# One and Two-individual Movements of Fish after Chemical Exposure

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Movement behavior of an indicator species, zebrafish (*Danio rerio*), was analyzed with one- and two-individual groups before and after treatment with a toxic chemical, formaldehyde, at a low concentration (1 ppm). After the boundary area had been determined based on experimental data, intermittency was defined as the probability distributions of the shadowing time during which data were above a pre-determined threshold and were obtained from experimental time-series data on the forces and the inter-distances for one and two individuals. Overall intermittencies were similar in the boundary and central areas. However, the intermittencies were remarkably different between the one- and the two-individual groups: the single line was used to fit the data for the one-individual group whereas two phases were observed with breakpoints (approximately 10 seconds in logarithm) in the exponential fitting curves for the two-individual group. A difference in the probability distributions of the shadowing time was observed "before" and "after" treatment for different areas. Intermittency patterns before and after treatment were contrasted in the center for the one-individual group whereas

the difference was observed in the boundary for two-individual group. The intermittencies for the inter-distances of two individuals in the boundary and the central areas were markedly different before and after treatment. When the differences between the intermittencies in the boundary and the central areas and between "before" and "after" treatment are considered, the distribution patterns of the shadowing time (scaling behaviors or intermittency patterns) should be a useful means of bio-monitoring to detect contaminants in the environment.

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# I. INTRODUCTION

The analysis of the response behaviors of animals has received considerable attention regarding *in situ* monitoring of indicator species since computational methods and interfacing techniques were introduced in the 1980's [1-4]. Monitoring by using behavioral changes is ecologically relevant, economical and faster than monitoring by using method of chemical detection [5-7]. Due to the high degree of complexity in behavioral data, however, various computational methods have been proposed to exploring time-series data on animal movements [1, 8]: parameterization with a fractal dimension [9] and permutation entropy [10, 11], statistical methods using correlation analyses [10, 12, 13], data transform including Fourier transforms [7, 14] and wavelet analysis [15]. Considering the complexity of behavioral data, informatics has been further applied to movement patterns, including self-

48  organizing map [7, 14, 16] and multi-layer perception [6, 15], and is capable of identifying specific

49  response behaviors of indicator species under chemical stress. Because of the uncertainties in

50  behavioral patterns, the hidden Markov model has been used to analyze behavioral state changes after

51  exposure to chemical treatment [16, 17]. However, the abovementioned reports mostly focused on data

52  for single individuals, and not many studies were conducted on the responses of multiple individuals.

53  Regarding group formation by multiple individuals, simulation models based on the equations of

54  motion have been  proposed to elucidate the collective behavior associated with self-propelled particle

55  systems according to the group (i.e., overall average orientation) and the neighbor (e.g., attraction,

56  repulsion) responses [18-23]. Group behavior models were also analyzed, and observed data were

57  evaluated; force components of individuals in collective motion were calculated in order to explain the

58  relationship between the individual itself, its neighbors and environmental factors [24, 25]; individual

59  fish movements were expressed by using the mass, drag coefficient, and external forces. Recently, the

60  importance of nearest-neighbor interactions in group formation was addressed [26, 27].

61  In this study, we focused on the physical forces produced by one and two individuals under stressful

62  conditions due to chemical exposure. In order to reveal the structure property in the movement data, we

63  addressed the probability distributions of the shadowing time in time-series force data on fish observed

64  in a confined area. Scaling behavior has been increasingly used in analyzing movement behavioral

65  patterns of animals in the wild and in the laboratory. Intermittency is defined as the probability

66  distribution of the shadowing time during which the data are consecutively higher than a threshold

67  number [28-31]. For time-series data generated from a chaotic system (e.g., attractor), intermittency

68  exhibits a universal algebraic scaling at high frequencies with a slope approximately $-3/2$ while it

69  exhibits an exponential scaling at lower frequencies [28, 30].

70  Intermittency is among the universal mechanisms that produce chaos from a periodic orbit in

71  a  continuous  way [32] and has been reported in various fields, including coordination of muscular

72  systems [33, 34], chemical kinetics [35, 36], laser models [37], and fluid dynamics [38]. In ecology,

73  flow intermittency regarding biodiversity determination in stream ecosystems has been recently

74  investigated [39, 40]. Intermittency has been further applied to behavior studies. Harnos *et al*. [41]

75  analyzed scaling and intermittency in the temporal behavior of nesting gilts, reporting that the time

76  spent by a gilt in a given form of activity had a power-law probability distribution, and showed the

77  intermittent occurrence of certain periodic behavioral sequences to indicate a critical state. Mashnova *et*

78  *al*. [42] investigated intermittency and a truncated power law in aphid movement and addressed the

79  alternate appearance of fast and slow movement phases that were distinguished by a threshold value of

80  velocity. However, intermittency in response behavior of animals under chemical stress has not been

81  extensively studied.

82  In addition to chemical response, we further observed individual movement at different locations in a

83  confined area. Although test animals can move around in a more-or-less straightforward manner over a

84  wide range, the individuals are constrained inside a confined arena within a boundary, especially for

85  behavior monitoring within an observation arena [16]. The boundary zone was considered to be the

86  area in which free movement would be minimally allowed, and is important for the life events,

87  including protection and exploitation, of animals [43, 44]. We showed that the scaling behaviors of

88  two individuals of *D. rerio* would be different at the boundary and the central areas of the observation

89  arena before and after chemical treatment. Specifically, we intended to characterize intermittency in

90  response behaviors in three different categories, 1) comparison of one and two individuals, 2) boundary

91  and central areas, and 3) before and after chemical treatment. We analyzed the probability distributions

92  of the shadowing time to address changes in the structure property in the movement data and found that

93  intermittency in individual and group movement could be used as a possible means of behavioral

94  monitoring.

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## II. EXPERIMENTS

### 1. Test Organisms

One and two individuals of zebrafish, *D. rerio*, were observed under chemical stress. Due to vulnerability to chemical stress and availability of biological information (e.g., genomics, physiological responses), the zebrafish is considered to be one of the most suitable vertebrate model organisms for various biological tests [45-47], including behavior assessment [7, 16, 48]. The species has a strong potential for being an indicator in risk assessment [16, 49]. Individuals of wild-type *D. rerio* were obtained from a local fish dealer for stock population (300 individuals) and were reared for 2 weeks before observation [50] at a temperature of $25 \pm 1^{\circ}C$ and pH of $7.1 \pm 0.3$ under a light/dark cycle of 14/10 h, light on at 7:00 h and off at 20:00 h [51]. Two fluorescent lights (26 J/s) were placed 50 cm above the rearing container. Tap water was filtered with air stones under air compression (DT - 10F, Chuang Xing Electric Appliances®) after dechlorination for three days. Fishes were fed dry food (Nutron Hi − Fi, PRODAC®) twice a day (once a day on weekends). Other rearing conditions are described below [16].

Test organisms (ages: 5 − 6 months; body lengths: 30 − 40 mm) were randomly chosen from the stock population and were placed individually in a glass aquarium (300 mm × 300 mm × 300 mm; water height of 20 mm). Before observation, organisms were acclimated to the observation system for 30 minutes [50]. To simplify observation and minimize noise, food and oxygen were not supplied to the arena during the observation period. Two 13J/s fluorescent lights were provided 50 cm above the water's surface and the two light sources were symmetrically 32 cm away from the center over the observation arena. Other rearing and observation conditions were the same as those used to rear the stock population.

Formaldehyde (HCHO, 37wt. % solution in water, A.C.S. reagent, Gamma−Aldrich®) was used as a source of stress to the test organisms. Formaldehyde is claimed to be one of most toxic environmental

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120   hormones and a possible carcinogenic agent through bioaccumulation [52]. The chemical was directly

121   added to the water in the observation aquarium at a concentration of 1 ppm. In order to minimize noise,

122   the chemical was delivered through an injector (Pipetman® P20) connected to the observation

123   aquarium through a flexible polyethylene tube (1.85 mm in diameter and 1 m in length) after dilution

124   with a proper amount of water.

