Identifying spawning events in the Japanese flounder *Paralichthys olivaceus* from depth time-series data

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Abstract

Vertical swimming events (VSEs) of the Japanese flounder, *Paralichthys olivaceus*, recorded by high-frequency depth data loggers were analysed to identify spawning events. In total 25907 VSEs from 10 adult fish were classified into 4 clusters using a *k*-means method. VSEs in a specific cluster (cluster-S) characterised by accelerated vertical swimming were identified as possible spawning events. Both the descent (0.43 ± 0.22 body length s^{-1}) and ascent rates (0.43 ± 0.24 body length s^{-1}) of VSEs in cluster-S were more than 4 times faster than in any other VSE. Our analyses indicated that 4 individuals exhibited the spawning events during the recording periods. The estimated spawning frequency ranged from 0.74 to 0.90 events day^{-1}. These values were comparable to those obtained in other field and laboratory studies. The spawning condition of fish at the time of recapture was confirmed by separate histological and anatomical observations, which supported the cluster analysis results. These results suggest that a clustering technique is successfully applied to behavioural time-depth data originating from free-swimming flatfishes that exhibit vertical swimming movements.

1. Introduction

Reproduction links primary population parameters such as natality, mortality, immigration, and emigration, which are vital to describe population dynamics (Krebs, 2001). Reproductive characteristics are highly variable, even within a species, and continue to evolve in response to environments. Because animals behave according to physiological state and their environmental condition, the observation of behaviour associated with reproduction is one of significant methods to understand reproductive characteristics of the species. In particular, spawning behaviour might provide informative data about reproductive ecology such as spawning season and spawning frequency on an individual basis. However, fine-scale measurements are often very difficult in behaviour of marine fishes in the wild. It may be for the reason that reproductive behaviour has received little attention but significant impact that they can have on population dynamics in marine fishes (e.g. Rowe and Hutchings 2003).

Flatfishes are a relatively diverse group and are widely distributed over the world waters (Munroe, 2005). Most flatfishes usually remain on the seafloor for most of their time, but they occasionally rise upward in the water column for activities such as foraging, releasing gametes, and travelling horizontally (Moyer et al., 1985; Manabe et al., 2000; Kawabe et al., 2004; also see review of Gibson, 2005). This may allow us to using electronic tags to study these behaviours. Each behavioural event of flatfishes can be recorded as instantaneous temporal changes in depth (e.g. Solmundsson et al., 2003; Hunter et al., 2004, 2009) if the registration frequency is sufficiently high (cf. Kawabe et al., 2009). However, previous flatfish studies using electronic tags focused on recording tracks over broad spatial scales and describing the general pattern of

movements (e.g. Hunter et al. 2003). Therefore, their swimming behaviour has been little studied (Gibson, 2005) and analytical method has been little developed. In this study, we attempted to identify spawning events of the Japanese flounder, *Paralichthys olivaceus*, an indeterminate multiple batch spawner, from depth time-series data recorded by an electronic tag.

2. Materials and methods

2.1. Field studies

On 18 December 2007 and 10 February 2010, tagging experiments were conducted on the west side of Kyushu Island, Japan. Japanese flounder were caught using commercial set-nets in 2007 and gill nets in 2010. We collected them from several fishermen and carefully selected individuals that were not injured. Basically, set-nets are set at the depths of 10-15 m and are hauled every day in the early morning (personal communication with Omura Bay fishermen's union). Gill nets are typically soaked for 2 days (personal communication with local fishermen) and less than 3.5 m in height, less than 1800 m in length, and about 150 mm in mesh size (Tashiro and Ichimaru 1995). The individuals of this study were fished at the depths of 90-120 m. A data logger (G5; Cefas Technology Ltd., Lowestoft, UK) was attached externally near the dorsal fin with plastic ties after anesthetises using a 0.04% 2-phenoxyethanol solution. G5 weight was 2.7 g in air and 1 g in seawater; length was 31 mm and diameter was 8 mm. Ten fish (body length [BL]: 41.6 - 47.5 cm) were released in 2007 and 13 fish (BL: 50.0 - 63.5 cm) in 2010. The frequencies of the depth records were every 10 s in 2007 and every 20 s in 2010. In the analysis, the time resolutions of the depth data were unified at 20 s by

47 using only every second registration of the 10 s data series. A high sampling frequency
48 compared to previous tagging studies of marine fishes was chosen as previous studies
49 suggested that low sampling frequencies would lead to inaccuracies in the number of
50 identified events and the statistics of various components (see Kawabe et al., 2009).
51 Each tagged fish that was recaptured was transported alive to the laboratory of
52 Institute for East China Sea Research, Nagasaki University in oxygenated seawater.
53 After euthanizing the fish, the gonads were removed and fixed in Bouin's fluid (picric
54 acid, formalin, and glacial acetic acid at a ratio of 15 to 5 to 1) for 24 h and were then
55 preserved in 70% ethanol or fixed in 10 % phosphate-buffered formalin.
56
57 **2.2. Analysis of behavioural data**
58 Visual inspection of the depth records revealed frequent vertical swimming events
59 (VSE) as illustrated in Figure 1. We defined the start and end times of a VSE as the time
60 when the vertical descent/ascent rate exceeded twice the depth resolution (i.e., 0.1 m s^{-1}).
61 VSEs were subsequently extracted automatically from the depth time-series data and
62 were broken down into following components: the duration (s), height of ascent (m),
63 ascent or descent rate (m s^{-1}), and time of occurrence (h:m) using a macro computed in
64 Igor Pro version 5.0 (WaveMetrics, Lake Oswego, OR, USA). The height of ascent was
65 defined as the distance between the depth at the start point and the shallowest depth
66 point during each VSE. Ascent or descent rates were defined as the vertical swimming
67 speed between the start or end point and the point of the highest ascent. The ascent and
68 descent rates were standardised to the body length of the fish (i.e., BL s^{-1}). The times of
69 occurrence were converted to angular directions, and the sine values of the angles were

used in the analysis. To confirm whether the macro successfully captured VSEs, we randomly selected more than 10 VSEs from the depth time series for each individual and compared outputs of the macro with results of visual analysis.

