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1	Diel migration pattern of pink salmon fry in small streams
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15	
16	Ethical Statement
17	All fish collection was legally conducted in accordance with the permit from the
18	Hokkaido Government. All animal handling in the surveys was conducted according to
19	the guidelines of animal care at Hokkaido University.
20	
21	Abstract
22	Downstream migration is a critical stage in the anadromous salmonid life cycle; however,
23	previous studies have shown different results between rivers or surveys for the diel
24	downstream migration pattern of the fry of the pink salmon Oncorhynchus gorbuscha.

25	We investigated the diel migration pattern of pink salmon fry in three small streams. Our
26	results showed that pink salmon fry migrate mainly within a few hours after sunset; 89.9%
27	of migration occurred between 18:00 and 23:00. Therefore, the results indicated that
28	sunset time influences the diel migration pattern of pink salmon fry in small streams. This
29	pattern could be a predator-avoidance behaviour.

31 Key Words: Salmonidae, salmonids, movement, behaviour, freshwater, stream.

Many animals exhibit "migration" as a behaviour to acquire more favourable feeding 32 grounds, habitats, and spawning sites (Dingle, 2014; Dingle & Drake, 2007). 33 Anadromous salmonids migrate according to the stages of their life cycles (Quinn, 2018): 34 "downstream migration" to rapidly grow using marine environments with better growing 35 conditions than those of most freshwater habitats and "homing migration" to return to 36 37 natal rivers to spawn. Downstream migration is a critical stage of the anadromous salmonid life cycle because the high mortality of salmon during downstream migration 38 affects adult populations and overall population persistence (Thorstad et al., 2012; 39 40 Williams *et al.*, 2001).

41 Several salmonids perform downstream migration at night (Hintz & Lonzarich, 2012; Johnston, 1997; Pavlov & Mikheev, 2017). In general, except for some large rivers, 42 high turbidity conditions, and polar-day conditions, the fry of the pink salmon 43 Oncorhynchus gorbuscha perform downstream migration at night (Ali & Hoar, 1959) and 44 45 finish their migration overnight (Heard, 1991); however, since the previous studies about diel migration of pink salmon have been conducted in rivers of various sizes (e.g., Kirillov 46 et al., 2018; Neave, 1955; Pavlov et al., 2015), these studies have shown different results 47 48 between rivers or surveys. Thus, a comprehensive understanding of the diel migration pattern of pink salmon fry has not been achieved. Here, we examined the diel migration 49 pattern of pink salmon fry in three small streams on the Shiretoko Peninsula, Japan. Since 50 small streams have less variation in environmental factors along their course, they are the 51 52 most suitable model system for examining the diel migration pattern of pink salmon fry.

We selected three small streams on the Shiretoko Peninsula as the study streams
(44.07°N, 145.12°E; Table 1): Chienbetsu stream, Funbe stream, and Syoji stream.
Hatchery programs have never been implemented in these streams, and natural spawning

sustains the pink salmon populations (T. Yamada, unpublished data). In each stream, we
established a sampling point for salmon fry that was located near the mouth of the stream
and was not affected by sea waves.

59 In May 2021, to determine the diel migration pattern of pink salmon, we 60 conducted fish collection over a 24-h period in each stream (Table 1). In Japan, the peak of downstream migration of pink salmon fry occurs in May. We placed two drift nets 61 (Matsui Corp., Tokyo, Japan; 50 cm squared opening, 100 cm long, 3 mm mesh) at a 62 sampling point for 15 minutes at hourly intervals. Since long-term sampling with drift 63 nets would result in the death of many fry, we limited the sampling effort. We used stakes 64 65 to fix the nets to the streambed so that the net openings ranged from the stream surface to the streambed. Drift nets have often been used to capture the fry of salmonids (Hintz & 66 Lonzarich, 2012; Johnston, 1997). We recorded the number of individuals without using 67 anaesthesia and then immediately released all individuals. All surveys started at 10:00 68 69 and finished at 09:15 the following day. To evaluate stream discharge at each sampling 70 point, we constructed a transect and measured the wetted width. Then, we measured the depth and current velocity at $0.2 \times$ depth and $0.8 \times$ depth at ten points along the transect. 71 72 We calculated the sectional areas between the measurement points or between the measurement point and the streambank, multiplied each sectional area by the average 73 velocity, and summed them to calculate stream discharge. To evaluate the water filtered 74 75 by drift nets, we measured the wetted sectional area of the net and current velocity at 0.2 76 \times depth and 0.8 \times depth at three points (left, right, and centre) on each net. We multiplied the wetted sectional area of each net by the average velocity and summed them to 77 calculate filtered water by drift nets. All measurements were taken between 15:00 and 78 79 17:00. Finally, we used the ratio of the water filtered by drift nets to stream discharge to

80 estimate the total migrants in each stream at each time point.