125   **2. Observations and Recording**

126   The observation system consisted of an observation aquarium, a camera (Logitech®Vid™HD), a PC

127   (Intel® Core ™ 2 Duo CPU E4500@ 2.20GHz ), and software for tracking the motion of multiple

128   individuals. The software was developed in the Ecosystem and Behavior Lab. at Pusan National

129   University based on stereo vision [53] after evaluation with a multiple individual tracking program

130   (SynthEyes, 2008, Anderson Technologies LLC). The *x-y* position of each individual was continuously

131   recorded at 30 frames per second from a top view in two dimensions before (30 minutes) and after (30

132   minutes) treatment. Five-minute segments were selected for analysis according to Suzuki *et al.* [27] and

133   Herbert-Read *et al*. [54]. After treatment, fish immediately responded to olfactory stimulus from the

134   chemical for approximately 5 minutes, showing abnormal behaviors including shaking and turning.

135   Afterwards abnormal behaviors occurred less frequently. Movement tracks for the initial five minutes

136   were analyzed before and after treatment.

137   Based on preliminary research [16, 55], a time segment of 0.2 s for recording movement was selected

138   for this study. Because we aimed to observe overall movement changes of the fish specimens in two

139   dimensions in response to the chemical treatment, the 0.2 s segment was sufficiently short for

140   presenting the displacement of organism location [7, 14]. Extremely short-time response behaviors due

141   to intoxication (e.g., compulsion, trembling) may be expressed in timer shorter than 0.2 s, but this type

142   of behaviors of extremely short duration was not analyzed in this study [16]. Each movement segment

143   was determined with three points with two consecutive 0.1 s segment (0.2 s in total). The observation

144    was repeated 20 times for each group of one and two individuals. Mean values of the linear and the

145    angular speeds were obtained from movement segments for each individual during the observation

146    period; subsequently, the mean values were calculated from the averages of all individuals (i.e., $n = 20$,

147    and 40 for the one- and the two-individual groups, respectively) before and after treatment.

148    **3. Computational Methods**

149    3.1.   Determination of boundary and central areas

150    Although test animals can move in a more-or-less straightforward manner (i.e., free run length), the

151    individuals are also located  inside a confined arena for monitoring the observation arena, as stated

152    above [16]. We defined the boundary and the central areas by measuring the velocity of single

153    individuals. Figures 1(a) and (b) show the distributions of the $x$- and the $y$-component of the velocity

154    along each coordinate before treatment, respectively. In order to determine the boundary area, we

155    inspected the cumulative sum of the velocity data. In Figures 1(c) and (d), the cumulative sums of the

156    two components of the velocity from the sides of the arena are presented.

157    Subsequently, the cumulative sum were fitted with the exponential function $A(1-e^{-\alpha x})$, here $A$ is a

158    proper amplitude and $\alpha$ is the damping parameter, which is taken as the inverse of the width of

159    boundary. By fitting the data with the exponential function, the boundary width in $x$-coordinates was

160    evaluated as 19.23 mm and 10.53 mm at the left and the right sides of the $x$-coordinate and 20.00 mm

161    and 10.53 mm at the left and the right sides of the $y$-coordinate. From the evaluated value of the width

162    of boundary area from the edge of each side, the largest value, 20.00 mm, was chosen to define the

163    boundary area. The obtained value was comparable to the boundary areas empirically based on the fish

164    size [16].

165    3.2.   Real forces of each individual

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166    Based on our empirical data, we measured changes in the forces on the test individuals before and

167    after treatment. Following the framework of classical mechanics, we defined the total force $\vec{f}_i$ on the

168    $i^{\text{th}}$ focal fish as the sum of two real forces, the frictional force $\vec{f}_i^{\,fric}$ and self-driven force $\vec{f}_i^{\,d}$ :

169    $$\vec{f}_i = \vec{f}_i^{\,d} + \vec{f}_i^{\,fric},$$  (1)

170    with

171    $$\vec{f}_i = m\vec{a}_i$$  (2)

172    $$\vec{f}_i^{\,fric} = \mu\vec{v}_i,$$  (3)

173    where $m$ is the mass of the fish, and $\mu$ is the friction coefficient in water. However, in our analysis, the

174    mass $m$ is set to unity, and $\mu$ is assumed to be 0.05 [21].

175    To calculate the self-driven force $\vec{f}_i^{\,d}$ , we calculated the velocity and the acceleration of $i^{\text{th}}$ individual

176    at time $t$ by using $\vec{v}_i = \dfrac{\Delta\vec{r}_i}{\Delta t}$ , $\vec{a}_i = \dfrac{\Delta\vec{v}_i}{\Delta t}$ from the movement tracks. We directly calculated the $x$- and the

177    $y$-components and the absolute force for the one-individual group while forces were calculated

178    according to center of mass, individual forces, and the relative coordinate between two individuals in

179    the two-individual group.

180    3.3.    Calculation of intermittency

181    The mean value of the absolute of the force measured before treatment was used as a criterion to

182    determine the threshold for the shadowing time (Figure 2). We used one fourth of the mean value as the

183    threshold, after testing various levels of the threshold from one eighth to 2 times the mean value. One

184    fourth the mean value was most suitable in characterizing the probability distributions of the shadowing

185    time in the boundary and the central areas, as well as "before" and "after" treatment. The threshold

186    value based on the absolute value of force was also used for the $x$-component and the $y$-component.

187    The shadowing times and their probability distributions were expressed on a logarithmic scale. The

188    slopes and the elevations were obtained using a regression analysis [56]. The probability distribution of

189    shadowing times of long duration was also fitted to an exponential curve when breakpoints occurred in

190    intermittency [28, 30].

## III.    RESULTS

192    Figure 3 shows the probability distributions of the shadowing time for forces on individuals observed

193    in the boundary and the central areas when the time duration was selected according to the threshold

194    (95.51 mm/s$^2$) in one- and two-individual groups. For the $x$- and the $y$-components of the forces, the

195    probability distributions of the shadowing time were overall similar between the boundary and the

196    central areas, but were different between one- and two-individual groups (Figures 3(a) – (b), (d) – (e),

197    (g) – (h), and (j) – (k)). Linearity across different shadowing times was observed for the one-individual

198    group (Figures 3(a) – (f)) whereas the linearity was not sustained and probability patterns appeared in

199    the curve for the two-individual group (Figures 3(g) – (l)). The slopes of the distribution became

200    steeper for long-time duration (i.e., right-hand side of the $x$-axis) for the short-time duration. For the $x$-

201    and the $y$-components, the probability distributions of the shadowing time in the boundary area

202    (Figures 3(a) – (b)) appeared to be slightly steeper than that in the central area (Figures 3(d) and (e)),

203    but no statistical difference was observed in the regression lines according to their slopes ($p > 0.05$) [56].

204    For the absolute forces, although the probability distributions of shadowing time were, in general,

205    similar to the $x$- and the $y$-components, a difference was observed to some degree in the shapes of the

206    probability distributions (Figures 3(c), (f), (i), and (l)). Before treatment in the boundary area, for

207    instance, the log abundance for the long-time duration appeared to spread over a broader range (i.e.,

208    long foot at the right bottom corner in Figure 3(c)) whereas this type of long foot was not observed in

209    the central area. In the two-individual group, similarly, distribution patterns were different in the

210    boundary and the central areas, as well as "before" and "after" treatment (Figures 3(i) and (l)). A

211  detailed description of the distribution patterns, however, is beyond the scope of this study and will be

212  reported elsewhere.