The 25907 VSEs performed by recaptured fish generated were classified using *k*-means clustering and five behavioural components (i.e., the duration, the height of ascent, the ascent or descent rates, and the time of day). *k*-means clustering is commonly used to partition a set of objects into a selected number of clusters (*k*). This method has frequently been used to categorise the behaviour of diving mammals and birds (e.g., Schreer and Testa, 1998; Lesage et al., 1999; Davis et al., 2003; Sakamoto et al., 2009). *k*-means cluster analysis is a non-supervised classification approach and is therefore partially subjective by the choice of *k*. To identify spawning-related clusters, supervised information from other flatfish and reef fish species that spawn pelagic eggs has been referenced. Although there are few quantitative measurements of swimming speed for spawning fishes (e.g. Colin, 1978), general observations are consistent with the predictions regarding swimming speed during spawning (Thresher, 1984); fish exhibit an accelerated ascent and/or descent swimming (Moyer et al., 1985; Donaldson and Colin, 1989; Colin and Bell, 1991; Manabe et al., 2000; Carvalho et al., 2003; Loher et al., 2008; also see review of Thresher, 1984). Therefore, our analysis aimed to detect clusters characterised by the maximal ascent and descent rate (hereafter referred to as cluster-S).

Sakamoto et al. (2009) suggested that by setting a larger number of *k* than is strictly necessary and to then combine the elements that the researcher identifies that represent the same behaviour. The aim of our analysis was to identify spawning-related

93 cluster (i.e., Cluster-S) rather than categorising all behavioural events. To understand
94 how results of cluster-S (i.e., mean values and coefficients of variations [CVs] for each
95 variables, in particular in ascent and descent rates, and number of events) varied with
96 the number of *k*, eighteen *k*-means analyses (i.e., $3 \leq k \leq 20$) were performed. The
97 clustering analyses were conducted using JMP Version 9.0 (SAS Institute Inc., Cary,
98 NC, USA).

99 To minimise the incidence of false detection during the mathematical
100 identification of spawning events, we considered the following two biological properties
101 of the spawning behaviour of the Japanese flounder. In aquarium, Japanese flounder
102 have shown a clear spawning periodicity (Hirano and Yamamoto, 1992; Kurita et al.,
103 2011), thus suggesting physiological restriction of successive spawning. We used 24 ± 2
104 and 48 ± 2 hours as the threshold values for the interval of spawning events (hereafter,
105 filter-1). The periodic interval was calculated based on successive VSEs in cluster-S.
106 Most Japanese flounder population in the study area spawn from mid-February to late
107 April (Tashiro and Ichimaru, 1995; Ozawa et al., 1996; Minami, 1997; Nakatsuka,
108 unpublished data), therefore, any events before the 1st of February were considered as
109 false detection (hereafter, filter-2). To examine the effect of these biological filtering
110 processes, we compared components between filtered and residual VSEs in cluster-S.
111 This comparison was performed by applying a standard least squares with a restricted
112 maximum likelihood in the Fit Model Platform of the JMP (SAS Institute Inc., 2010).
113 Each component was defined as a dependent variable. A categorical variable (i.e.,
114 filtered or residual) was fitted as the fixed effect, and individual identity as the random
115 effect.

116 Using this approach, we estimated the total number of spawning events,
117 spawning period, and spawning frequency (day^{-1}; the number of spawning events per
118 spawning period in a day) for each recaptured fish.
119
120 **2.3. Observation of gonads**
121 Histological sections of the testes and ovaries were prepared at a 4-μm
122 thickness using conventional techniques. Methacrylate resin (Technovit 7100, Heraeus
123 Kulser Co. Ltd., Wehrheim, Germany) was used as the embedding medium and 2%
124 toluidine blue and 1% borax for staining. Sections of testes were scored for the presence
125 of spermatids and sperm. Males were defined as *spawning* when both spermatids and
126 sperm were observed together. Ovary sections were scored for the most advanced
127 oocyte stage and for the presence of ovulated eggs (OVs), postovulatory follicles
128 (POFs), and atresia. Following Kurita et al (2011), the developmental stages of the
129 oocytes were classified as follows: the early yolk granule stage (EYG), late yolk granule
130 stage (LYG), migratory nucleus stage (MN), and the hydrated stage (HD). Recaptured
131 fish were classified as: (1) *spawning* - females exhibiting oocytes in the final maturation
132 (MN or HD), OVs, or POFs stages; (2) *non-spawning* - females without MN, HD, OVs,
133 or POFs were classified as inactive spawners; and (3) *irregularly spawning* - females
134 with few OVs or POFs with few LYG oocytes.
135
136 **3. Results**
137 **3.1. Tag return**
138 We recovered 10 loggers and 8 tagged fish (Table 1). Fish numbers JF1, JF2, JF5

and JF7 were recaptured using set nets, whereas JF12, JF16, JF17 and JF18 were recaptured in gill nets. For fish JF4 and JF8 only the loggers were recovered. The number of recording days for each logger ranged from 13 to 77. Three loggers (JF5, JF7 and JF8) were recovered after they reached the limit of their recording capability.

