



## CAN SWIMMING DEPTH DATA FROM MULTIPLE PACIFIC HERRING INDIVIDUALS BE USED TO ESTIMATE CHARACTERISTICS OF THEIR SCHOOL? VERIFICATION BY MICRO BIO-LOGGERS

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## Acknowledgements

We thank Drs. Y. Mitani, K. Minami, M. Kobayashi who provided with invaluable and critical comments during this study. We sincerely thank Dr. M. Ichimura, and all members of Shibetsu Fisheries Cooperative Association who kindly helped the fieldwork. We also thank Natalie Kim, Ph.D., from Edanz Group ([www.edanzediting.com/ac](http://www.edanzediting.com/ac)) for editing a draft of this manuscript. Finally, we are grateful to anonymous reviewers of this manuscript. This work was supported by JST CREST Grant Number JPMJCR13A5, and Grant-in-Aid for JSPS Research Fellows (17J02165).

# CAN SWIMMING DEPTH DATA FROM MULTIPLE PACIFIC HERRING INDIVIDUALS BE USED TO ESTIMATE CHARACTERISTICS OF THEIR SCHOOL? VERIFICATION BY MICRO BIO-LOGGERS

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Key words: fish school, Pacific herring, bio-logging, neighbor distance.

## ABSTRACT

Bio-logging and bio-telemetry are effective methods for monitoring the activity of aquatic animals. Tagging technology has recently advanced, and it is now possible to use tracking devices on small fish and multiple individuals. For species that live in schools such as small pelagic fish, analyzing the activity of multiple individuals may help to further our understanding of school activity in nature. In this study, we tracked the swimming depth of multiple individuals of schooling Pacific herring and examined whether the school's characteristics and activity can be estimated from the individuals' swimming depth in the laboratory and a set net experiment. In the laboratory, herring formed dense schools during the day and scattered at night. The change in swimming depth in four tagged individuals often synchronized. A high frequency of short vertical neighbor distance (VND: 0-0.5 m) for a long duration (> 200 s) was typical in dense schools. In contrast, scattered schools were typically reflected by a wide distribution of VND for a short duration. Therefore, some school characteristics and activity in the laboratory can be estimated by VND and duration. However, in the set net, the diurnal difference was only observed in the VND distribution, and school characteristics could not be estimated clearly. Herring school characteristics and activity are influenced by school density and external stimuli such as light intensity

and predator presence. For detailed monitoring in natural habitats, experiments controlling school density and the introduction of additional devices such as acceleration or inter-communication loggers may be necessary.

## I. INTRODUCTION

In the past, the activity and movement of aquatic animals have been directly observed for relatively brief time periods using underwater acoustic methods (Masse et al., 1996), underwater cameras (Urquhart and Stewart, 1993; He, 2003) and the capture-and-release method (Hay et al., 2001). However, recent advances in bio-logging and bio-telemetry methods have made it possible to obtain continuous data on the activity of individual specimens. These methods, which involve attaching a micro bio-logger and acoustic or radio transmitter to the animal's body to obtain data, could previously only be used on large mammals and birds due to the large size of the devices and a low data recovery ratio (Boyd et al., 2004; Shillinger et al., 2012). The newer loggers are smaller and multifunctional, increasing the versatility of the bio-logging and bio-telemetry method. Researchers have started using them on fish, and methods for tagging and minimizing the influence of the attachments on the fish have been developed (Bridger and Booth, 2003; Brown et al., 2006; Jepsen et al., 2015).

In addition, the number of individuals that can be fitted with bio-loggers or transmitters in a study is gradually increasing as a result of growing support from large-scale bio-logging and bio-telemetry research projects (Welch et al., 2003; Cooke et al., 2012; Miyashita et al., 2014). As more activity data from multiple individuals become available, a better understanding of the relationships between individuals and their schools will be obtained in the near future. Mitsunaga et al. (2013) tracked the swimming depth of schooling yellowfin tuna, *Thunnus albacares*, around a fish-aggregating device using an acoustic transmitter and investigated school structure from the synchronized vertical movement of multiple individuals. Meanwhile, Noda et al.