213  Intermittency was further contrasted before and after treatment in Figure 4, and the probability

214  distributions of the shadowing time were fitted to lines and exponential curves because intermittency

215  exhibited a universal algebraic scaling at high frequencies and an exponential scaling at lower

216  frequencies [28, 30]. Table 1 summarizes the slopes of the lines based on regression analyses, and

217  Table 2 lists the coefficients and fittings to the exponential functions in the boundary and the central

218  areas for one- and two-individual groups. For the one-individual group, the probability distributions

219  were fitted to single lines (Figures 4(a) and (b), (d) and (e)). The slopes were similar and were in the

220  range of -1.89 – -1.91 for the $x$- component and -1.75 – -1.76 for the $y$-components before and after

221  treatment, but the difference in the slopes of the regression lines were not statistically significant

222  ($p>0.05$; Figures 4(a) and (b)) [56]. In the central area, however, the slopes were different for the $x$-and

223  the $y$-components of the forces. The slopes were statistically steeper for both components of the forces

224  after treatment (-1.79 – -1.80) than "before" treatment (-1.13 – -1.32) ($p<0.05$; Figures 4(d) and (e)),

225  indicating that the phase change in the shadowing time was more sensitive in the central area under

226  chemical stress.

227  For the absolute forces, the probability distributions of the shadowing time for one individual (Figures

228  4(c) and 4(f)) were more spread compared to those for the $x$-and the $y$-components of the forces in the

229  boundary and the central areas.  The slopes appeared to be different, with statistical significance, before

230  and after treatment (Table 1). At the boundary area, slopes were steeper after treatment (-1.38) than

231  before treatment (-1.02) whereas slopes were less steep in the central area after treatment (-1.34) than

232  before treatment (-1.66) (Table 1).

233  Forces on the center of mass for the two-individual group were also calculated (Figures 4(g) – (l)).

234  Compared with the forces on the one-individual group (Figures 4(a) – (f)), the probability distributions

of the shadowing time were different, showing two phases as stated above. In both the *x*- and the *y*-

components, intermittency appeared to be curved with a breakpoint in the boundary (Figures 4(g) – (h))

and the central (Figures 4(j) – (k)) areas whereas single lines were fitted to the intermittency curves for

the case of the one-individual group (Figures 4(a), (b) and (d), (e)), as stated above. The breakpoint was

found to be around 10 seconds, and intermittency was overall similar between the boundary and the

central areas for the two-individual group. It was remarkable that the difference in intermittency before

and after treatment was more clearly observed in the boundary area (Figures 4(g) and (h)), contrary to

the case of the one-individual group where the difference was only observed in the central area (Figures

4(d) and (e)) (Table 1). It is also noteworthy that after treatment, the elevation of the intermittency (i.e.,

intercepts of regression lines) was lower in both the *x*- and the *y*-components in the boundary area

(Figures 4(g) and (h)). A statistical difference between the boundary and the central areas was observed

for the absolute forces (Figures 4(i) and (l), Table 1).

  For the absolute forces on two individuals, curves were also formed in the boundary area, more

strongly for "after" treatment (Figure 4(i)) although higher variation was observed in probabilities

compared to the *x* and the *y*-components of the forces (Figures 4(g) and (h)). The breakpoint appeared

to slightly move toward long time duration, a little over 10 seconds (Figure 4(i)). The lines fitted to the

probability distributions at high frequency (i.e., before breakpoint) were statistically different before (-

0.89) and after (-1.19) treatment in the boundary area (Table 1). In the central area, however, single

lines were fitted to probability distributions across the shadowing time, and the slopes (-0.94 and -0.96)

were not statistically different (Table 1). We also fitted the intermittency at lower frequency (i.e., a

long shadowing time after breakpoint) to an exponential function [28, 30]. The coefficients were in the

range of 0.30 – 0.34 and exponential curves before and after treatment were not statistically different

when the goodness of fit between the two curves was tested according to the chi-square test [56] (Table

2). Although not presented in the figures, intermittency curves for velocities observed at the boundary

and the central areas were similar to the case of forces (center of mass) both "before" and "after"

treatment. However, the intermittency of velocities was weaker in expressing the difference between

"before" and "after" treatment.

We also calculated the relative forces between two individuals in the two-individual group (Figures

5(a) – (f)). Similar to force of center of mass (Figures 4(g) – (l)), two phases were observed around the

breakpoint of 10 seconds. The shapes of the probability distributions before and after treatment were

different in the boundary area while the shapes were similar in the central area. Statistical significance

was observed in the lines fitted to the intermittency in the $x$- and the $y$-components for the short-time

duration before and after treatment (Table 1) (Figures 5(a) and (b)). The slopes ranged from -0.90 to -

1.10 before treatment and from -1.09 to -1.63 after treatment for the $x$- and the $y$-components in the

boundary and the central areas. The slopes were statistically different before and after treatment in the

boundary area (Table 1). Although the slopes were not different in the central area, the elevations (i.e.,

$y$-intercepts of the regression lines) were statistically different before and after treatment (see Ref. 56

for the statistical significance of the elevation in a regression line).

For absolute forces, the probability distributions were also different before and after treatment

(Figures 5(c) and (f)). A breakpoint was observed, and the point appeared to move more toward the

long-time duration, approximately matching 25 seconds in the boundary area. The slopes (-0.76 – -0.89)

of the regression lines for the absolute force were less steep compared to those of the $x$- and the $y$-

components (-0.90 – -1.63) in the boundary area and were comparable to those of the absolute forces (-

0.94 –-0.96) on the center of mass in the center area (Table 1). The coefficients ($\alpha$) of the exponential

functions were also fitted to the probability distribution of the long shadowing time and ranged from

0.30 to 0. 35. The exponential curves before and after treatment were not statistically different when

the goodness of fit between two the curves was tested according to the chi-square test [56].

282    We also checked the distribution pattern for forces for all individuals in the two-individual group

283    (Figures 5(g) – (l)). Similar to the case of intermittency of the relative force, overall probability

284    distributions were observed in two phases, single lines for short shadowing times and exponential

285    curves for long shadowing times (Figures 5(g) – (h) and (j) – (k)). According to this figure, the

286    probability distributions for the shadowing time tended to be slightly steeper in both the boundary and

287    the central areas after treatment. Difference in the slopes and the elevations were observed before and

288    after treatment for the $x$- and the $y$-components, as well as the absolute forces, and these differences

289    were statistically significant (Table 1). In the absolute forces, however, breakpoints were not clearly

290    observed in the central area (Figure 5(l)). Overall, the difference in intermittency appeared to be more

291    clearly observed in the boundary area (Table 1). Similar to the case of the relative force, intermittency

292    of individual forces was fitted to an exponential function with $\alpha$ values ranging from 0.30 to 0.35, and

293    the exponential curves before and after treatment were not statistically different, similar to two cases

294    above [56] (Table 2).

295    We also calculated the probability distributions of the shadowing time for two individuals' inter-

296    distance. The difference was outstanding in the boundary area before and after treatment; the curve

297    became rapidly steeper after the breakpoint (Figure 6(a)). Similar to the case of forces, the break point

298    was formed around 10 seconds. In the center, however, single lines were fitted both "before" and "after"

299    treatment. The slopes of the intermittency appeared to be flat, ranging from 0.22 to -0.26 in the

300    boundary area. The slope after treatment, however, became steeper (-0.80) than the slope before

301    treatment (-0.47) in the central area (Figure 6(b)). The slopes before and after treatment were

302    statistically different for both the boundary and the central areas (Table 1, Figure 6). Exponential

303    functions were fitted to the intermittency after treatment, with $\alpha = 0.26$ ($R^2$=0.71) in the boundary area,

304    according to chi-square-test goodness of fit [56] (Table 2).