3.2. Classification of vertical swimming events

A total of 25907 VSEs performed by the 10 fish were detected. Both ascent and descent rates were principal parameters to characterise VSEs of Japanese flounder (see electronic supplementary materials Table S1). The mean values and CVs of behavioural components and number of events in cluster-S were the highest when k = 3 and decreased as k increased (see electronic supplementary materials Table S2). Clustering results were roughly consistent when $4 \leq k \leq 7$ in terms of cluster-S, suggesting that VSEs in cluster-S were greatly different from the rest events in terms of vertical swimming speeds. When $8 \leq k \leq 10$, the cluster-Ss were distinguished a cluster characterised by the maximal ascent from a cluster characterised by the maximal descent. When k was more than 11, irrelevant results were emerged. Because we did not have sufficient reasons to select a single cluster when $8 \leq k \leq 10$, a 4-cluster analysis was selected that successfully distinguished between the spawning cluster-S and the other VSEs (Figs. 1 and 2). The mean descent rate of the VSEs in cluster-S was 0.43 ± 0.22 BL s^{-1} (n = 199), which was more than 4 times greater than the VSEs of all other clusters. The mean ascent rate in cluster-S (0.43 ± 0.24 BL s^{-1}) was also clearly faster than those of all other clusters. The VSE durations (115 ± 126 s) and the highest ascents (8.67 ± 4.7 m) were intermediate among all clusters. Cluster-2 (n = 113) was

characterised by both the longest VSE duration (1868 ± 1054 s) and highest ascent (26.39 ± 16.34 m). Cluster-3 was the most frequently occurring event (n = 22886). The values of all components of the VSEs of cluster-3 were by far the lowest among all clusters (descent rate: 0.01 ± 0.13 BL s^{-1}, ascent rate: 0.02 ± 0.02 BL s^{-1}, duration: 65 ± 0.29 s, height of ascent: 0.29 ± 0.46 m). The values of all components of the VSEs of cluster-4 demonstrated intermediate values among all of the clusters (n = 2709).

Not all cluster-S events occurred during the known spawning period or occurred in a periodically-explicit manner, More than half of the l events were removed by filter-1 (i.e., application of a periodical constraint) and applying filter-2 (i.e., spawning period thresholds), reduced the number of spawning fish to 4 individuals (JF5, JF8, JF16 and JF18: Fig 3). The filtered cluster-S events showed an orderly periodic behaviour during the general spawning season in this region (Table 2). For four individuals, 17 to 19 spawning events were observed, and the spawning frequencies ranged from 0.74 to 0.90 events per day; the number of spawning events and the spawning frequency for one male were 19 events and 0.79 events per day, respectively. These events occurred with concentration in the daytime regardless of individuals. Mean vector and length of mean vector for the time of day were 13:50 and 0.967 for JF5, 12:46 and 0.972 for JF8, 11:07 and 0.87 for JF16, and 10:00 and 0.961 for JF18, respectively (Fig. 4). Note that the length of mean vector expresses a measure of concentration (see Zar, 2009). It has no units and it may vary from 0 (when there is so much dispersion that a mean the time of day cannot be described) to 1.0 (when all the data are concentrated at the same the time of day).

Least square means ± standard errors of duration was 80.37 ± 19.59 for filtered

events and 132.94 ± 16.15 for residual events, respectively. There was a difference in the durations between them (ANOVA test: $F_{1, 115.3} = 6.5042$, $p = 0.0121$). The height of ascent was 7.25 ± 0.94 for filtered events and 9.37 ± 0.81 for residual events, respectively. A slight difference in the height of ascent ($F_{1, 175.4} = 7.6442$, $p = 0.0063$) was seen. The coefficients of variation of the residual events were greater than those of filtered events, suggesting little consistency in behavioural components between the residual events. Ascent rate was 0.48 ± 0.04 for filtered events and 0.41 ± 0.03 for residual events, respectively. Descent rate was 0.40 ± 0.03 for filtered events and 0.45 ± 0.02 for residual events, respectively. No differences in either the ascent or descent rates were observed (descent, $F_{1, 138.4} = 1.8780$, $p = 0.1728$; ascent, $F_{1, 145} = 3.6344$, $p = 0.0586$).

3.3. Reproductive condition

Based on histological observations (Table 3), three females (JF5, JF7 and JF18) and one male (JF16) were defined as *spawning*, and two females (JF1 and JF2) were identified as *non-spawning*. Only fish that were found to be histologically mature were also found to display cluster-S VSEs. Two females (JF12 and JF17) were defined as non-spawning or *irregularly spawning*; these fish had very few normal OVs and POFs and did not exhibit oocytes that were undergoing the final maturation process (MN or HD). Additionally, an intensive atresia was observed in JF12 (Fig. 5). Therefore, we concluded that if the individuals could, the individuals spawn irregularly and with very few eggs. For the other two fish (JF4 and JF8), only the data loggers were retrieved, and the spawning conditions at recapture were unknown.

208

209 **4. Discussion**

210 This is the first study that successfully applied a clustering technique to study
211 vertical swimming behaviour from time-depth recordings of free-swimming tagged
212 flatfish. Flatfishes have long fascinated scientists (Berghahn and Bennema, this volume).
213 However, very little is known about their behaviour, especially in adult stages (Gibson,
214 2005). Both short-term and long-term time-depth profiles recorded by electronic tags
215 may be characteristic for certain behavioural modes (cf. Kawabe et al., 2004; Hunter et
216 al., 2004, 2009; Seitz et al., 2005; Takagi et al., 2010; Yasuda et al. 2010; Loher, 2011),
217 and may provide new insights into flatfish biology and their fisheries stock
218 management.

