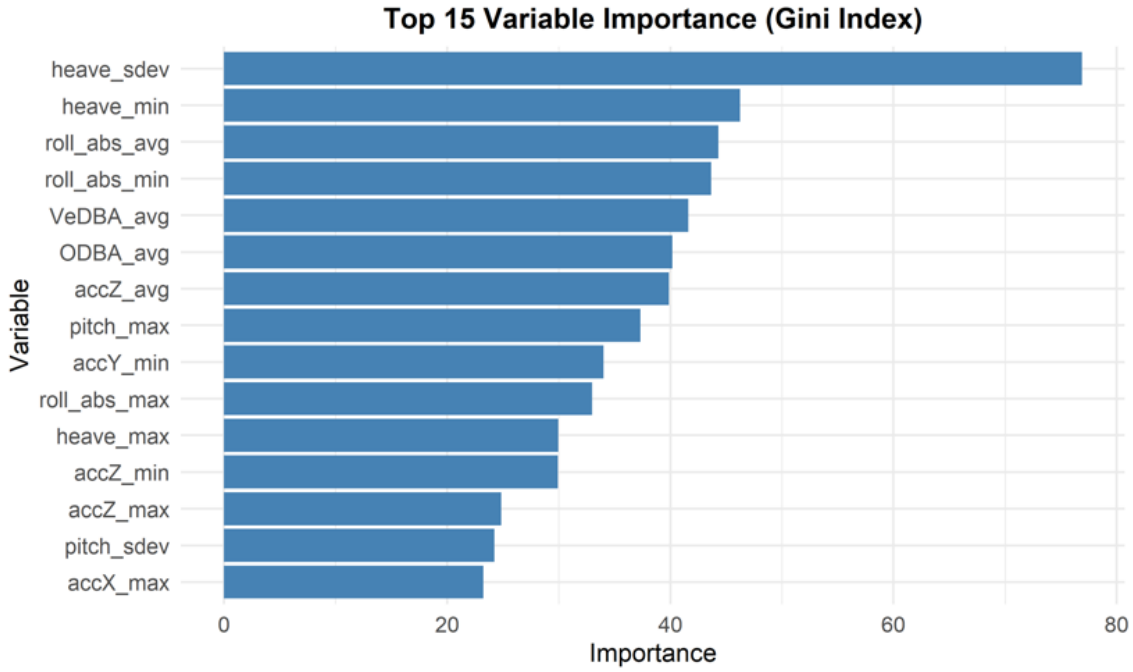


Fig. S1

The variable importance based on the decrease in Gini impurity in the RF model. The 15 most important predictor variables for behavioral classification are shown. Variables related to heave and roll acceleration, as well as ODBA and VeDBA, showed relatively high importance.

Experimental setup, logger attachment, acceleration data, and individual information

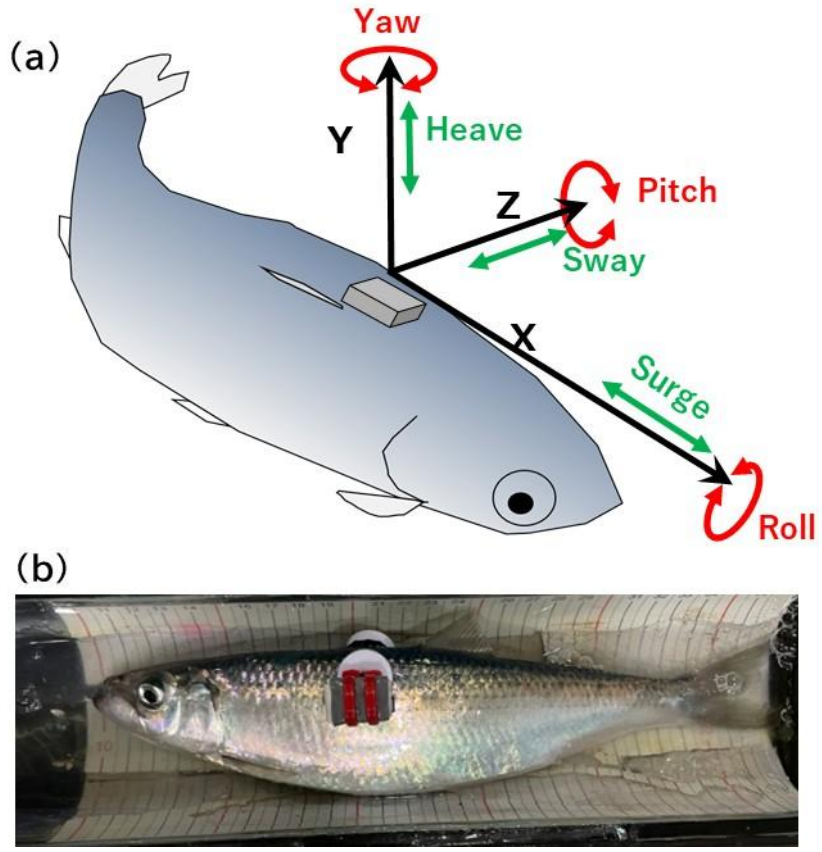


Fig. S2

(a) Illustration of the tri-axis acceleration.