305                        **IV.   DISCUSSIONS AND CONCLUSIONS**

306　　It was remarkable that the data structure was fundamentally different between single and two

307　　individuals. The breakpoints with two phases in intermittency were observed for short and long

308　　shadowing times in the two-individual group (Figures 3(g), (h), (j) and (k)) whereas single lines were

309　　presented in the one-individual group (Figures 3(a), (b), (d), and (e)). The linearity and the breakpoints

310　　were consistently observed both "before" and "after" treatment (Figures 3 – 5). This indicates that

311　　pairwise interaction between two individuals played a key role in determining movement data structure.

312　　Recently, the importance of the nearest-neighbor relationship in group behavior was reported. Herbert-

313　　Read *et al*. [54] demonstrated the importance of repulsion and response to a single nearest neighbor in

314　　fish group–behavior dynamics. Pairwise interactions are important in qualitatively capturing the correct

315　　spatial interactions in small groups of fish when compared with the observed data [57]. Our study

316　　indirectly supports the significance of two-individual interactions in group formation.

317　　In addition, the intermittency patterns were substantially different "before" and "after" chemical

318　　exposure for different areas in the observation arena (Figures 4 – 6). The probability distributions of the

319　　shadowing time were different before and after treatment in the center area for the one-individual group

320　　whereas the difference was observed in the boundary area for the two-individual group. Regarding

321　　behavioral-state changes (i.e., transition probability of different movement patterns), no qualitative

322　　difference was observed between the boundary and the central areas [16]. Indeed, the overall patterns

323　　of intermittency were similar in the boundary and the central areas (Figure 3). However, response to

324　　chemical stress appeared differently according to the organism's location in the arena. Especially, the

325　　inter-distances between two individuals were markedly different "before" and "after" treatment (Figure

326　　6). This further indicates that pairwise interactions are strongly reflected in the spatial dynamics in the

327　　boundary area, suggesting emergence of new property in the movement data structure in responding to

328　　neighbors nearby edge areas under stressful conditions. To the best of the authors' knowledge, this is

329　　the first report observing differences in the intermittency of forces on individuals in the boundary and

330  the central areas. Further study, however, is required in both the computational and the biological

331  aspects.

332  The existence of breakpoints in two-individual groups (Figures 3 − 5) also reflects critical time

333  duration for characterizing collective motion. Considering that the slopes for intermittency at short

334  times were near -1.5 and the slopes became steeper, the time duration of 10 seconds may be due to

335  behaviors stemming from the association of two individuals in a confined area (e.g., approach,

336  communication). The breakpoints moved toward longer time duration in the case of absolute forces

337  (Figures 5(c) and (i)). Currently, the mechanism of breakpoint formation is not known. This time

338  duration may also be due to an output from physiological networks [58]. In biological aspects,

339  physiological and/or molecular genetics networks could be investigated; how stereotypic changes in

340  behavioral patterns could originate from integrative actions of neural and endocrine systems [50].

341  However, the detailed mechanism is currently unknown and more research may be required in this

342  direction in the future.

343  Considering the difference in the intermittency patterns at different locations before and after

344  treatment, especially in the boundary area, the probability distributions of the shadowing time could be

345  utilized as a useful means of monitoring chemical stress. Intermittency in the inter-distance between

346  two individuals was remarkably different between "before (i.e., strong curves with a breakpoint)" and

347  "after (i.e., single line)" treatment in the boundary, as shown in Figure 5(a).

348  In this study, we did not use the abundance data for the minimal time duration (i.e., the first

349  probability matching to the shortest shadowing time); the points were not maximal in all cases (Figures

350  3 − 6). For instance, the point matching minimum time duration in Figure 4(a) showed abundance less

351  than the abundance shown by the second shortest shadowing time. Considering the negative value (-

352  1.5) of the intermittency [30], the abundance should be theoretically maximized at the shortest

353  shadowing time. The somewhat lower abundance at the minimum shadowing time indicates that the

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354 shortest shadowing time is expressed in a reserved manner biologically, and this may stem from the

355 physiological and behavioral nature of the organisms. However, the reason is currently unknown. In

356 some cases, sufficient data to evaluate intermittency were not recorded. For instance, the intermittency

357 applied to the relative force in the central area, insufficient data points were collected in the central area

358 (Figures 5(d) and (e)). This may be due to the fact that more data points were recorded in the boundary

359 area. Considering that an acute response due to the olfactory stimulus of formaldehyde were generally

360 observed within 5 minutes as stated above, the observation time may not be extended due to weaker

361 response behaviors after 5 minutes, but the replication number may be increased. More data need to be

362 accumulated in a future study.

363   In this study, only one concentration of the chemical was tested. More research is needed at different

364 concentrations of chemicals in order to determine the fish's behavioral response to an increase in stress

365 levels. In the future, more than two individuals could be tested, and the contributions of additional

366 neighbors to group formation could be more closely investigated.

367   In conclusion, the intermittency of forces and inter-distances in one- and two-individual groups

368 effectively addressed the structural changes in collective motion. Whereas linearity was observed in the

369 probability distributions of the shadowing time for the one-individual group, two phases with

370 breakpoints were measured for two-individual group consisting linearity (the short shadowing time)

371 and exponential function (the long shadowing time). Furthermore, the effect of chemical stress was

372 demonstrated by using difference between the intermittencies in the boundary and the central areas.

373 Differences in the intermittency patterns appeared more clearly in the center for the one-individual

374 group, but the differences were more effectively presented in the boundary for the two-individual

375 group. Changes in the probability distributions of the shadowing time suggested that the pairwise

376 association between two individuals is essential in collective motion and group formation. The

377 sensitivities in the intermittencies evaluated for the one- and the two-individual groups in response to

378 toxic chemicals can be utilized as a means of behavioral monitoring to detect contaminants in the

379 environment.

380 **ACKNOWLEDGMENT**

384 **REFERENCES**

385 [1] A. D. Lemly and R. J. F. Smith, Ecotox. and Environ. Safe. **11**, 211 (1986).

386 [2] H. Dutta, J. Marcelino, and C. Richmonds, Arch. Int. Phys. **100**, 331 (1992).

387 [3] T. S. Chon, N. Chung, I. S. Kwak, J. S. Kim, S. C. Koh, S. K. Lee, J. B. Leem, and E. Y. Cha, Environ.

388 Monit. Assess. **101**, 2 (2005).

389 [4] J. A. Macedo-Sousa, J. L. T. Pestana, A. Gerhardt, A. J. A. Nogueira, and A. M. V. M. Soares,

390 Chemosphere **67**, 1665 (2007).

391 [5] A. Gerhardt, *Biomonitoring of polluted water: reviews on actual topics* (Trans Tech Publications, Uetikon-

392 Zuerich, 1999).

393 [6] I. S. Kwak, T. S. Chon, H. M. Kang, N. I. Chung, J. S. Kim, S. C. Koh, S. K. Lee, and Y. S. Kim, Environ.

394 Pollut. **120**, 672 (2002).

395 [7] Y. S. Park, N. I. Chung, K. H. Choi, E. Y. Cha, S. K. Lee, and T. S. Chon, Aquat. Toxicol. **71**, 217 (2005).

396 [8] B. J. Lawrence and R. J. F. Smith, J. Chem. Ecol. **15**, 210 (1989).

397 [9] C. W. Ji, S. H. Lee, K. H. Choi, I. S. Kwak, S. G. Lee, E. Y. Cha, S. K. Lee, and T. S. Chon, Int. J. Ecodyn.

398 **2**, 28 (2007).

399 [10] Y. Liu, T. S. Chon, H. K. Baek, Y. H. Do, J. H. Choi, and Y. D. Chung, Mod. Phys. Lett. B **25**, 1135

400 (2011a).

