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1 **Acoustic discrimination between juvenile walleye pollock and pointhead flounder**

2 Naizheng Yan^a, Tohru Mukai^b, Jun Yamamoto^c, Kohei Hasegawa^b

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4 ^aGraduate School of Fisheries Sciences, Hokkaido University, 3-1-1 Minato, Hakodate,
5 0418611, Japan.

6 ^bFaculty of Fisheries Sciences, Hokkaido University 3-1-1 Minato, Hakodate, 0418611,
7 Japan.

8 ^cField Science Centre for Northern Biosphere, Hokkaido University 3-1-1 Minato, Hakodate,
9 0418611, Japan.

10

11 E-mail addresses

12 N.Y., ynz_1992@outlook.com

13 J.Y., yamaj@fish.hokudai.ac.jp

14 K.H., kohase@fish.hokudai.ac.jp

15

16 *Corresponding author: Tohru Mukai. Faculty of Fisheries Sciences, Hokkaido University 3-
17 1-1 Minato, Hakodate, 0418611, Japan. Tel & Fax: +81-138-40-8853. E-mail:
18 mukai@fish.hokudai.ac.jp

19

20 **Abstract**

21 When using acoustic methods to investigate fish biomass, it is important to distinguish the
22 target species from other organisms. Previous limitations to acoustic information in surveys
23 meant it was not possible to discriminate juvenile walleye pollock from their predator,
24 pointhead flounder, in Funka Bay, Japan, possibly leading to overestimates in population size.
25 Thus, here, an acoustic method was developed to distinguish the two species based on
26 relative frequency response. This method was used to survey juvenile walleye pollock to
27 evaluate capture potential around Funka Bay, Japan, where pointhead flounder is reportedly
28 its most abundant predator. Volume backscattering strength of pointhead flounder was highest
29 at 200 kHz, while that of juvenile walleye pollock was highest at 38 kHz. Therefore, the
30 relative frequency response at 38 kHz and 200 kHz was successfully used to distinguish
31 between the two species. This method could be more extensively applied to other species in
32 other aquatic environments globally.

33

34 **Keywords:** walleye pollock, pointhead flounder, predator-prey relationship, frequency
35 characteristic, bladderless fish

36

37 **1 Introduction**

38 Acoustic methods have been widely used in biomass surveys of fish since the 1970s, as
39 these facilitate the investigation of a wide oceanographic area in a short period of time at
40 lower costs than with the traditional trawl method (Foote, 2001; Simmonds and MacLennan,
41 2005). However, acoustical discrimination is an issue for acoustic surveys. Marine organisms
42 have inherent frequency characteristics based on body size and body composition, and the
43 relative frequency response is useful to differentiate different organisms on echograms
44 (Simmonds and MacLennan, 2005; Fernandes, 2009; Korneliussen, 2018). Some published
45 studies have reported on this method to distinguish between organisms; further, the method
46 has been successfully used for discrimination between fish and zooplankton, e.g., between
47 walleye pollock (*Gadus chalcogrammus*) and isada krill (*Euphausia pacifica*) (Miyashita *et*
48 *al.*, 1998) or copepods (Kim *et al.*, 2016), or between different species of fish, such as
49 juvenile walleye pollock and capelin (*Mallotus villosus*) (Logerwell and Wilson, 2004), or
50 herring (*Clupea harengus*) and juvenile capelin (Pedersen and Korneliussen, 2009).

51 Walleye pollock is broadly distributed throughout the North Pacific and plays both
52 important ecological and economic roles (Livingston, 1993; Bailey, 2000; Yamamura *et al.*,
53 2002; Sigler and Csepp, 2007). In Japan, the Pacific stock of walleye pollock off the coast of
54 Tohoku and Hokkaido is the largest (Tsuji, 1989) and one of the most important fishery
55 resources. The Pacific stock spawns around the mouth of Funka Bay, in Northern Japan, from
56 December to March (Maeda *et al.*, 1976; Maeda, 1986; Honda *et al.*, 2004). The larvae and
57 juveniles primarily inhabit the surface and subsurface layers of the bay waters from January
58 through June (Kamba, 1977; Nakatani, 1988). During the day, small juveniles (< 30 mm) are

59 distributed in shallow, warm, and low-salinity water, whereas larger ones are found in deeper,
60 cold, and saline water, but migrate to shallower depths at night (Kawauchi, 2014). Juveniles
61 that have grown to approximately 7 cm in length start to navigate out of Funka Bay and move
62 to the seabed (Nakatani and Maeda, 1987). A study by Funamoto et al. (2013) suggests that
63 predation on juvenile walleye pollock in the nursery ground, outside of Funka Bay, affects the
64 recruitment variability of the Pacific stock. However, predation by a flatfish, pointhead
65 flounder (*Cleisthenes pinetorum*), is likely the cause of the predation pressure that affects the
66 recruitment success of the 0-age walleye pollock (Yan et al., 2018).