(b) Fish after tagging. To identify the individuals, each fish was marked with a unique combination of colored cushions and plastic ties.

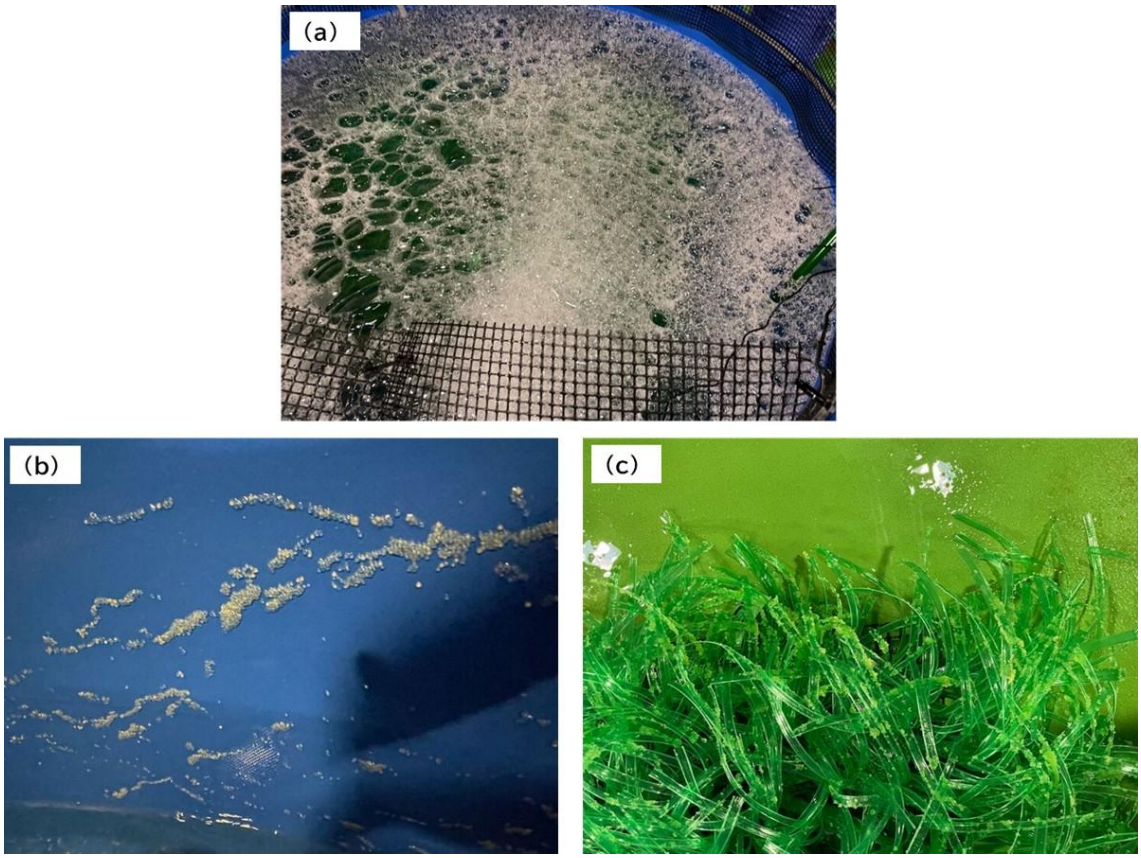


Fig. S3

Spawning environment after first experiment.

(a) Experimental tank after the spawning event.

(b) Eggs that adhered to the tank wall.

(c) Eggs that adhered to artificial seaweed.