401 [11] Y. Li, J. M. Lee, T. S. Chon, Y. Liu, H. Kim, M. J. Bae, and Y. S. Park, Mod. Phys. Lett. B **27**, 135 (2013).

402 [12] P. C. Tobin and O. N. Bjørnstad, J. Ani. Ecol. **72**, 461 (2003).

403 [13] S. Dray, M. Royer-Carenzi, and C. Calenge, Ecol. Res. **25**, 675 (2010).

404    [14] T. S. Chon, Y. S. Park, K. Y. Park, S. Y. Choi, K. T. Kim, and E. C. Cho, Appl. Entomol. and Zool. **39**, 79

405    (2004).

406    [15] C. K. Kim, I. S. Kwak, E. Y. Cha, and T. S. Chon, Ecol. Model. **195**, 62 (2006).

407    [16] Y. Liu, S. H. Lee, and T. S. Chon, Ecol. Model. **222**, 2192 (2011b).

408    [17] T. V. Nguyen, Y. Liu, I. H. Jung, T. S. Chon, and S. H. Lee, Mod. Phys. Lett. B **25**, 1144 (2011).

409    [18] T. Vicsek, A. Czirók, E. Ben-Jacob, I. Cohen, and O. Shochet, Phys. Rev. Lett. **75**, 1227 (1995).

410    [19] S. V. Viscido, M. Miller, and D. S. Wethey, J. Theor. Biol. **208,** 315 (2001).

411    [20] J. K. Parrish, S. V. Viscido, and D. Grünbaum, Biol. Bull. **202**, 296 (2002).

412    [21] S. H. Lee, H. K. Pak, and T. S. Chon, J. Theor. Biol. **240**, 250 (2006).

413    [22] A. S. Kane, J. D. Salierno, G. T. Gipson, T. C. A. Molteno, and C. Hunter, Water Res. **38**, 3993 (2004).

414    [23] S. Y. Ha, S. Jung, and M. Slemrod, J. Differ. Equations **252**, 2563 (2012).

415    [24] N. S. Matuda, Bull. of the Jpn. Soc. Sci. Fisher. **46**, 689 (1980).

416    [25] S. H. Lee, J. of Asia-Pac. Entomol. **16**, 12(2013).

417    [26] K. N. Tsutomu, T. Takagi, K. Yamamoto, and T. Hiraishi, Nippon Suisan Gakkaishi **59**, 1280 (1993).

418    [27] K. Suzuki, T. Takagi, and T. Hiraishi, Fish. Res. **60**, 4 (2003).

419    [28] J. E. Hirsch, B. A. Huberman, and D. J. Scalapino, Phys. Rev. A **25**, 519 (1982).

420    [29] W. C. B. David, K. Sauer, and R. J. Otis, J. Environ. Eng. Div. **102**, 790 (1976).

421    [30] Y. Do, Y. C. Lai, and Z. Liu, E. J. Kostelich, Phys. Rev. E **67**, 202 (2003) .

422    [31] Y. Do and Y. C. Lai, Europhys. Lett. **67**, 915 (2004).

423    [32] P. Manneville and Y. Pomeau, Phys. Lett. A **75**, 1 (1979).

424    [33] N. F. S. Lawrence, Biophys. J. **8**, 252 (1968).

425    [34] P. Gawthrop, I. Loram, M. Lakie, and H. Gollee, Biol. Cybern. **104**, 32 (2011).

426    [35] Y. Pomeau, J. C. Roux, A. Rossi, S. Bachelart, and C. Vidal, J. Physique Lett. **42**, 272 (1981).

427    [36] I. M. De la Fuente, L. Martinez, and J. Veguillas, Biosystems **39**, 88 (1996).

428    [37] G. J. de Valcárcel, E. Roldán, V. Espinosa, and R. Vilaseca, Phys. Lett. A **206**, 360 (1995).

429    [38] Y. Pomeau and P. Manneville, Commun. in Math. Phys. **74**, 190 (1980).

430    [39] T. Datry, D. Arscott, and S. Sabater, Aquat. Sci. **73**, 454 (2011).
18

431    [40] M. T. Bogan, K. S. Boersma, and D. A. Lytle, Freshwater Biol. **10**, 1111 (2013).

432    [41] A. Harnos, G. Horváth, A. B. Lawrence, and G. Vattay, Physica A **286**, 312 (2000).

433    [42] A. Mashanova, T. H. Oliver, and V. A. A. Jansen, J. R. Soc. Interface **7**, 199 (2010).

434    [43] R. Jeanson, S. Blanco, R. Fournier, J. L. Deneubourg, V. Fourcassié, and G. Theraulaz, J. Theor. Biol. **225**,

435    443 (2003).

436    [44] M. Martin, F. Bastardie, D. Richard, F. Burel, and C. R. Acad. Bulg. Sci. **324**, 1029 (2001).

437    [45] J. R. Fetcho and K. S. Liu, Ann. NY Acad. Sci. **860**, 334 (1998).

438    [46] G. R. Blaser R, Behav. Res. Methods **38**, 456 (2006).

439    [47] E. Levin, Z. Bencan, and D. Cerutti, Physiol. and Behav. **90**, 55 (2007).

440    [48] S. Kato, J. Neurosci. Methods **134**, 2 (2004).

441    [49] H. A. Swain, C. Sigstad, and F. M. Scalzo, Neurotoxicol. and Teratol. **26**, 726 (2004).

442    [50] R. Gerlai, V. Lee, and R. Blaser, Pharmacol. Biochem. Behav. **85**, 753 (2006).

443    [51] N. Miller and R. Gerlai, Behav. Brain Res. **184**, 157 (2007).

444    [52] N. Noordiana and  Y. C. B. Farhana, Int. Food Res. J. **18**, 125 (2011).

445    [53] C. Xia, Y. Li, T. S. Chon, and J. M. Lee, Indust. Elec. ISIE, 909 (2009).

446    [54] J. E. Herbert-Read, A. Perna, R. P. Mann, T. M. Schaerf, D. J. T. Sumpter, and A. J. W. Ward, P. Natl. A.

447    Sci. **108**, 18726 (2011).

448    [55] R. D. Collins, R. N. Gargesh, A. D. Maltby, R. J. Roggero, M. K. Tourtellot, and W. J. Bell, Physiol.

449    Entomol. **19,** 166 (1994).

450    [56] J. H. Zar, *Biostatistical analysis* (Prentice hall, Upper Saddle River, New Jersey, 1999).

451    [57] Y. Katz, K. Tunstrøm, C. C. Ioannou, C. Huepe, and I. D. Couzin, P. Natl. A. Sci. **108**, 18720 (2011) .

452    [58] I. D. Couzin, J. Krause, R. James, G. D. Ruxton, and N. R. Franks, J. Theor. Biol. **218**, 2 (2002).

453      Table 1 Estimates of the slopes and elevations by applying a regression analysis to the intermittency

454      of forces for movement of zebrafish in one- and two-individual groups in the boundary and central

455      areas before and after chemical treatment.

| No. of indi | Type | Treat | Boundary | | | Center | | |
|---|---|---|---|---|---|---|---|---|
| | | | X | Y | Absolute | X | Y | Absolute |
| One | Indi | Before | -1.89±0.20 | -1.76±0.19 | -1.02±0.27 | -1.32±0.16 | -1.13±0.16 | -1.66±0.49 |
| | | After | -1.91±0.19 | -1.75±0.19 | -1.38±0.26* | -1.79±0.11* | -1.80±0.18* | -1.34±0.11* |
| Two | Center of mass | Before | -1.11±0.17 | -1.10±0.11 | -0.89±0.11 | -1.08±0.15 | -1.17±0.18 | -0.94±0.26 |
| | | After | -1.61±0.17* | -1.48 ±0.14* | -1.19±0.16* | -1.26±0.32* | -1.14±0.26* | -0.96±0.31 |
| | Relative | Before | -1.09 ±0.13 | -1.1±0.09 | -0.79±0.18 | -0.90±0.19 | -1.07±0.15 | -0.76±0.32 |
| | | After | -1.63±0.16* | -1.41±0.09* | -0.89±0.14[①] | -1.30±0.22[②] | -1.09±0.20[③] | -0.87±0.40* |
| | Indi | Before | -1.35±0.11 | -1.28±0.08 | -0.71±0.15 | -0.94±0.10 | -1.07±0.12 | -0.79±0.06 |
| | | After | -1.61±0.17* | -1.52±0.09* | -0.89±0.16* | -1.03±0.19[④] | -1.02±0.17[⑤] | -1.03±0.07* |

456

457      * Indicates statistical significance "before" and "after" treatment based on the different slopes of the regression lines ($p<0.05$) [56].