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(2016) found that different-sized Pacific bluefin tuna, *Thunnus orientalis*, synchronized their swimming into school units by gliding at different times. In line with these reports, we expect to be able to visualize the activity of multiple individuals and schools by focusing on the similarities and differences in activity data for certain individuals. For small pelagic schooling fish in particular, being able to estimate the activity and characteristics of the school may lead to a deeper understanding of migration patterns and activity characteristics in natural habitats. Past studies have only presented data from multiple individuals of a school without any direct observation, and none have compared the activity of schooling individuals with the activity and characteristics of the school.

Bio-logging and bio-telemetry methods have been used successfully on small pelagic fish such as Pacific herring, *Clupea pallasii*, and Atlantic herring, *Clupea harengus*, which make up one of the most important fishery resources, especially in the far northern latitudes (Corten, 2002; Dickey-Collas et al., 2010). Since 2015, several bio-telemetry studies have been conducted in Norway, Alaska, and Japan (Langård et al., 2015; Bishop and Eiler, 2017; Tomiyasu et al., 2018). These studies reported on the spawning migration and behavior of these species based on the tracked movement of individuals; however, the relationships between individual movement and school characteristics, structure, and behavior were not identified. Understanding such relationships may allow for the estimation of school activity and characteristics from the tracking data of individuals, ultimately revealing more details on specific behaviors including spawning and aggregation in nature.

In this study, we tracked the swimming depth and vertical neighbor distance (VND) of multiple individuals of Pacific herring using micro bio-loggers. We compared these metrics with data on school activity and characteristics that were obtained via direct observation in a laboratory experiment. The same assessment was conducted in a set net, without the direct observation. Finally, we evaluated the potential of using the tracking data from multiple individuals to estimate Pacific herring school activity and characteristics.

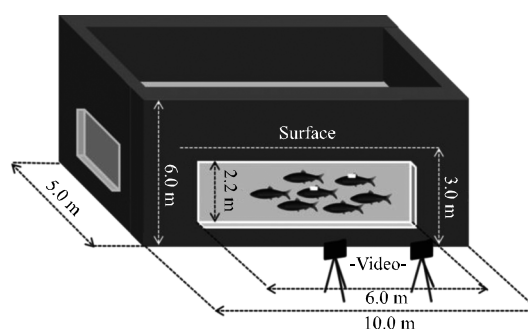
## II. MATERIALS AND METHODS

### 1. Laboratory Experiment

A laboratory experiment was conducted using a large experimental water tank (10 m width × 5 m length × 3 m water depth) at the Hakodate Research Center for Fisheries and Oceans (Hokkaido, Japan) on 3-7 June, 2016 (Fig. 1). Pacific herring ( $n = 138$ ;  $211 \pm 28$  mm: Mean length  $\pm$  SD) were sampled using a coastal set net at Shibetsu in eastern Hokkaido on 6 June, 2016. After collection, the animals were placed in a recovery tank for 3-h and then transported to the experimental water tank within 10-h. During transportation, the water temperature was kept under 10°C to control the activity of the fish. The experiment began after a 24-h recovery period from the tagging and release of all fish. Fish that died ( $n = 36$ ) during transportation

**Table 1. Fork length of tagged individuals in each experiment.**

Experiment	Individuals	Fork length (mm)
Water tank	HR_01	215
	HR_02	220
	HR_03	220
	HR_04	250
Set net	Set net HR_01	262
	Set net HR_02	245



**Fig. 1. The large experimental water tank at the Hakodate Research Center for Fisheries and Oceans. Two digital cameras were installed in front of the tank window.**

were excluded from analysis. During the experiment, day and night were defined as 8:00-20:00 and 20:00-8:00, respectively, and the diurnal light intensity was set to 50-100 and 0-10 lx, respectively.