219 To our knowledge, the first study to recognise the potential of electronic
220 tagging for the identification of spawning behaviour in flatfishes was performed by
221 Seitz et al. (2005). In deploying a time-depth tag on the Pacific halibut, *Hippoglossus*
222 *stenolepis*, visual observation of the depth time series suggested a conspicuous routine
223 of VSEs (Seitz et al., 2005). This finding has been substantiated by further investigation
224 of the biometrics of reproductive traits, such as size at maturity (Loher et al., 2008).
225 However, the identification of specific events relied on visual inspection of the data.
226 Hence,an accurate method for objectively identifying and quantifying spawning
227 behaviour is needed. Here, we demonstrated that *k*-means clustering is a statistically
228 reliable method for the identification of spawning events in a time series of vertical
229 activity patterns.

230 The cluster analysis results are compatible with previous information of

231 spawning behaviour of Japanese flounder. The frequency of filtered events estimates for
232 both spawning fish (JF5, JF16 and JF18) and unknown fish (JF8) ranged from 0.74 to
233 0.90 events per day. The most active Japanese flounders in laboratory experiments have
234 been shown spawn almost daily for 2–3 months (Hirano and Yamamoto, 1992) with a
235 spawning frequency ranging from 0.66 to 0.88 events per day. Our estimates are also
236 comparable to the spawning frequency of 0.37 to 0.80 events per day observed in a
237 recent study of wild fish during their active spawning season (June-August) in their
238 northern region of Japan using more detailed histological analyses (Kurita, 2012).
239 Our fine-scale measurements of swimming behaviour demonstrated that the vertical
240 swimming speeds of possible spawning events were clearly faster than in any other
241 events. This result strongly indicates an advantage of electronic tagging methods and
242 our analyses presented. Although the rushing vertical swimming might be common in
243 spawning flatfishes (*Crossorhombus kobensis*, Moyer et al., 1985; *Engyprosopon*
244 *grandisquama*, Manabe et al., 2000; *Bothus podas* Carvalho et al., 2003; *H. stenolepis*,
245 Seitz et al., 2005), comparative measurement values are surprisingly limited (Thresher,
246 1984). Therefore, our approach can be applied in other species that exhibit vertical
247 spawning movements as well.
248 We observed that the duration of possible spawning events to be were
249 relatively short. This implies that the use of data loggers to study spawning behaviour
250 high recording frequencies (seconds) are required. In contrast, to describe the long-term
251 depth change over their life cycle (Hunter et al., 2004, 2009; Seitz et al., 2005; Loher et
252 al., 2008; Loher, 2011), the sampling frequency has to be programmed in increments of
253 minutes because both battery and memory capacities of tags are often limited. Burst

sampling might be effective to capture annual life cycle and consecutive spawning events simultaneously.

Possible discrepancies were observed between the results obtained through electronic tagging and histological observations for three of the females (JF7, JF12 and JF17; Table 3). No spawning events were detected for JF7, despite the fact that females would be expected to have been spawning regularly at the time of recapture. We hypothesise that the reason for the discrepancy observed for JF7 is that described previously by Yasuda et al. (2010). In short, the tag revealed that JF7 experienced low temperatures during the monitoring period until 5 March (the limit of recording). However, the location at recapture on 1 April indicated that JF7 was exposed to high temperatures, due to migration, after reaching the tags recording limit and that VSEs associated with spawning would also have occurred thereafter. Therefore, it seems reasonable to infer that OV and POF would have developed in JF7 after 5 March. Although females JF12 and JF17 presented ovulated eggs and POFs, they were few in number; in addition, one of these two fish (JF12) contained many atretic yolked oocytes. From observation of females in captivity, stressed individuals contained few ovulated eggs that were not released or only few eggs were released, but without normal courtship behaviour (Kurita, unpublished data). Such females could experience high levels of stress, and do not adapt regular courtship behaviours. Although tag attachment was performed under the most rigorous conditions, it is conceivable that the fishing and the tagging processes may result in a high level of stress on the fish.

We could detect orderly periodic behaviour from the male as well as females. However, it is wonder if an application of the same periodic filtering process was

277 adequate for male. For plaice, Solmundsson et al. (2003) reported that males were more
278 active than females during the time of spawning. Male Japanese flounder could be
279 engaged in several spawning events every day. The appropriateness of application of
280 filter-1 (24 or 48 h as spawning interval) for males is the future study.

281 Our findings suggest that electronic tagging methods progress understanding of
282 reproductive traits in exploited marine fishes. Fecundity which is the potential
283 reproductive capacity of an organism (Krebs, 2001) is known to vary with both age and
284 size of individuals but is highly variable. Many fisheries biologists have a concern about
285 divergences between the potential fecundity (i.e., the number of yolked oocytes
286 produced in the ovary) and the realised fecundity (i.e., the number of eggs released), in
287 particular in “indeterminate” spawners (e.g., Somarakis et al., 2004, 2006; Motos, 1996).
288 Recent developments in computer-aided semi-automatic measurements and counting
289 have enabled the rapid and accurate estimation of fish fecundity (Thorsen and Kjesbu,
290 2001; Witthames et al., 2009; Kurita and Kjesbu, 2009). Nevertheless, the number of
291 batches, or spawning frequency, remains one of the most difficult reproductive traits to
292 estimate (Stratoudakis et al., 2006). Our study shows that electronic tags can record
293 consecutive spawning events, suggesting an additional reliable and independent new
294 method to validate estimates obtained using standard methods. Moreover, our technique
295 can be further extended to record the ambient physical environment over an extended
296 period of time allowing the study of the realised fecundity in indeterminate multiple
297 batch spawning fish such as Japanese flounder.