458      Numbers in circles present statistical significances "before" and "after" treatment based on the different elevations in the regression

459      lines ($p<0.05$) [56] ①-2.07/-1.50, ②-1.15/-0.97, ③-1.08/-0.81, ④-1.40/-1.17 and ⑤-1.47/-1.10, before /after treatment, respectively.

460

461     Table 2 Estimates of the coefficient ($\alpha$) of the exponential function and the goodness of fit (chi-square test)

462     applied to the intermittency of forces of zebrafish in one- and two-individual groups before and after treatment.

| Type | Treat Test | Boundary | | Center | |
|---|---|---|---|---|---|
| | | X | Y | X | Y |
| Center of mass | Before † | 0.31 (0.42) | 0.30 (0.41) | 0.30 (0.59) | 0.30 (0.36) |
| | After † | 0.35 (0.61) | 0.34 (0.62) | 0.30 (0.30) | 0.30 (0.25) |
| | $\chi^2$ †† | 10.66 (0.15) | 10.14 (0.18) | 0.63 (1.00) | 1.79 (0.97) |
| Relative | Before | 0.30 (0.64) | 0.31 (0.45) | 0.30 (0.56) | 0.31 (0.27) |
| | After | 0.35 (0.79) | 0.33 (0.85) | 0.31 (0.30) | 0.31 (0.56) |
| | $\chi^2$ | 10.28 (0.17) | 11.72 (0.11) | 5.61 (0.59) | 11.89 (0.10) |
| Indi | Before | 0.33 (0.48) | 0.33 (0.52) | 0.30 (0.34) | 0.30 (0.29) |
| | After | 0.35 (0.58) | 0.35 (0.52) | 0.30 (0.61) | 0.31 (0.41) |
| | $\chi^2$ | 3.79 (0.80) | 3.19 (0.87) | 5.30 (0.63) | 7.67 (0.47) |

463

464     † Numbers in parentheses indicate the $R^2$ value according to the coefficient estimate of the functions (exponential decay [56]).

465     †† Numbers in parentheses present the probability according to chi-square test's goodness of fit between two exponential functions, one

466     before and one after treatment [56].

467
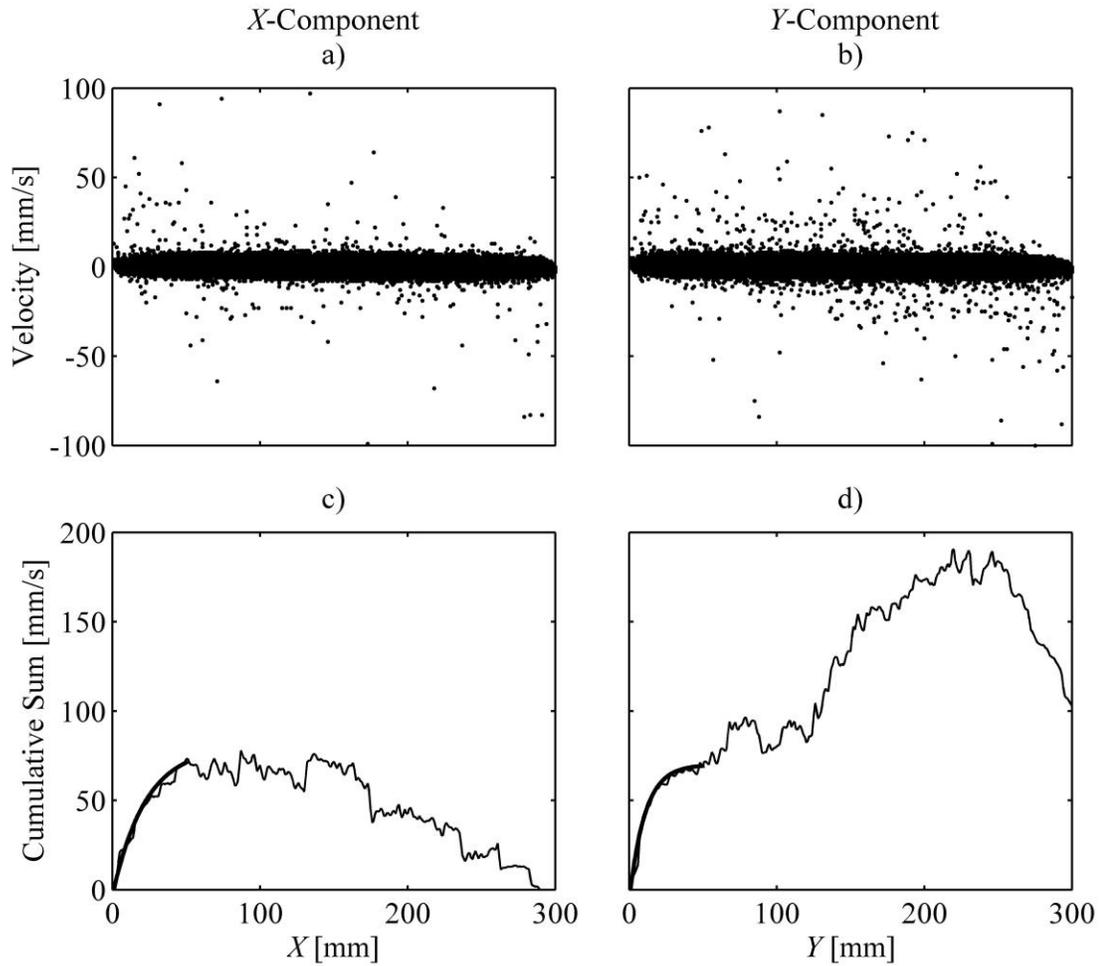
Fig. 1. Velocity distribution and its cumulative sum along each axis of fish movement in the observation arena in defining the boundary area: (a) *x*-component, (b) *y*-component, (c) cumulative sum of the *x*-component, and (d) cumulative sum of the *y*-component. Solid curves at the boundary (c) and (d) indicate the exponential curves fitting the data from 0 mm to 50 mm.