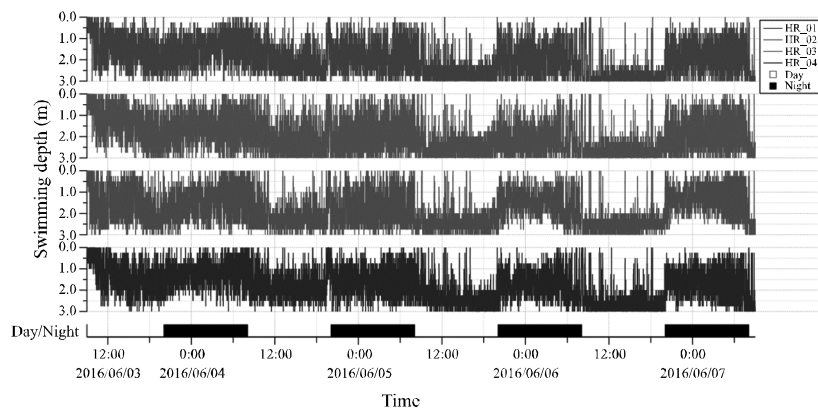
### 2. Measurement of the Swimming Depth of Individuals and School Characteristics

Micro bio-loggers (11 × 35 mm, 5.1 g, 20-s interval; LAT1400; LOTEK Inc., Newmarket, ON, Canada) were attached to ten herring individuals to track swimming depth. The external attachment method was used due to the sensitivity of the herring to handling (Jepsen et al., 2015; Tomiyasu et al., 2018). To attach the logger, the upper side of the body in front of the dorsal fin was pierced using a 3-mm-radius needle, and the logger was attached using a cable tie. Four individuals, HR\_01-04, displayed normal swimming without lurching and sinking during the experiment, and were thus used for analysis (Table 1).

For comparison with the tracked swimming depth of the individuals, the school structure of the sample fish in the experimental water tank was recorded from the front observation window using a digital video camera (HDR-CX900; HDR-SR12; SONY Inc., Tokyo, Japan). Two video cameras were installed to continuously record the entire area of the observation window and the swimming behavior of all fish (Fig. 1). Additional direct observations were made in 3-h intervals throughout the day.

### 3. Set Net Experiment

To assess an environment in which it was not possible to observe the school, the swimming depths of three herring individuals



**Fig. 2.** Swimming depth of the four tagged individuals in the laboratory experiment. The black bars at the bottom show the period at night (20:00-08:00) when the light intensity was 0-10 lx.

were tracked using micro bio-loggers (11 × 35 mm, 5.1 g, 10-s interval; AZBL003-100; AI Technology Inc., Tokyo, Japan) in a set net with a trap net depth and width of about 6 m each. Approximately 200 herring were initially caught in a set net on 10 June, 2017. After sampling, the bio-loggers were attached to three fish in the same manner as in the laboratory experiment, and only tagged individuals were released into the set net. On the morning of 11 June, the tagged individuals and approximately 200 herring were caught. Two specimens, Set net HR\_01 and Set net HR\_02, were used for analysis (Table 1).

#### 4. Analysis

The continuous swimming depth of tagged individuals in each experiment was plotted on a time-series profile and compared with the frequency of the respective swimming depth and vertical movement distance. The vertical movement distance was calculated from the sum of the absolute difference between adjacent swimming depth data in the time-series profile. To compare the activity of the individuals in the laboratory and set net, vertical movement distance was converted into meters per each hour. The VND time-series was calculated from the absolute difference between the swimming depth of two tagged individuals at the same time point (Mitsunaga et al., 2013). In the laboratory experiment, which yielded swimming depth data from four individuals, a total of six different VND time-series were calculated. Finally, the diurnal pattern of the frequency distribution of VND and the duration of the respective VNDs were calculated from the respective VND time-series. Igor Pro ver. 6.1.2.2 (WaveMetrics Inc., Lake Oswego, OR, USA) was used for the time-series swimming data analysis.

From the video recording snapshots collected at 3-h intervals, the school structure was quantified using the ImageJ processing program (Schneider et al., 2012). For the image analysis, a school was defined as at least three fishes having the same swimming direction and a neighbor distance between adjacent individuals shorter than two fork lengths (Partridge and Pitcher, 1980; Torisawa et al., 2007; Larsson, 2012). In video recordings taken from only one direction, the size of an object changed

relative to its position from the camera. In this study, the size of an object was estimated along the length of the water tank by comparing the video records of objects with the known lengths of tagged individuals in the snapshot used for analysis. The factors related to school structure (i.e., width (m), height (m), depth (m), number of individuals (Ind), area (m<sup>2</sup>), aspect ratio (%), density (Ind/m<sup>2</sup>), and nearest neighbor distance (NND; mm)) were measured for individuals in the upper, lower, and side portions of the tank. The depth of the school was defined as the crossing position of the lines extending from the respective ends of individuals in the school. The aspect ratio was calculated as the percentage of the value of width over height of school. NND was calculated as the length between the mouth of the central individual and that of the adjacent individual. R ver. 3.4.0 was used for all statistical analyses (R Development Core Team, 2017).