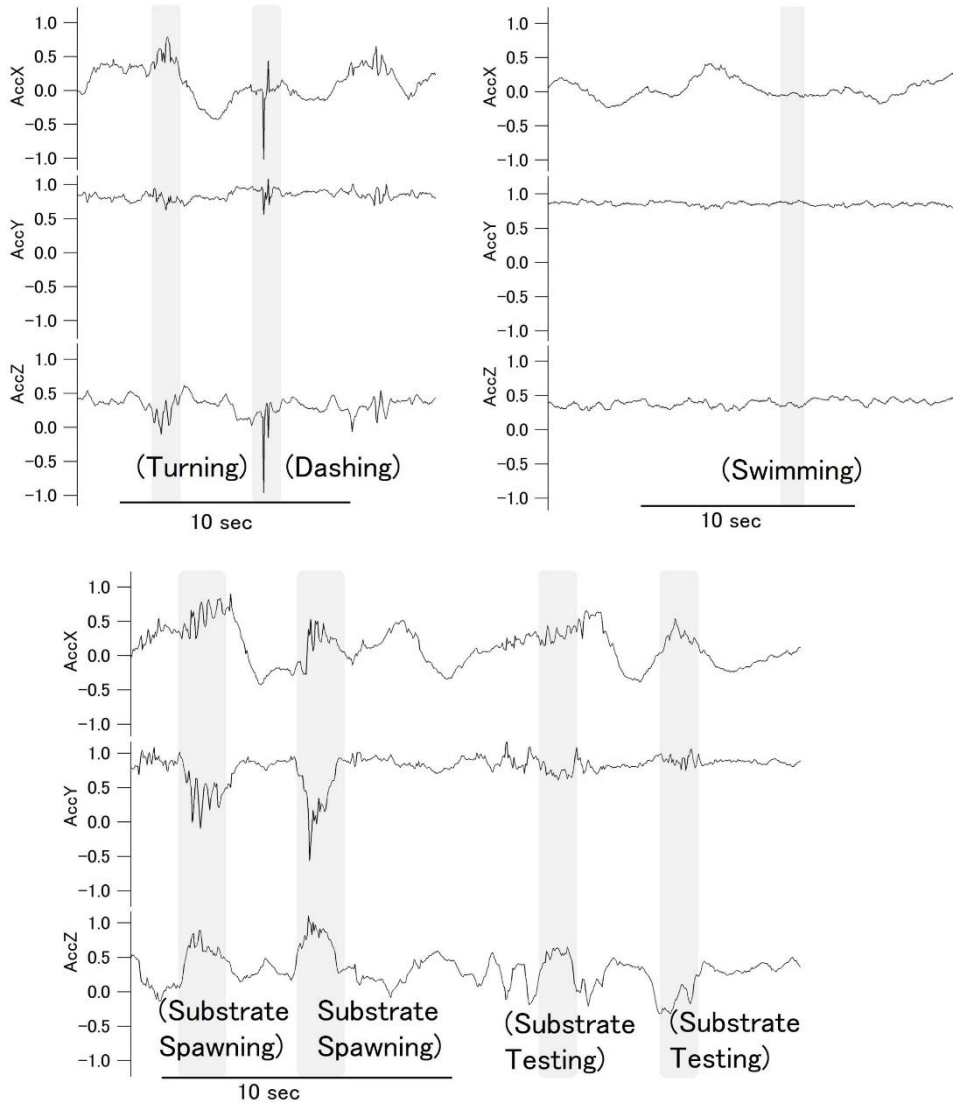


Fig. S4

Examples of raw acceleration data on each axis for five classified behaviors: swim, turn, dash, substrate spawning, and substrate testing. Gray areas indicate 1-s time windows. When a behavior lasted longer than 1 s, it was divided into consecutive 1-s segments.

Table S4

Individual data from the two small-scale experiments, in which 26 fish were used: 10 in the first experiment and 16 in the second. FL, fork length; BW, body weight; GW, gonad weight; GSI (Gonadosomatic Index) represents the percentage of the total body weight (after the experiment) of the gonads.

ID	FL (cm)	BW before Experiment (g)	BW after Experiment (g)	GW (g)	GSI	Sex	Logger equipment	Behavior labeling
1_1	26.5	251	185.5	31.8	17.1	M	○	○
1_2	25.8	184	156.5	2.5	1.6	F	○	
1_3	27.4	286	208.5	46.0	22.1	F	○	
1_4	25.5	226	151.6	2.2	1.5	M	○	○
1_5	27.5	298	196.5	4.7	2.4	F	○	○
1_6	25.0	202	164.0	27.7	16.9	M	○	
1_7	26.0	246	166.8	1.9	1.1	M	○	○
1_8	27.0	279	197.0	2.0	1.0	F	○	○
1_9	25.7	231	168.6	19.8	11.7	M	○	○
1_10	24.5		169.9	17.4	10.2	F		
2_1	30.2	285	249.5	29.0	11.6	M	○	
2_2	27.0	251	215.0	29.0	13.5	M	○	○
2_3	24.3	217	147.8	24.0	16.2	M	○	○

2_4	27.9	234	185.0	2.8	1.5	F	○	
2_5	30.0	412	237.0	3.6	1.5	F	○	○
2_6	29.5		265.5	15.8	6.0	M		
2_7	23.7		143.7	37.5	26.1	F		
2_8	27.0		188.0	2.5	1.3	M		
2_9	28.4		194.0	1.5	0.8	M		
2_10	25.0		157.0	4.0	2.5	M		
2_11	26.5		157.5	0.8	0.5	M		
2_12	31.5		240.0	3.0	1.3	M		
2_13	27.8		189.5	1.5	0.8	M		
2_14	28.6		252.0	62.5	24.8	F		
2_15	30.5		340.5	64.2	18.9	M		
2_16	31.0		283.0	57.2	20.2	M		

Movie. S1

Video of sperm egg behavior during the small-scale experiment. The fish that frequently released egg near the center of the tank was identified as female individual 2_5.