472
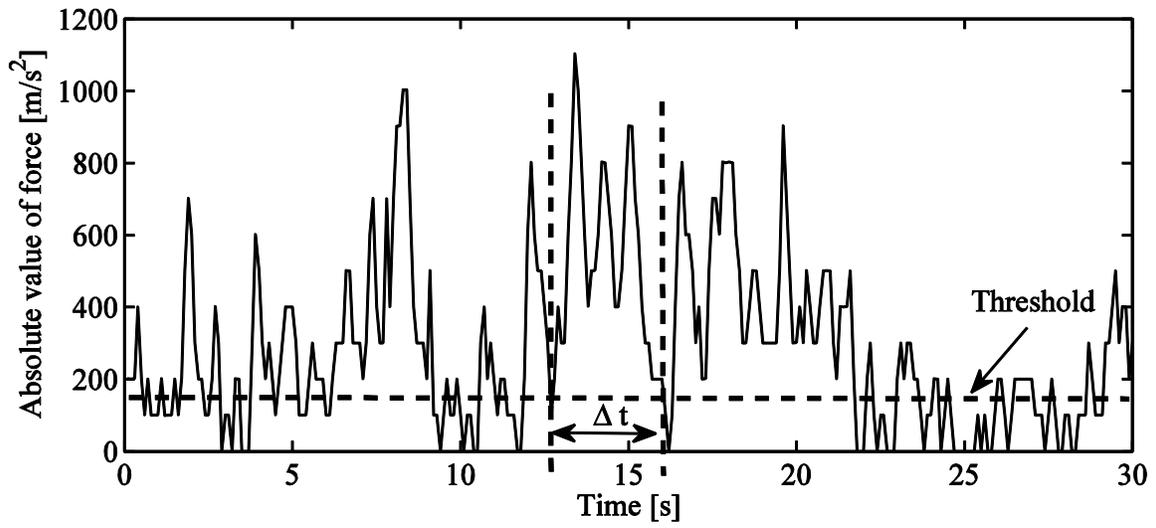
Fig. 2. Time series of the absolute value of force for one individual before treatment in the center for various shadowing times ($\Delta t$) and a threshold (dashed line) to determine the shadowing time.
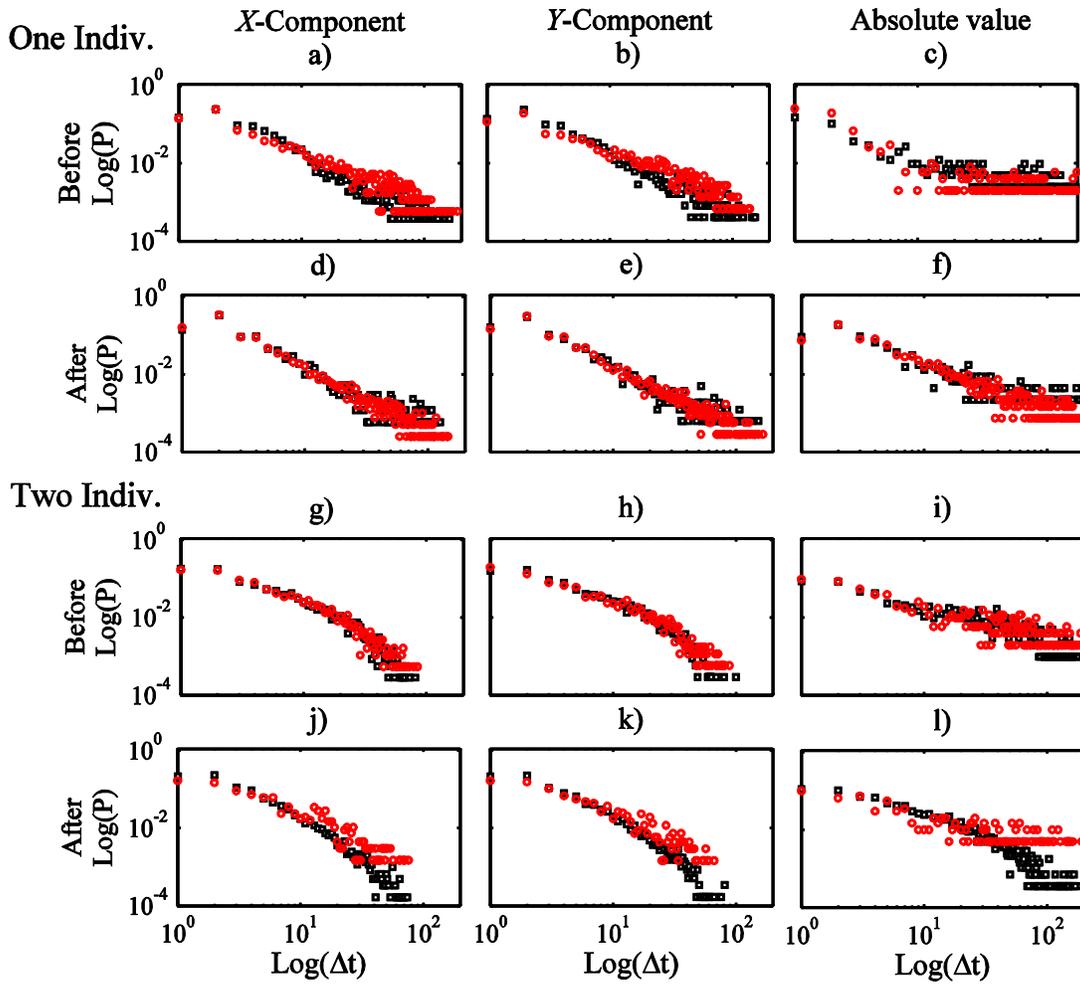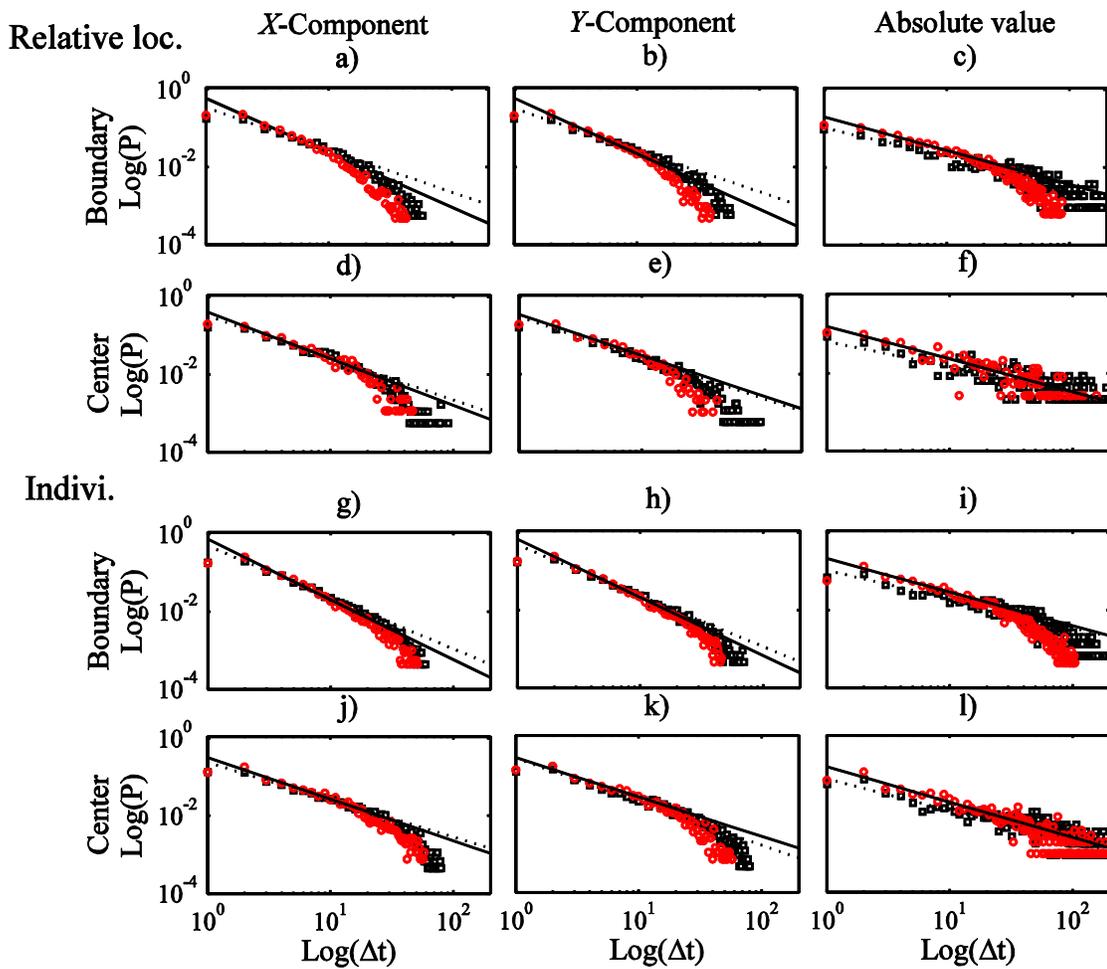
Fig. 3. Intermittency patterns for forces on one individual before (a, b, and c) and after (d, e, and f) treatment, and those for two individuals before (g, h, and i) and after (j, k, and l) treatment in the boundary (blank squares) and the central (red circles) areas. The probability distributions of the shadowing time were fitted to single lines for the one-individual group whereas they were matched to exponential curves for the two-individual group.