### III. RESULTS

#### 1. Swimming Depth of Tagged Individuals in the Laboratory Experiment

During the laboratory experiment, a school consisting of 40-70 individuals and several individuals swimming alone was observed. A diurnal change in behavior of the school was observed, forming a dense school and synchronized swimming during the day, and scattering and unsynchronized swimming at night. HR\_01-04 swam together in the school with other untagged individuals. The mean swimming depth of tagged individuals during the day and at night were  $2.11 \pm 0.79$  m and  $1.68 \pm 0.72$  m (Mean depth  $\pm$  SD), with fish concentrating around the bottom of the tank during the day and moving toward the surface at night (*t*-test,  $p < 0.05$ ; Fig. 2). The vertical movement distance was greater at night than during the day, e.g.  $326.96 \pm 23.55$  m each h and  $435.93 \pm 27.04$  m each h (Mean  $\pm$  SD; Mann-Whitney U test,  $p < 0.05$ ).

A 49.3% frequency was observed for VNDs of 0.0-0.5 m during the day, which declined to 34.5% at night (Fig. 3). Meanwhile, VNDs over 1.0 m were more frequent at night

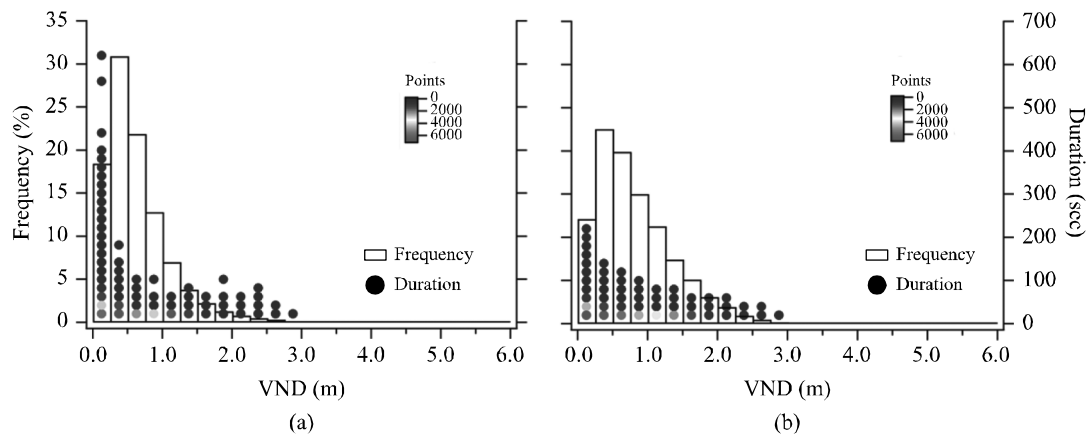
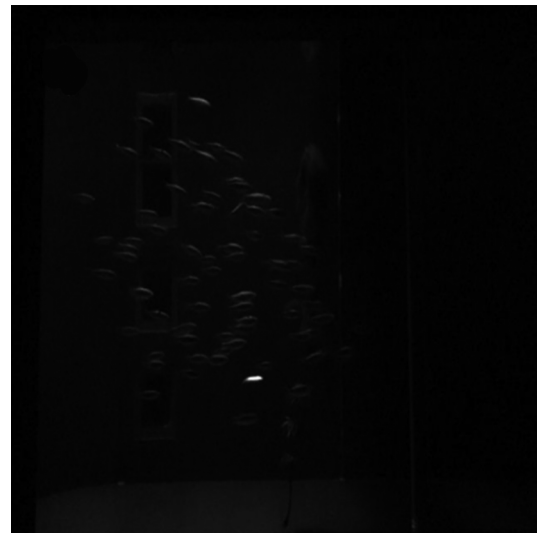


Fig. 3. Frequency distribution of the vertical neighbor distance (VND) and duration of each VND in the laboratory experiment during the day (08:00-20:00; left) and at night (20:00-08:00; right). The color indicates the frequency of the duration of each VND.