67 The pointhead flounder is a middle-sized flatfish (adult total length; ca. 200 to 400 mm)
68 and widely distributed from Sakhalin southward to the coastal waters of the Sea of Japan, the
69 East China Sea, the coastal area of Hokkaido, and the northern Pacific coast of Honshu, Japan
70 (Tominaga et al., 2000). Around the Funka Bay, the pointhead flounder, a major species of the
71 demersal fish community, is found in abundance (Hiraoka et al., 2005). Pointhead flounder
72 swim actively and forms schools in the middle of the water column to capture prey, including
73 juvenile walleye pollock (Yan et al., 2018). This suggests that echosounders could detect
74 them, as well as juvenile walleye pollock. Acoustic discrimination between these two species
75 in acoustic surveys for juvenile walleye pollock conducted around Funka Bay is essential to
76 accurately estimate population abundance. At the same time, the echoes from the pointhead
77 flounder have presumably contaminated acoustic surveys when evaluating the distribution
78 and abundance of juvenile walleye pollock around the Funka Bay. In particular, pointhead
79 flounder preying on pollock are liable to cause an overestimation because of the distribution
80 overlap.

81 Quantitative echosounders at 38 kHz have been successfully used to survey the
82 distribution and abundance of adult (Honkalehto et al., 2011; Miyake, 2012) and juvenile
83 walleye pollock to evaluate global capture potential (40 mm–110 mm by Brodeur and
84 Wilson, 1996; age-0 by Swartmzan et al., 1999; 30–92 mm by Schabetsberger et al., 2000).
85 Acoustic information for flatfish species is limited because they are believed to occur in the
86 dead zone of echosounders, i.e., the region near the seabed, where fish cannot be resolved
87 acoustically. For this reason, pointhead flounder has not been previously studied using
88 echosounders. In addition, pointhead flounder lack swim-bladders and their acoustic
89 characteristics are unknown, however, their acoustic characteristics can be assumed to be
90 similar to those of other swim-bladderless fish such as Atlantic mackerel. Korneliussen
91 (2010) showed that Atlantic mackerel can be identified from multi-frequency data by means
92 of the standard 200 kHz (3.5 ± 1 times stronger backscatter) compared to 38 kHz. This
93 suggests that 200 kHz is a good frequency to observe swim-bladderless fish. Moreover,
94 pointhead flounder swim in the middle of the water column in schools, which signifies that
95 echosounders can easily detect them.

96 We hypothesized that the relative frequency response should effectively discriminate
97 pointhead flounder from juvenile walleye pollock, as there are large differences in size and
98 body components between the two species. Therefore, we investigated the acoustic
99 characteristics and effectiveness of acoustic discrimination of pointhead flounder and juvenile
100 walleye pollock on echograms, and confirmed the prey-predator relationship between them.

101 **2 Material and methods**

102 **2.1 Data collection**

103 Surveys were conducted in and near Funka Bay (Fig. 1), Hokkaido, Japan, on board T/S
104 USHIO-Maru (179 tons, 39.39 m) of Hokkaido University, for four or five days each April
105 and May of 2015 to 2018. This period followed the spawning season, when juveniles were
106 still present, before migrating out of the bay. Acoustic data were obtained from a split-beam
107 quantitative echosounder (frequency 38, 120, 200 kHz; EK 60, Simrad, Norway) with three
108 transducers (ES38B, ES120-7C, ES200-7C, Simrad, Norway). All echosounders were
109 calibrated on the first day of each survey with a 38.1-mm diameter sphere made from
110 tungsten carbide with 6% cobalt binder. The echosounders were configured with the
111 parameters listed in Table 1 (Foote et al., 1987; Demer et al., 2015). The pulse width was set
112 at 1.024 ms.