298 Information from a single time-depth data and a mathematical method may be
299 limited in explanation capability. By applying previous knowledge of the flounder

behaviour to guide the mathematical interpretation of our results, we demonstrate how biologging studies may be optimised to gather more detailed information in future deployments. Hunter et al. (2004) and (2009) reported that seasonal swimming patterns of fish may be related to their annual life cycle. Loher (2011) suggested long-term maximum depth profile also provided seasonal migration of a flatfish. The above ecological information derived from tagging data may be useful to determine the spawning season (i.e., filter-2). Ambient water temperatures and locations, which are recorded using traditional electronic tags, may also be useful for determining the spawning seasons of individual fish (Yasuda et al., 2010). By smart programming of advanced sensors such as acceleration sensors (Kawabe et al., 2004; Sakamoto et al., 2009) and camera sensor (Kudo et al., 2007), varying recording rates (Kawabe et al., 2009) could be set may further aid in evaluating performance regarding the identification of spawning events.

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Table 1. Summary of release and recapture data of Japanese flounder tagged with electronic data loggers.

ID	Date of tag recovery	Recording days	BL (cm)	Weight (kg)	Sex
Released on 18-Dec-2007 in Omura Bay					
JF1	15-Jan-08	28	45	1.4	F
JF2	17-Jan-08	30	44	1.4	F
JF4	25-Feb-08	69	45	1	-
JF5	13-Mar-08	77*	43	1.4	F
JF7	1-Apr-08	77*	44	1.6	F
JF8	24-Nov-08	77*	45	1.4	-
Released on 10-Feb-2010 off Hirado Island					
JF12	22-Feb-10	13	59	3.5	F
JF16	14-Mar-10	32	50	2.2	M
JF17	22-Mar-10	40	55	3.2	F
JF18	23-Mar-10	41	57	2.9	F

* Maximum number of recording days

Table 2 Summary of the analysis of Japanese flounder swimming behaviour, as extended from time series of depth data using *k*-means clustering analysis. Detailed information regarding cluster-S and filtering processes are provided in the text.

ID	Number of vertical swimming events					Emergence period of filtered cluster-S		Estimated spawning frequency (events/day)
	All	Cluster-S	Filtered events (Spawning)	Residual events of Filter-1	Residual events of Filter-2	Date	Days	
JF1	996	4	0	0	4	-	0	-
JF2	3222	23	0	21	2	-	0	-
JF4	415	0	0	0	0	-	0	-
JF5	3630	33	17	14	2	11-Feb-08 - 4-Mar-08	23	0.74
JF7	1216	7	0	5	2	-	0	-
JF8	3948	72	19	38	15	1-Feb-07 - 21-Feb-08	21	0.90
JF12	1608	0	0	0	0	-	0	-
JF16	4534	39	15	24	0	20-Feb-08 - 10-Mar-08	19	0.79
JF17	3559	1	0	1	0	-	0	-

JF18	2389	20	18	2	0	2-Mar-08 - 21-Mar-08	20	0.90

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Table 3. Summary of histological and anatomical analysis of Japanese flounder with electronic tags at the time of recapture. Detailed information regarding developmental stages and spawning conditions are provided in the text. EYG, early yolk granule; LYG, late yolk granule; MN migratory nucleus; HD, hydrated; OV ovulated eggs; POF, postovulatory follicles.

Fish ID	Developmental stage	Maximum oocyte diameter (μm)	Presence of OV or POF	Presence of Atresia (Y/N)	Spawning condition
JF1	EYG	<300	N	N	Non-spawning
JF2	EYG	<350	N	N	Non-spawning
JF4	-	-	-	-	-
JF5	MN	560	OV, POF	N	Spawning
JF7	HD	900	OV, POF	N	Spawning
JF8	-	-	-	-	-
JF12	LYG	567	OV, POF*	Y**	Non-spawning or irregularly spawning
JF16	Matured sperm	-	N	N	-
JF17	LYG	550	OV, POF*	Y	Non-spawning or irregularly spawning
JF18	LYG	613	OV, POF	N	Spawning

* The number of normal OVs and POFs were relatively small.

** Many old atresia originating from yolked oocytes occurred.

Figure Captions;

Figure 1. (a) An example of a single vertical swimming event and behavioural components for JF4. (b) and (c) Examples of vertical swimming profile sequences that correspond to the *k*-means clustering analysis ($k = 4$). (b) An exploratory swimming event (cluster-2) among short swimming events (cluster-3 or cluster-4) for fish JF18, (c) possible spawning event (cluster-S) among short swimming events (cluster-3 or cluster-4) for fish JF18. The number of clusters is shown above each event.

Figure 2. Results of *k*-means clustering analysis ($k = 4$) of vertical swimming events of Japanese flounder. Means ± standard deviations of the vertical swimming event components (a: descent rate, b: ascent rate, c: duration, and d: height of ascent) for each cluster are presented. The numbers of events are plotted (a). The cluster representing spawning (i.e., cluster-S) is shaded in each graph.

Figure 3. Time-series of depths during the overall monitoring periods that correspond to *k*-means clustering analysis and filtering processes for spawning Japanese flounder. Shaded, black, white squares indicate filtered vertical swimming events (i.e., possible spawning events), residual events of filter-1 (i.e., application of a periodical constraint) and residual events of filter-2 (i.e., spawning period thresholds), respectively.

Figure 4. Enlarged time-series of depths for spawning Japanese flounder. Arrows indicate filtered vertical swimming events (i.e., possible spawning events).

Figure 5. Photographs of oocytes showing each spawning condition. (a) non-spawning fish JF1, (b) spawning fish JF18, and (c) irregular spawning fish JF12. Black bars indicate a scale of 500 μm. EYG: early yolk granule stage, LYG: late yolk granule stage, POF: post ovulatory follicle, AT: atresia.