(a) Day



(a) Night

Fig. 4. Snapshots of school structure during the day (a) and at night (b).

(day: 16.0%; night: 30.4%). A longer duration of the same VND was found for VNDs of 0.0-0.25 m (day: 20-620 s; night: 20-220 s), with durations over 200 s often seen during the day (day:  $n = 205$ ; night:  $n = 2$ ). The shortest VND duration, 20 s, was found more frequently at night in all VND ranges (day: 24.1%; night: 31.1%).

## 2. Measurement of School Structure

A diurnal change in school structure was observed in the laboratory experiment (Fig. 4). School structure, height, aspect ratio, and area differed significantly between day and night (Table 2). The individuals formed a dense school with a horizontal shape during the day, but then scattered vertically and horizontally at night. Because the number of individuals in the school did not change remarkably, the density was lower and VND was larger at night (Table 2).

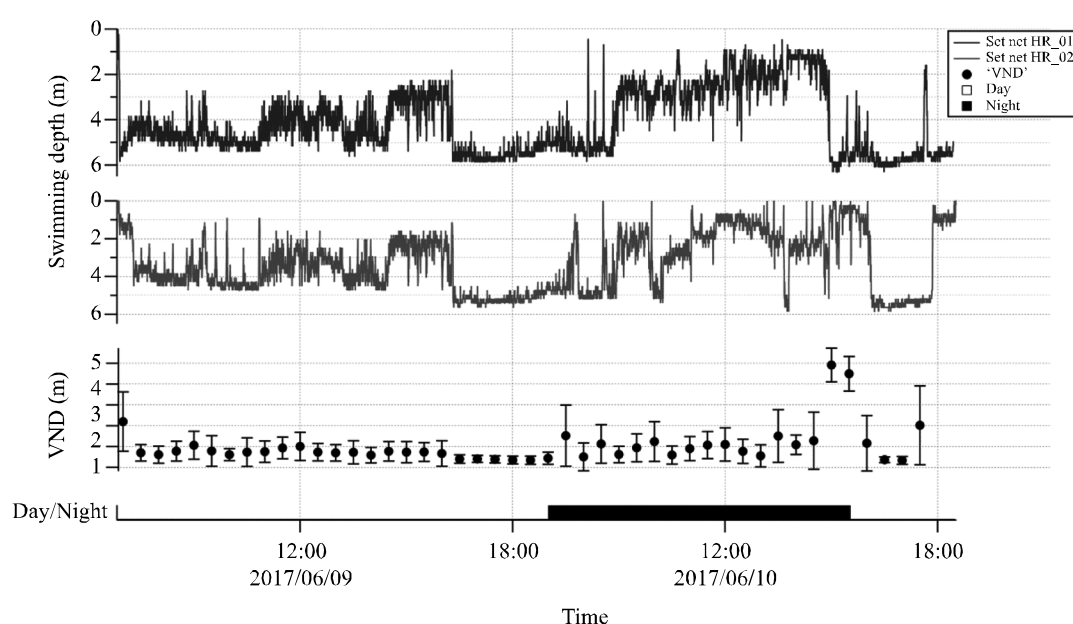
## 3. Swimming Depth of Tagged Individuals in the Set Net

As in the laboratory experiment, the swimming depth of Set net HR\_01 and Set net HR\_02 tended to be around the bottom of the set net during the day and close to the surface at night, e.g.,  $4.22 \pm 1.31$  m and  $2.85 \pm 1.51$  m (Mean  $\pm$  SD; Mann-Whitney U test,  $p < 0.05$ ; Fig. 5). However, the vertical movement distance was shorter than that seen in the laboratory experiment and did not differ significantly between day and night, e.g.,  $77.7 \pm 9.9$  m each h and  $76.9 \pm 3.4$  m each h (Mean  $\pm$  SD; Mann-Whitney U test,  $p > 0.05$ ).