113 A frame-type midwater trawl net (FMT; black frame; mouth: 2 m × 2 m; mesh opening:
114 1.5 mm; Itaya et al., 2001; Itaya et al., 2007; Lu et al. 2018) was used to collect juvenile
115 walleye pollock during the daytime and at night. The FMT was towed horizontally at 3 to 4
116 knots for 10 min once reaching the juvenile walleye pollock layer water depth. A catch
117 monitoring system (PI50, Simrad, Norway) was used to monitor the real-time depth of the
118 FMT to ensure that the trawler fished at the depth of the targeted layers, with a PI depth catch
119 monitoring sensor fastened to the head of the FMT.

120 Pointhead flounder were sampled by rod fishing with six rods drifting alongside the ship.

121 **2.2 Data analysis**

122 **2.2.1 Biological data**

123 Biological samples from FMT were preserved in 5% formalin solution immediately after
124 towing. After the cruise, samples were classified, the number of individuals was counted, and

125 wet weight was measured. For each survey, more than 100 juvenile walleye pollocks were
126 randomly selected for fork length measurements.

127 Sampled pointhead flounder were measured on board. To verify the prey-predator
128 relationship between pointhead flounder and juvenile walleye pollock, the stomach contents
129 of the flounder were collected from 30 specimens at sites sampled in the 2015–2018 surveys.
130 Undigested contents were classified according to species, and wet weight was measured.
131 Total wet weight of digested contents was recorded.

132 **2.2.2 Acoustic data**

133 When scatters are uniformly irregular and widely distributed with respect to beam
134 opening, the volume backscattering strength (S_V) is shown in the linear notation as:

$$135 S_V = 10 \log s_v$$
$$136 = 10 \log n + TS \quad (1)$$

$$137 TS = 10 \log \sigma_{bs} \quad (2)$$

138 Where s_v is the volume backscattering coefficient and the linear value of S_V , n is the
139 distribution density (ind./m³) of fish. TS is target strength, a prime factor of fish for
140 quantitative and qualitative assessments of their stocks, and σ_{bs} is acoustic cross-section.

141 Three frequencies were used to delineate the acoustic characteristics of juvenile walleye
142 pollock and pointhead flounder. Using equation (1), the difference in S_V between any two
143 frequencies is the difference in TS between these two frequencies when the beam width of
144 each transducer is the same, is as follows:

$$145 S_V(f_high) - S_V(f_low) = TS(f_high) - TS(f_low) \quad (3)$$

146 Thus, we used acoustic data gathered during FMT and rod fishing from 2015 to 2017 to

147 extract the S_V of the two species at three frequencies to delineate their frequency
148 characteristics. Acoustic data were processed using Echoview software (Version 9, Echoview
149 Software Pty. Ltd, Australia). One juvenile walleye pollock echo cell was defined as 5 min
150 (horizontal) \times 5 m (vertical). The echo cell of a pointhead flounder school was detected along
151 the echo shape. Then, every certain fish school on the echogram was extracted to calculate
152 average S_V at three frequencies.

153 For the data from 2018, we separated the two species in the echograms using the
154 differences in the S_V frequency characteristics. To reduce the position deviation of the echoes
155 due to transducer position (ES120-7C and ES200-7C were 20 cm apart and 54 cm away from
156 the ES38B), the 10 ping \times 10 cm analysis cell was used to average S_V values.

157 **2.3 Digestion observation experiments**

158 Digestion observation experiments were conducted to find out how long it takes the
159 pointhead flounder to digest juvenile walleye pollock after feeding on it. The fish samples
160 used for the experiment were collected by rod fishing (pointhead flounder) and FMT
161 (juvenile walleye pollock) at the Funka Bay on the 25th and 26th of April 2017. Pointhead
162 flounders were reared in a rectangular seawater tank (2 tons, 2 m \times 1 m \times 1 m; approximate
163 temperature 10 °C, approximate salinity 32 psu) and juvenile walleye pollock were reared in
164 a circular seawater tank (1 ton, 1.5 m in diameter, 1 m in height; approximate temperature
165 10 °C, approximate salinity 32 psu) in Hokkaido University until just before the experiment.
166 Both fish species were reared on krill fed to them once a day.