482
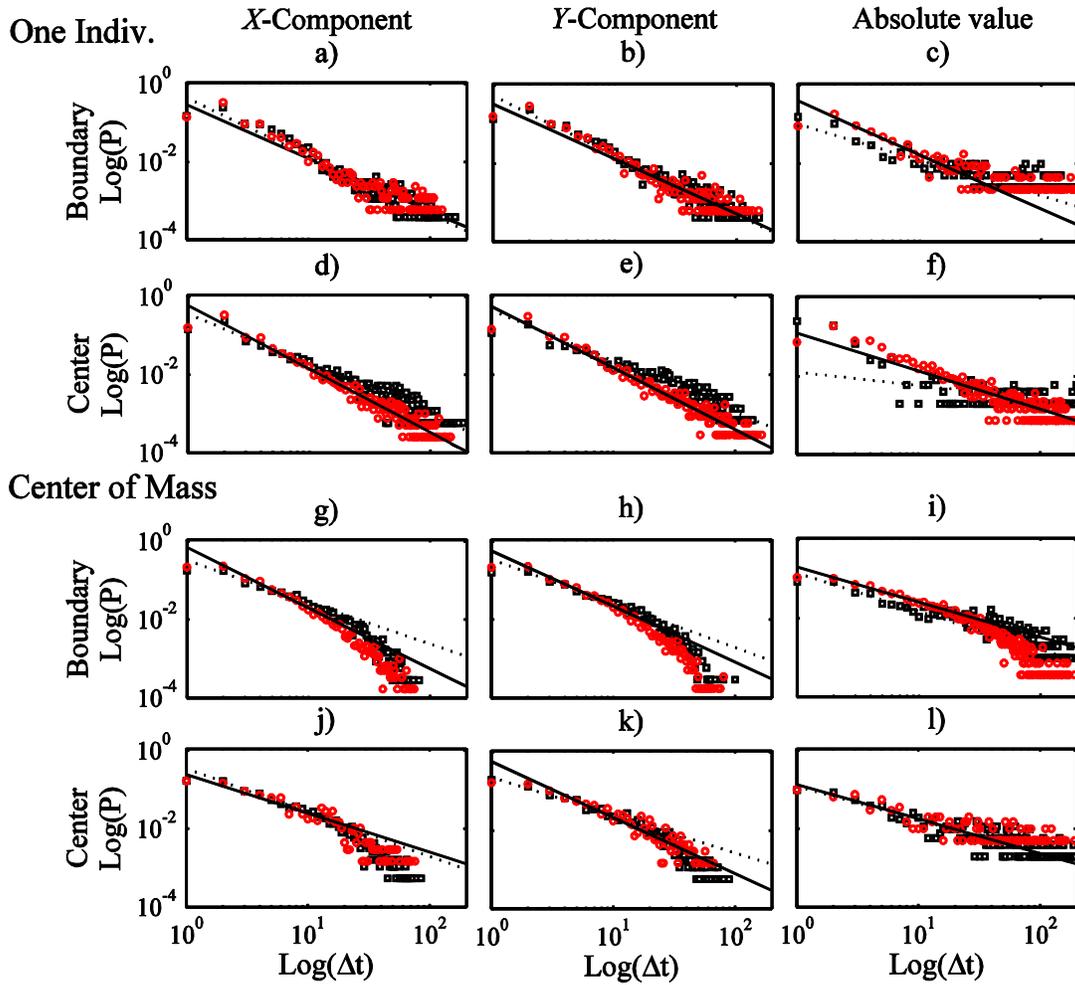
Fig. 4. Intermittency patterns for the forces on the one-individual group in the boundary area ((a) *x*-components, (b) *y*-component, and (c) absolute value) and in the central area ((d) *x*-component, (e) *y*-component, and (f) absolute value), and those for force at the center of mass of the two-individual group in the boundary area ((g) *x*-component, (h) *y*-component, and (i) absolute value) and the central area ((j) *x*-component, (k) *y*-component, and (l) absolute value). Intermittency patterns before and after treatment were different in the center for the one-individual group whereas the difference was observed in the boundary for the two-individual group. Solid and dotted lines fitting "before" and "after" treatment, respectively.

Fig. 5. Intermittency patterns for the forces before (blank squares) and after (red circles) treatment in the two- individual group. The relative force on individuals in the boundary area ((a) *x*-component, (b) *y*-component, and (c) absolute value) and in the central area ((d) *x*-component, (e) *y*-component, and (f) absolute value), and those on two individuals in the boundary area ((g) *x*-component, (h) *y*-component, and (i) absolute value) and in the central area ((j) *x*-component, (k) *y*-component, and (l) absolute value). Differences in the intermittency patterns before and after treatment were more clearly observed in the boundary for relative forces whereas the difference was equally observed in the boundary and the center for individual forces. Solid and dotted lines fitting "before" and "after" treatment, respectively.
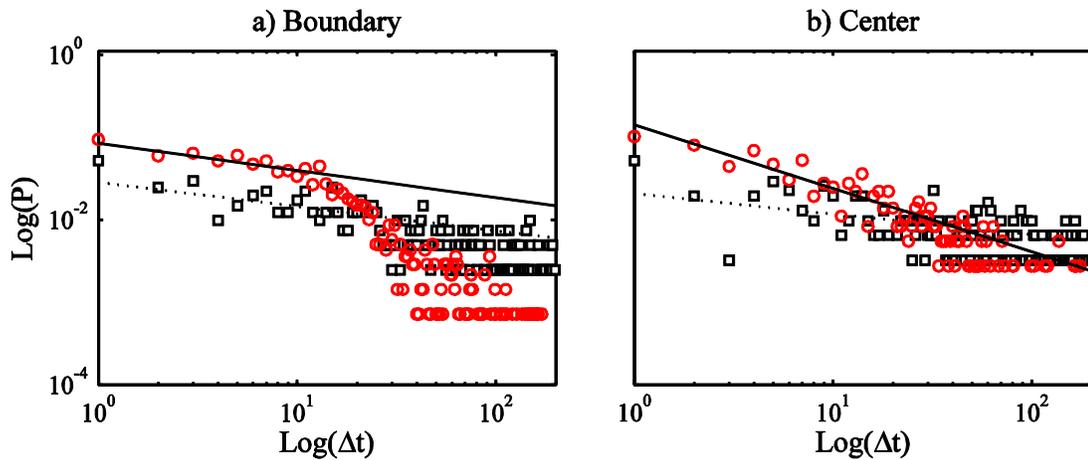
Fig. 6. Intermittency patterns for the inter-distance between two individuals before (blank squares) and after (red circles) treatment in (a) the boundary (slopes before (-0.22 ± 0.08) and after (-0.26 ± 0.12) treatment), and (b) the center (slopes before (-0.47 ± 0.06) and after (-0.80 ± 0.11) treatment). The intermittency pattern was markedly different after treatment in the boundary with a breakpoint clearly separating flat (for short shadowing time) and steep (for long shadowing time) slopes. Solid and dotted lines fitting "before" and "after" treatment, respectively.