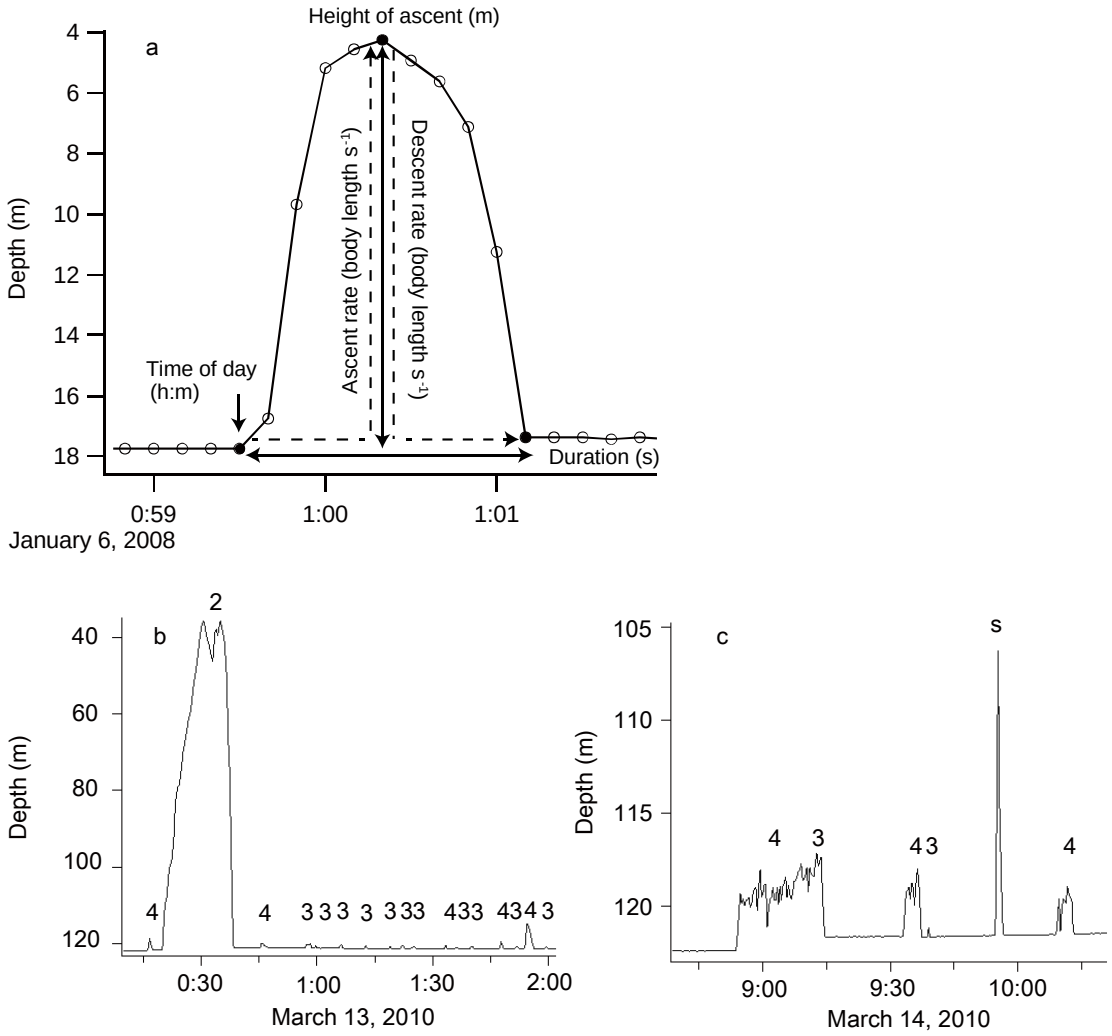
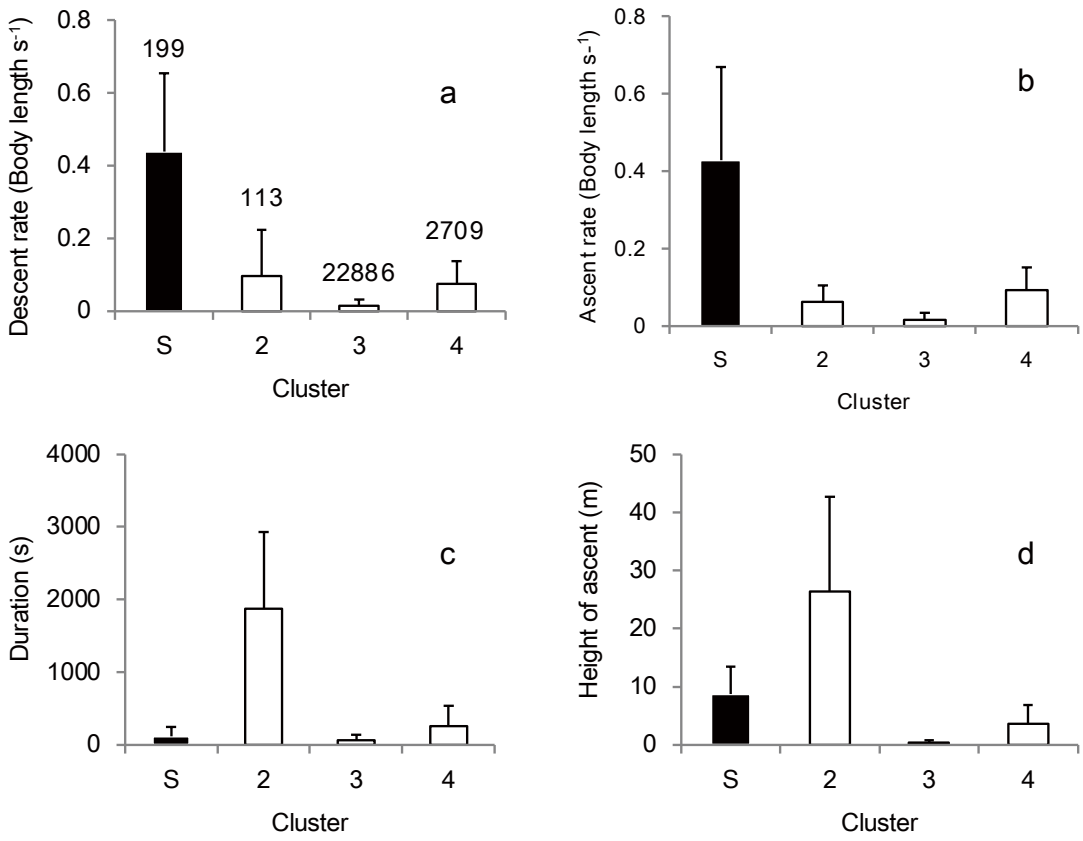