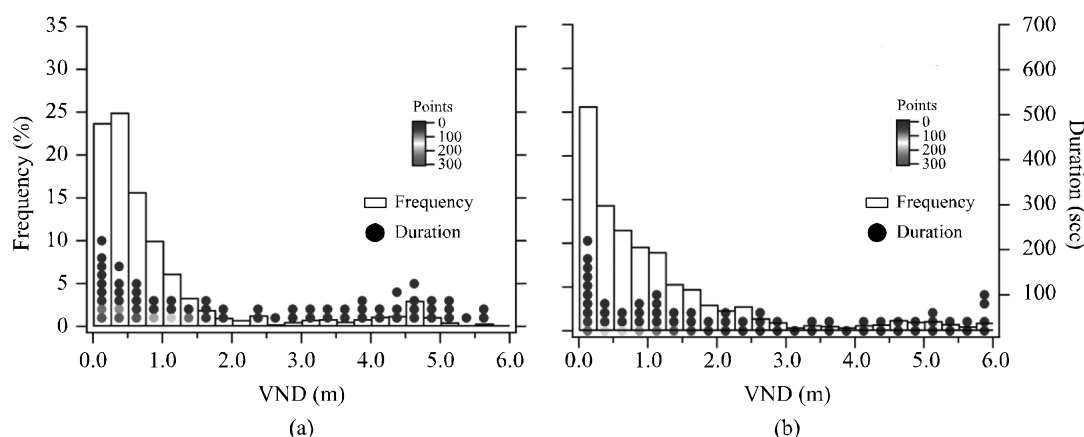
A 49.3% frequency was found for VNDs of 0.0-0.5 m during the day, which declined to 38.9% at night (Fig. 6). VNDs over 1.0-3.0 m were more frequent at night (day: 15.5%; night: 30.2%). Moreover, VND values were concentrated between 0.25-0.5 m at specific times around dawn (4:30-5:00) and dusk

**Table 2. Factors affecting school characteristics during day and night. The paired *t*-test and Wilcoxon signed-rank test were used for the statistical analysis.**

		Feature values		Significance
		Day (8:00-20:00)	Night (20:00-8:00)	
Height	(m)	1.44 ± 0.61	2.00 ± 0.39	Day < Night; <i>P</i> < 0.05
Width	(m)	2.30 ± 0.55	2.12 ± 0.46	N/A
Aspect rate	(%)	70.17 ± 38.74	98.28 ± 29.71	Day < Night; <i>P</i> < 0.05
Area	(m <sup>2</sup> )	1.62 ± 0.43	2.34 ± 0.63	Day < Night; <i>P</i> < 0.05
Number of individuals	(n)	57.29 ± 11.51	53.63 ± 13.22	N/A
Density	(Ind/m <sup>2</sup> )	37.13 ± 10.23	24.06 ± 7.90	Day > Night; <i>P</i> < 0.05
NND	(m)	0.16 ± 0.05	0.23 ± 0.06	Day < Night; <i>P</i> < 0.05



**Fig. 5. Swimming depth and average VND per 30 minutes (Mean ± SD) for the two tagged individuals in the set net experiment. The black bars at the bottom show the night period (19:00-03:30) from sunset to sunrise.**



**Fig. 6. Frequency distribution of the vertical neighbor distance (VND) and duration of each VND in the set net experiment during the day (03:30-19:00; left) and at night (19:00-03:30; right). The color indicates the frequency of duration of each VND.**

(16:30-19:00) (Fig. 5). While a longer duration of the same VND was found for VNDs of 0.0-0.25 m, which was similar to the laboratory experiment, the actual duration was shorter than that

observed in the laboratory experiment (day: 10-130 s; night: 10-230 s) (> 200 s: day: *n* = 0; night: *n* = 1). During the day, some VND values between 4.0-6.0 m were observed, with the most

frequent duration being 60-90 s.