167 The experiment was conducted in the rearing tank on the 12th of June 2017, using 15
168 pointhead flounders (mean total length, TL = 31.4 \pm 2.1 cm; mean body weight, W = 247.4 \pm

169 50.0 g). The time of observation of digestion was 0, 3, 6, 12, 18, and 24 hours after eating the
170 juvenile walleye pollock. In sequence, 2, 2, 3, 3, and 3 individuals were used in the
171 experiments. One to seven juvenile walleye pollocks (fork length, FL = 5.1 ± 0.6 cm) were
172 eaten per pointhead flounder. After the specified time, the size was measured, the body was
173 dissected, the stomach was opened, and the state of digestion of the juvenile walleye pollock
174 was confirmed.

175 In order to investigate how long the swim-bladder will be left in the body of the pointhead
176 flounder if the juvenile walleye pollock are eaten, the stomach contents of the pointhead
177 flounder that ate the juvenile walleye pollock in the digestion-observation experiments were
178 confirmed by soft X-rays. Additionally, in order to compare the changes of the swim-bladder
179 of juvenile walleye pollock with those that were eaten by the pointhead flounder, at the same
180 time as feeding the juvenile walleye pollock to the pointhead flounder, another three juvenile
181 walleye pollocks were fixed with 5% formalin, and after a specified time, soft X-ray images
182 were taken.

183 All procedures for the use of the fish were approved by Institutional Animal Care and Use
184 Committee of the National University Corporation, Hokkaido University.

185 **3 Results**

186 **3.1 Population levels and stomach contents**

187 Juvenile walleye pollock were caught by FMT in every survey both in daytime and night.
188 Fig. 2 shows the distribution of their total length in April and May 2015–2018. Total length
189 ranged from 9 to 62 mm (overall mean total length was 25.0 mm; mean \pm SD: 18.7 ± 6.8 and

190 28.9 ± 5.8 in April and May, respectively). Juvenile walleye pollock accounted for over 70%
191 of the FMT samples in wet weight. Aside from juvenile walleye pollock, some zooplankton
192 were also collected, including amphipods (under 7% in April, under 23% in May), krill
193 (under 13% in April, under 4% in May), and other juvenile fishes and zooplankton (under
194 2%).

195 Pointhead flounder were collected by rod fishing in every survey only at daytime, except
196 for those conducted in May 2016. The total length of 740 individuals ranged from 160 to 390
197 mm (mean ± SD: 261.0 ± 36.0), with almost no difference (p-value > 0.05) between
198 measurements from April and May (Fig. 3). Nevertheless, more swimming pointhead
199 flounder were found in April than in May. Aside from pointhead flounder, there was no other
200 species captured from the middle layers during rod fishing.

201 The stomach contents of pointhead flounder confirmed the prey-predator relationship
202 between the two species. Juvenile walleye pollock were the main prey of pointhead flounder
203 in April and May 2015–2018. In 2015, juvenile walleye pollock accounted for 47.9% (i.e. the
204 highest proportion) of the stomach contents of pointhead flounder specimens. In 2016,
205 juvenile walleye pollock only accounted for 5.9% of pointhead flounder stomach contents. In
206 contrast, in 2017, almost all stomachs were empty and no juvenile walleye pollock were
207 found. In 2018, again, juvenile walleye pollock made up 31.5% of the stomach contents. We
208 consider that the content was dependent on the occurrence of pointhead flounder interacting
209 with juvenile walleye pollock. Notably, more juvenile walleye pollock were predated by
210 pointhead founder in April. Pointhead flounder also preyed on some zooplankton, such as
211 amphipods, krill, copepod, and other organisms, as well as the juveniles of other species,

212 including flounder and shrimp.

213 **3.2 Echo characteristics on echograms**

214 Representative echograms of juvenile walleye pollock during trawling at night are shown
215 in Fig. 4. Echoes from juvenile walleye pollock were strongly scattered at all three
216 frequencies (38, 120, 200 kHz). The echoes typically exhibited a layered shape, regardless of
217 the time of day; however, the layers at night were shallower than those during the daytime,
218 probably due to diel vertical migration (Schabetsberger et al., 2000). The same echo shapes
219 and vertical distributions were observed by Kawauchi et al. (2014).