a
Height of ascent (m)
Depth (m)
4
6
8
10
12
14
16
18
Ascent rate (body length s-1)
Descent rate (body length s-1)
Time of day
(h:m)
Duration (s)
0:59
1:00
1:01
January 6, 2008
b
2
40
60
80
100
120
4
4
3 3 3 3 3 33
433
4343
0:30
1:00
1:30
2:00
March 13, 2010
c
s
105
110
115
120
4 3
4 3
4
9:00
9:30
10:00
March 14, 2010

Figure 1



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37 Figure 2

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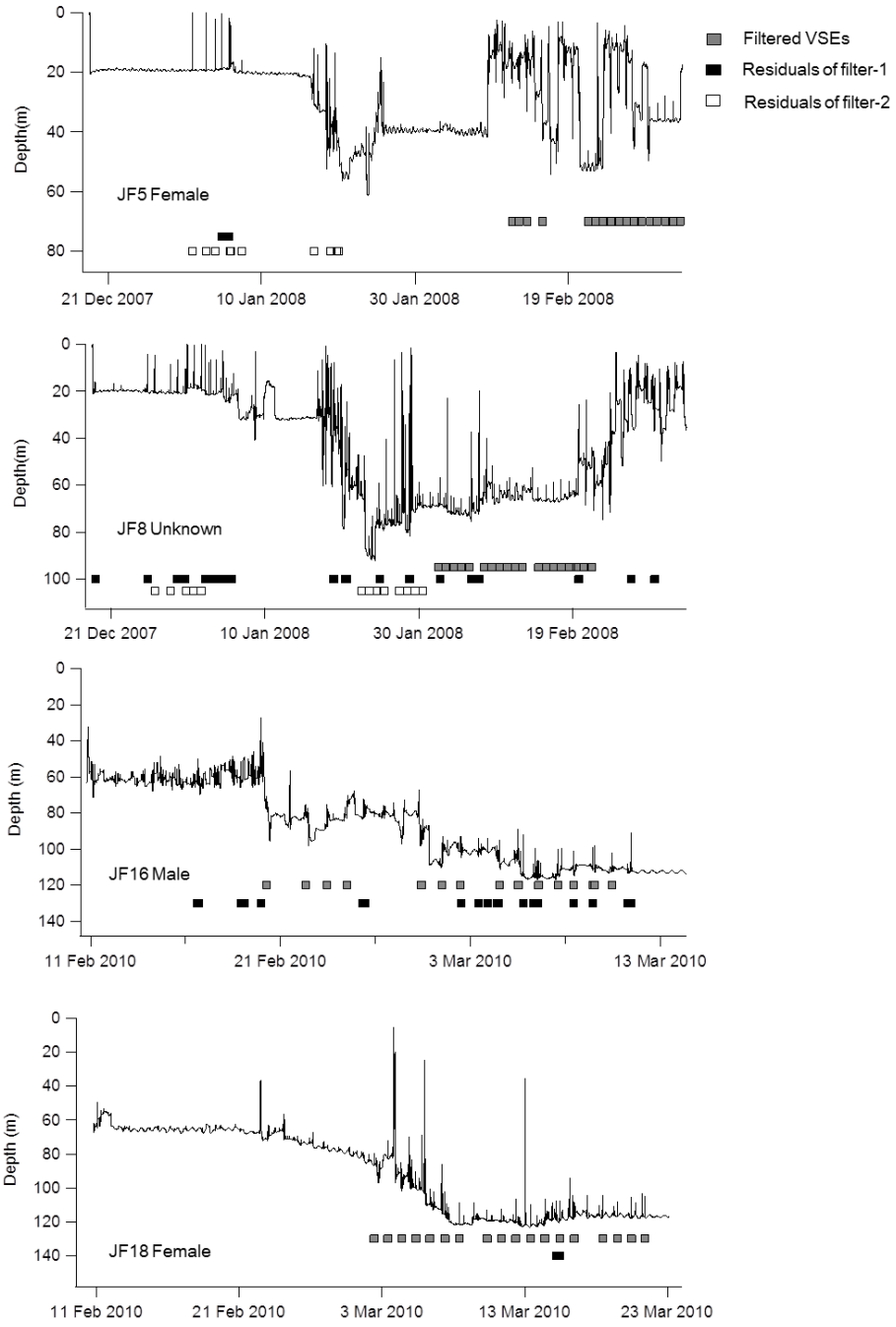
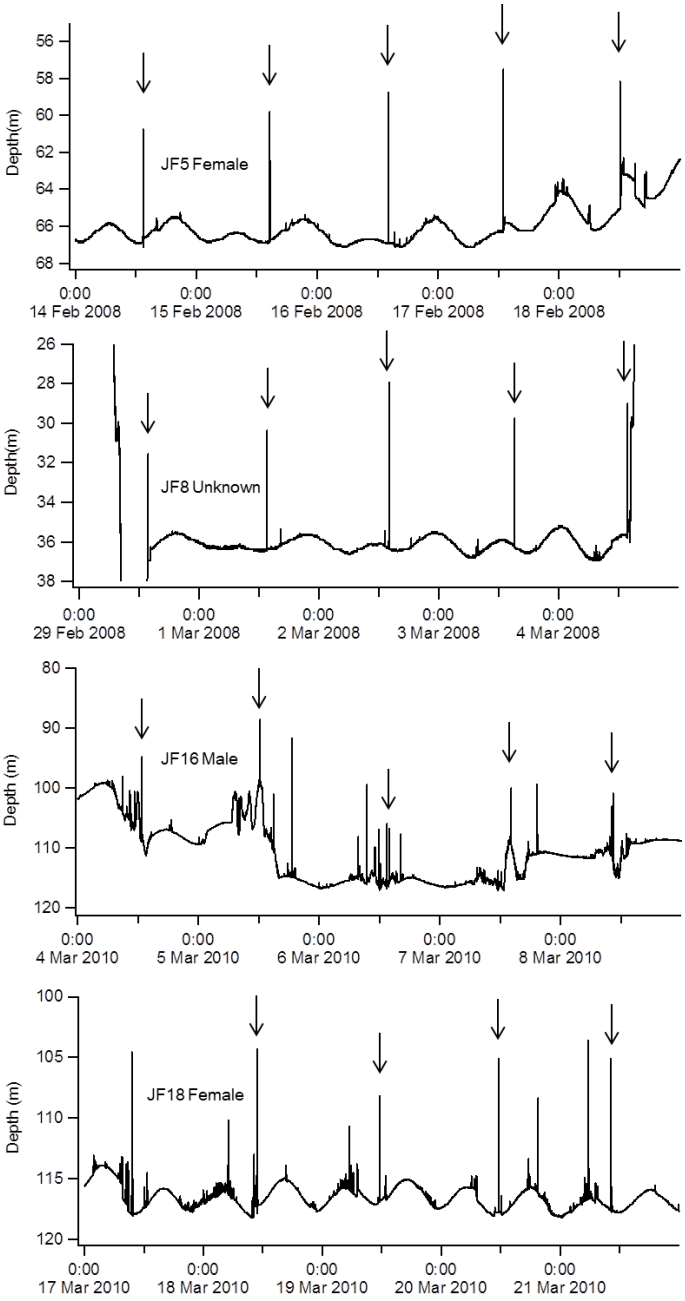