#### IV. DISCUSSION

##### 1. Behavior of Herring Individuals and Influence of Bio-Logger Attachment

With the recent developments in bio-logging and bio-telemetry technology, many studies have discussed the influence of attaching electric tags onto target animals (Bridger and Booth, 2003; Brown et al., 2006; Jepsen et al., 2015). It is essential to select a suitable size, weight, and tagging method for the target animal to enable the precise evaluation of behavior and reduce the influence of handling and attaching tags. To decrease the influence of tag weight, we used small and light tags that were less than 2% of the herring's body weight, based on the suggestion of Winter (1983) and Makiguchi and Kojima (2017). Moreover, the length of the tagging and handling period was decreased using the external tagging method, which involves attaching the tags relatively quickly as detailed in Tomiyasu et al. (2018) and Eiler and Bishop (2016). During the laboratory experiment, tagged individuals swam with untagged individuals to form a school, confirming that tagged herring individuals schooled together with untagged individuals in a laboratory environment. Moreover, the school showed the same behavioral tendencies seen in the natural habitat, forming a dense school during the day and scattering at night (Huse and Korneliussen, 1995). This was reflected in the logger data, which also indicated that tagged individuals swam normally in the same manner as untagged individuals.

##### 2. Diurnal Changes in School Structure and Individual Swimming in the Laboratory Experiment

In the laboratory experiment, the structure of the school changed from a dense one with a horizontal shape during the day to a vertically and horizontally scattered one at night. Schooling behavior in aquatic animals including fish generally provides benefits in terms of reduced energy consumption (Weith, 1973; Hemelrijk et al., 2015), avoidance of predators (Partridge 1982), and increased probability of encountering prey (Pitcher et al., 1982). The school structure can change and is influenced by the behavioral strategy and physiology of individuals. In situations where animals prioritize the avoidance of predators, individuals can form dense and strongly synchronized schools (Nottestad et al., 2002; Parrish et al., 2002). When prioritizing prey foraging, individuals generally form low-density and unsynchronized schools because their own benefits take precedence (Pitcher et al., 1982; Calovi et al., 2014). In contrast, species such as the Arabesque greenling *Pleurogrammus azonus* show strongly synchronized schooling that induces vortex flows, which concentrates prey from the surface and forces them downward, creating an ample feeding environment (Kitagawa et al., 2011). In the case of Pacific herring, Huse and Korneliussen (1995) and Misund et al. (1997) reported that Atlantic herring migrate in large, dense schools at 300-400 m depth during daytime. Such school structure and deep swimming depth in daytime may indi-

cate that the herring prioritize to minimize predation risk (Fernö et al., 1996). A large part of population migrates vertically to upper water layer and scatters at night when the risk of predation from visual predators is decreased. This migration and swimming depth change for variation in external influence such as distribution of food (Mackison, 1999). However, it observed during non-feeding hibernation period, herring may maintain their established diurnal vertical migration in order to keep contact with potential navigational cues at the surface (Misund et al., 1997). In this study, a diurnal change in behavior of the school was observed, forming a dense school and synchronized swimming during the day, and scattering and unsynchronized swimming at night. A longer vertical movement distance was observed at night. Therefore, the behavior and structure of school was likely influenced by such diurnal differences in activity and the prioritized tactics of individuals, it showed the similar behavioral tendencies seen in the natural habitat even though there were no prey and predator in the small-scale experimental tank.

In addition to the influence of individual behavioral strategy, the diurnal difference in the prioritization of sensory organs might have influenced the school characteristics in this study. Fish use different sensory organs for communication within schools, with light intensity being an important environmental factor (Partridge and Pitcher, 1980; Larsson, 2012). During the day, they tend to mainly use their sense of sight and form schools with a short NND, while at night, they mostly use their sense organs in the lateral line and form schools with a long NND.

Diurnal differences were also observed in the VND of multiple individuals and the durations of their respective VNDs. During the day, individuals tended to form a dense school with a high frequency and long duration of VNDs between 0.0-0.25 m. Short VND durations were not often observed, except at night. These results likely reflect the characteristics and activity of schools in daytime. The times when schools are dense and synchronized can probably be estimated using the frequency and VND duration data of multiple individuals. Herring are highly sensitive to external stimuli such as light intensity and predators and swim in a strongly synchronized manner (Nottestad et al., 2002; Parrish et al., 2002); therefore, it may be possible to estimate periods when schools prioritize predator avoidance and quick movement.