220 Echograms at the site where pointhead flounder were collected during the day are shown
221 in Fig. 5. Patch-shaped echoes that also scattered strongly at all three frequencies in the
222 middle of the water column represented schools of pointhead flounder. Such patch shaped
223 echoes were frequently detected in the middle of the water column during the daytime. It is
224 noted that we only fished pointhead flounder and could only find their echoes during the
225 daytime.

226 **3.3 Distribution of S_v**

227 The frequency characteristics of each species are shown in Fig. 6. Comparison of $S_v(38)$
228 and $S_v(120)$ for juvenile walleye pollock showed that most data were distributed along the
229 line; therefore, $S_v(120)$ was almost the same as $S_v(38)$, with a mean difference $S_v(120) -$
230 $S_v(38) = -0.16$ dB (p-value > 0.05; 95% confidence interval: -0.35–0.03 dB). Similarly,
231 $S_v(200)$ was lower than $S_v(120)$, with $S_v(200) - S_v(120) = -2.96$ dB (p-value < 0.01; 95%
232 confidence interval: -3.09–2.83 dB); further, $S_v(200)$ was also lower than $S_v(38)$, with

233 $S_V(200) - S_V(38) = -3.12$ dB (p-value < 0.01; 95% confidence interval: -3.35--2.89 dB).

234 Therefore, juvenile walleye pollock showed lower S_V at a higher frequency.

235 Comparison of $S_V(38)$ and $S_V(120)$ for pointhead flounder showed that most data were
236 distributed above the line. Therefore, $S_V(120)$ was higher than $S_V(38)$, with a mean difference
237 $S_V(120) - S_V(38) = 3.45$ dB (p-value < 0.01; 95% confidence interval: 3.22–3.68 dB);
238 similarly, $S_V(200)$ was higher than $S_V(120)$, with $S_V(200) - S_V(120) = 1.77$ dB (p-value <
239 0.01; 95% confidence interval: 1.57–1.96 dB), and also $S_V(38)$, with $S_V(200) - S_V(38) = 5.22$
240 dB (p-value < 0.01; 95% confidence interval: 4.88–5.55 dB). Therefore, pointhead flounder
241 showed higher S_V at a higher frequency, which is the opposite to the S_V pattern described by
242 juvenile walleye pollock.

243 **3.4 Acoustic discrimination**

244 Based on the results shown in Fig. 6, we decided to use 38 and 200 kHz to distinguish
245 between the two species, because the data for each species showed the least overlap at these
246 two frequencies. The mean differences between these two frequencies had the greatest
247 distance. To determine the data range for each species, the distribution of the differences
248 between $S_V(200)$ and $S_V(38)$ between the two species were examined (Fig. 7). Based on our
249 test, the data range of $S_V(200) - S_V(38)$ for juvenile walleye pollock was designated as -8~0
250 dB (i.e. 92% of juvenile walleye pollock echoes were obtained), while the range was 1~10 dB
251 for pointhead flounder (i.e. 83% of pointhead flounder echoes were obtained). We attempted
252 to separate the two species using this method on the data, as shown in Fig. 8 and Fig. 9. In
253 Fig. 8*d*, juvenile walleye pollock were almost completely separated in both the towed layer
254 and for all scatters. There were no patch-shaped echoes (Fig. 8*e*), indicating that mixing with

255 pointhead flounder occurred at that time. In Fig. 9e, all patch shaped echoes (pointhead
256 flounder schools) were separated and there were no layered echoes, which indicated that there
257 were no juvenile walleye pollock school (Fig. 9d). Therefore, this method was effective in
258 detecting and differentiating between two species.

259 To test whether frequency differences could accurately distinguish the two species when
260 they overlapped, we analysed the data collected at 15:00 to 15:20, 19th of April 2018 (Fig.
261 10), when we caught more than 150 pointhead flounder containing many juvenile walleye
262 pollock in their stomachs, where the reflection on the echograms should represent both
263 species. Figure 10c clearly shows that the distribution of the two fish overlapped. The echoes
264 of the two fish were separated according to their frequency characteristics shown in Fig.
265 10d,e. In Fig. 10d, there were layered echoes around 50–60 m of depth caused by juvenile
266 walleye pollock. In Figure 10e, the echoes at 30–40 m were derived from pointhead flounder.
267 The surveys from 2015 to 2017 aimed to acquire the echoes of single-fish schools, i.e. survey
268 areas were chosen where the two species overlapped the least. In the 2018 data, we focused
269 on the search echoes where the two species overlapped.