Filtered VSEs
Residuals of filter-1
Residuals of filter-2
Depth(m)
JF5 Female
21 Dec 2007
10 Jan 2008
30 Jan 2008
19 Feb 2008
JF8 Unknown
Depth (m)
JF16 Male
11 Feb 2010
21 Feb 2010
3 Mar 2010
13 Mar 2010
JF18 Female
23 Mar 2010

Figure 3



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43 Figure 4

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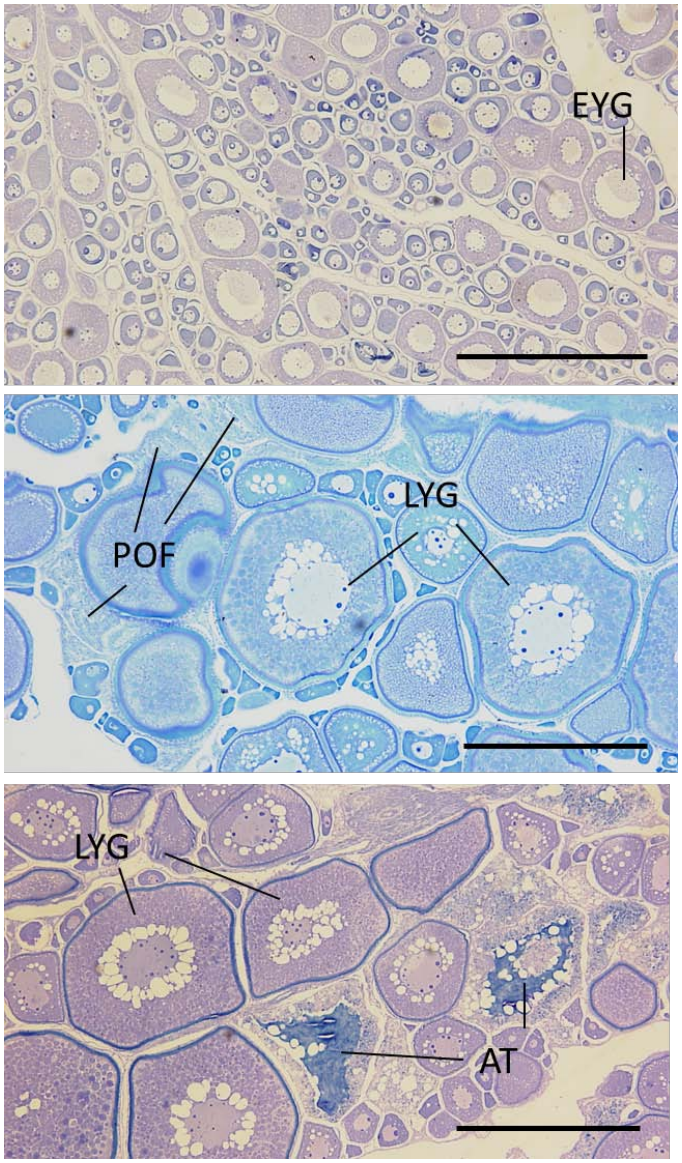

Figure 5