Then, we analysed the data using a generalized additive mixed model (GAMM) with a binomial distribution and logit link function. We used the migration activity of pink salmon fry as a response variable and the cumulative time from survey start time as a smooth term and stream ID as a random effect. Migration activity represents the proportion of the estimated number of migrants at each time to the estimated total number of migrants obtained through each 24-hour survey. All data analysis was conducted with R v. 4.1.2 (R Core Team, 2021) using "mgcv" v. 1.8.38 (Wood, 2004) for GAMM.

From April to June 2021, we conducted a "peak survey" to confirm whether the 88 downstream migration pattern obtained from the 24-hour survey described above is 89 90 universal. This peak survey was conducted six, six, and three times in the Chienbetsu 91 stream, Funbe stream, and Syoji stream, respectively. The survey was conducted between 18:00 and 22:15 based on a previous study conducted in a stream with a runnable reach 92 93 length close to those in our study streams (Neave, 1955). We used the same method to 94 catch pink salmon fry as that used in the 24-hour survey. Additionally, we estimated the total number of migrants using the same method as that used in the 24-hour survey. We 95 96 conducted all surveys, including 24-hour surveys, when the water in the streams was transparent and flooding conditions were not observed. In addition to pink salmon, the 97 fry of chum salmon O. keta and juveniles of Dolly Varden Salvelinus malma were also 98 99 collected in these surveys.

100 The 24-hour survey showed that pink salmon fry mainly migrated downstream 101 within a few hours after sunset (Figure 1A). The model indicated that time was significant 102 $(P < 0.0001, \text{ adjusted } \mathbb{R}^2 = 0.564)$, with 89.9% of the migrations occurring between 18:00 103 and 23:00 (Figure 1A). Additionally, all peak surveys indicated that the peak for 104 downstream migration of pink salmon fry was between 19:00 and 22:00 (Figure 1B). The results indicated that sunset time influences the diel migration pattern of pink salmon fry 105 in small streams. Pink salmon fry emerged from the riverbed and moved actively to the 106 107 current for downstream migration in the first two (maximum three) hours after nightfall 108 (Pavlov et al., 2019). Therefore, our study shows that individuals emerging in small 109 streams quickly migrated to the sea. Other salmonids show nocturnal downstream migration: the brown trout Salmo trutta, coho salmon O. kisutch, Atlantic salmon S. salar, 110 and rainbow trout O. mykiss (Hintz & Lonzarich, 2012; Johnston, 1997; Pavlov & 111 112 Mikheev, 2017). Nocturnal migration may be a strategy to avoid predation (Ibbotson et al., 2011; Johnston, 1997; Kennedy et al., 2018). Selection may have favoured the 113 114 nocturnal migration strategy in pink salmon fry, as observed in other salmonids.

There are several reasons why the peak of downstream migration for pink salmon 115 fry occurs within a few hours after sunset. First, early night may be the best time to avoid 116 117 predation. The Dolly Varden, which occasionally preys on fish (Denton et al., 2009), is 118 dominant in the studied streams (T. Yamada, unpublished data). The foraging activity of the Arctic charr S. alpinus, which is closely related to Dolly Varden, declines just after 119 120 sunset (Björnsson, 2001). If Dolly Varden have the same feeding pattern as that of Arctic charr, then the best time for pink salmon fry to avoid predation is just after sunset. An 121 alternative explanation for the timing of peak migration is the strategy of completing 122 123 downstream migration during the night in rivers with various channel lengths. If pink 124 salmon spawned far from the mouth of the river, then fry would need a longer time to 125 reach the mouth of the river. Therefore, by starting downstream migration immediately 126 after sunset, pink salmon fry can reach the river mouth at night in small streams when 127 predation pressure is low. Pavlov et al. (2019) suggested that pink salmon in a longer

river would repeat this behaviour for several nights. Pink salmon, with their higher straying rate among Pacific salmon (Hendry *et al.*, 2004), may have adapted to complete their downstream migration at night even if they were born in rivers different from their natal rivers.

132 This study revealed the diel migration pattern of pink salmon fry; however, 133 previous studies have shown different results between the studied rivers or between surveyed dates (Kirillov et al., 2018; Neave, 1955; Pavlov et al., 2015). Genetic drift 134 between neighbouring river populations of pink salmon often occurs (Hendry *et al.*, 2004) 135 136 due to their higher straying rate. Therefore, genetic differences between neighbouring river populations in the same year class are small (Aspinwall, 1974; Gharrett et al., 1988; 137 138 Hendry et al., 2004; Shaklee et al., 1991); thus, the differences in diel patterns between neighbouring rivers should be determined by factors other than genetic factors. By 139 selecting rivers as study sites with different lengths of spawnable reaches between the 140 141 studied rivers (range 1.6–16.1 km) or longer spawnable reach lengths (maximum distance 142 21 km), these studies may not have identified the diel migration patterns of pink salmon fry. In the case of differing spawnable reach lengths between rivers, even if pink salmon 143 144 fry start their downstream migration at the same time, the time for the fry to reach the mouth of the river would vary among rivers. Additionally, when rivers have longer 145 spawnable reach lengths, the location of the spawning redds makes a difference in terms 146 147 of the time it takes to reach the mouth of the river. In this study, by selecting streams that 148 have the same spawnable reach lengths between streams and short spawnable reach lengths, we were able to elucidate the diel migration pattern. 149