At night when the individuals scattered, a wide distribution of VND frequencies was observed, with a relatively short duration of 20 s accounting for 30% of all continuous VND measurements. As with the daytime observations, these results likely reflected the characteristics and activity of the school at night when individuals showed unsynchronized swimming and used the lateral line as the main sensory organ. Huse and Ona (1996) reported that the distribution of tilt angle in herring became wider at night because they showed rise and glide swimming strategy during vertical migration at night; therefore, such active movements by individuals probably led to the wider distribution and shorter duration of a given VND at night in this study. Overall, the periods when the school is scattered and unsynchronized can probably be estimated by the width of the distribution and



shortness of a particular VND duration. However, these tendencies may be observed in swimming data from multiple individuals forming either several or no schools, for more precise estimations, it is necessary to confirm this using data from non-schooling fish. It is also important to note that the resolution of the depth loggers used in this study was 0.25 m. For more precise monitoring, a depth logger with a greater resolution should be used, because it is possible that herring individuals may have VNDs less than 0.25 m.

### 3. Estimation of School Characteristics in the Set Net

As in the laboratory experiment, diurnal differences were observed in the swimming depth and VND of the two tagged individuals in the set net experiment. This may reflect the behavioral tendency of Pacific and Atlantic herring in natural habitats, which form dense schools in the deeper layers during the day and rise closer to the surface and scatter at night (Huse and Korneliussen, 1995; Misund et al., 1997). However, individuals had brief durations of short VNDs measuring 0.0-0.25 m during the day and a large VND of over 1.0 m and brief duration at night. Around dawn and dusk, VND values were concentrated between 0.25-0.5 m. This indicates that individuals tended to aggregate during the day, albeit with poorly synchronized swimming except for specific periods around dawn and dusk. The strength of swimming synchronization is influenced by swimming speed (Patridge et al., 1980), and recent studies have indicated that it is also influenced by the number of individuals in a school (Cambui and Rosas, 2012; Calovi et al., 2014). With a small number of individuals, individuals tend not to constitute a three-dimensional school, but rather tend to track each other (Patridge et al., 1980). In all type of set net fishery, the number of fishes in the set net fluctuates throughout the day (Akiyama and Arimoto, 1997). In our experiment, only three tagged individuals were released in the set net after the morning operation of set net. From these, the number of fish in the set net was estimated to be small when the tagged individuals released, which probably influenced the swimming synchronization of individuals. In contrast, around dawn and dusk when fish activity changes, the number of fishes in the set net might have fluctuated considerably and induced the strongly synchronized swimming of tagged individuals. In addition, the volume of the set net was about four times that of the experimental water tank; therefore, it is possible that the space was too large for the tagged fishes to meet frequently and school.

Our results suggest that it is possible to estimate the school characteristics and activity from the swimming data of multiple herring individuals in a limited environment with a controlled number of fish and light intensity. The distribution and duration of the VND likely reflects the school structure when individuals aggregate and strongly synchronize their behavior. Yet the influence of the available space and fluctuations in fish numbers must be considered when monitoring wild populations. Pacific and Atlantic herring tend to aggregate and scatter during their spawning migration (Skeret et al., 2002). In the future, it may become possible to detect these behavioral events during spawn-

ing migrations using the swimming data of multiple individuals. Recent advancements in electric tagging technology and the growing number of studies using it have led to an increase in the versatility of these methods (Welch et al., 2003; Cooke et al., 2012; Miyashita et al., 2014). Still, it is not easy to track the behavior of multiple individuals in their natural habitat because of the high cost. The depth logger used in this study is the most basic device available for bio-logging and bio-telemetry and has a relatively low cost; therefore, this technology will likely be used widely for behavioral monitoring of multiple individuals and school. However, it is important to consider the limitations of the swimming depth data. To verify the relationship between the horizontal position of respective individuals in the school, and the school structure, it is necessary to apply tags with additional functions including acoustic transmitters, acceleration logger and inter-communication loggers (Miyashita et al., 2014). Further verification of the relationship between school characteristics and the swimming data of target species will lead to more precise behavioral monitoring of schools in their natural habitats.

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