This study indicated that pink salmon fry exhibit a diel downstream migration pattern that depends on the time of sunset in the small streams. The diel pattern of

152	organisms is affected by light pollution (Longcore & Rich, 2004). Pink salmon fry avoid
153	light (Hoar et al., 1957); therefore, the diel pattern of pink salmon fry may change
154	dramatically with light pollution, and the survival rate of salmon fry may decline as
155	optimal downstream strategies cannot be selected. Future studies should examine the
156	effect of light pollution on the diel pattern and survival of pink salmon fry.
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162	their helpful comments on the sampling method.
163	
164	
165	Contributions
166	T. Y. and H. U. conceived the ideas. T. Y. conducted the field survey, performed the
167	analyses, and led the writing. All authors read and commented on the manuscript.
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247

248Table captions

- 249 Table 1: Physical characteristics and environmental conditions on the dates of the 24-
- 250 hour survey of the three studied streams.
- 251 *Note*: RunnableL represents the reach length from the sampling point to the unpassable
- dam or fall; DistFromSG represents the distance between the upstream edge of the main
- spawning grounds and the sampling points.

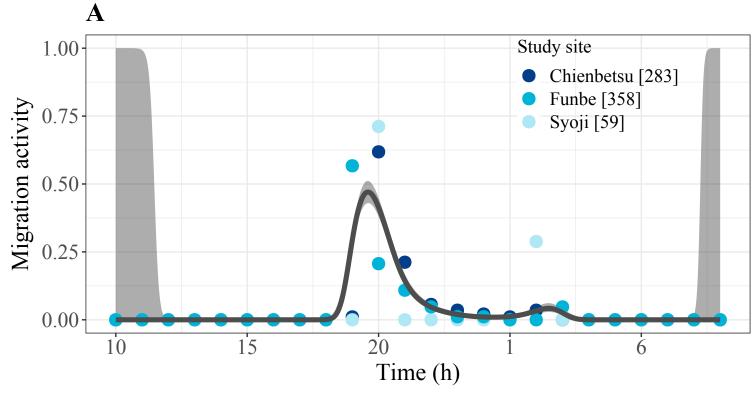
Metric	Chienbetsu stream	Funbe stream	Syoji stream
RunnableL (m)	409.1	348.2	211.3
DistFromSG (m)	326.5	348.2	169.2
Start date	2021-05-15	2021-05-05	2021-05-21
Sunset time	18:38:17	18:27:43	18:44:42
Sunrise time	3:51:50	4:04:24	3:46:06
Sunshine duration (min / 24 h)	334	411	0
Weather condition at night	Cloudy	Cloudy then clear	Cloudy
Precipitation (mm / 24 h)	0.0	0.0	0.0
Lunar age	3.3	23.0	9.3
Average water tempereture (C°)	8.1	6.3	7.8
Stream discharge (m ³)	65.1	91.6	166.8
Filtered water by drift nets (m ³)	20.5	21.2	19.7

Table 1 Physical characteristics and environmental conditions on the dates of the 24-hour survey of thethree studied streams.

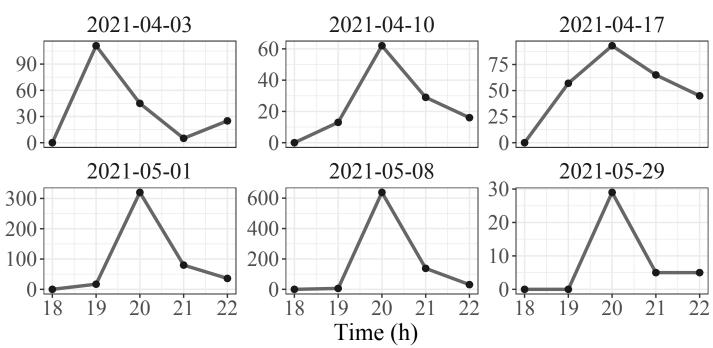
Note : RunnableL represents the reach length from the sampling point to the unpassable dam or fall; DistFromSG represents the distance between the upstream edge of the main spawning grounds and the sampling points.

1 Figure captions

Figure 1: (A) Estimate of migration activity in relation to time in three streams.
Confidence interval (95%) is shown as the shaded area. The numbers in the brackets
indicate the estimated total number of pink salmon *Oncorhynchus gorbuscha* fry. (B)
Results of the peak survey in the Chienbetsu stream (1), Funbe stream (2), and Syoji
stream (3).







B-2