270 **3.5 Digestion-observation experiments**

271 The digestion of juvenile walleye pollock up to 24 h after predation is summarised as
272 follows:

273 0h: 100% remaining

274 3h: 60%~80% remaining

275 6h: 5%~50%; remains can still be confirmed as fish

276 12h: 0%~10%; remains can no longer be confirmed as fish, only some white flesh remains

277 18h: under 5% remaining

278 24h: nothing remaining

279 At any given time after eating, more of the body of juvenile walleye pollock remained
280 when pointhead flounder preyed on a larger number of juvenile specimens, and after 6 h,
281 pointhead flounder had digested most of the juvenile walleye pollock body. As the tank was
282 too small for pointhead flounder to swim, this may have caused slow digestion. We believe
283 pointhead flounder will digest faster at sea.

284 Predatory activity is not believed to occur at night because pointhead flounder was
285 confirmed to use the eyes while preying. Additionally, rod fishing is done always around
286 sunrise, when pointhead flounder started to prey on walleye pollock during the surveys; thus,
287 we cannot say that pointhead flounder were not actually feeding on juvenile walleye pollock
288 even when their stomach was empty.

289 The results of soft X-ray imaging showed that juvenile walleye pollock can only be
290 detected by the otoliths in the stomach of pointhead flounder, but soft X-ray imaging cannot
291 confirm the swim-bladder, except for the results of 0 h after predation. Fig. 11 is a soft X-ray
292 photo of a pointhead flounder immediately after predation (0 h), two juvenile walleye
293 pollocks were confirmed, as their swim-bladders could be seen clearly. Therefore, when the
294 digestive activity begins, the gas in the swim-bladder will be discharged. On the other hand,
295 the soft X-ray photographs of juvenile walleye pollock showed there was no change in the
296 swim-bladder even 24 h after death; this indicates that predator activity caused the swim-
297 bladder of juvenile walleye pollock to burst. These findings confirm that there is no gas left
298 in the pointhead flounder.

300 **4 Discussion**

301 Zooplankton was always mixed with juvenile walleye pollock but their distribution
302 density and TS were much lower in the survey area, so we did not consider the frequency
303 characteristics of juvenile walleye pollock to be influenced by zooplankton. For pointhead
304 flounder, there was no other bladderless fish swimming in the middle layers in Funka Bay,
305 and we were able to fish them at the depths shown by observed echoes. More than 100
306 pointhead flounder were always fished within one hour and no other species was caught;
307 therefore, we concluded the target echoes for pointhead flounder.

308 We used FMT to catch the juvenile walleye pollock in our surveys, despite findings by Lu
309 et al. (2018) that juveniles over 60 mm are difficult to be caught by FMT. However, in May,
310 Nishimura et al. (2007) used the midwater trawl to collect juvenile walleye pollock with a
311 sample size distribution of 16 – 64 mm at the mouth of Funka Bay. Nakatani and Maeda
312 (1987) reported that juvenile walleye pollock move to the seabed when they reach a total
313 length of 7 cm; therefore, we consider that FMT was suitable to collect the juveniles from the
314 middle water layers during April and May.

315 In April, the majority of walleye pollock had already left Funka Bay after spawning, and
316 pointhead flounder became the dominant fisheries in Funka Bay (around 40% during April
317 and May 2013 to 2017, data from Fisheries statistics in Hokkaido prefecture). Other acoustic
318 target species, walleye pollock (under 5%), and cod (around 10%), were reported swimming
319 near the ocean bottom but were not caught during rod fishing. Therefore, we believe that
320 there is a minimal number of other acoustic fish swimming with pointhead flounder and

321 juvenile walleye pollock when they overlap, but this shall be investigated further in future
322 studies.

323 After preying on a swim-bladder fish, will target strength of swim-bladderless fish
324 change? From the results of the digestion experiments with pointhead flounder and juvenile
325 walleye pollock, we concluded there was not a great influence on the scatter of pointhead
326 flounder after preying on juvenile walleye pollock during the surveys reported herein. Our
327 conclusion is based, firstly, on the fact that no gas of the swim-bladder was left in pointhead
328 flounder; additionally, pointhead flounder exhibits a fast digestion; lastly, there is a large
329 difference in the body size between the two species.

330 Using echosounders, we effectively detected flatfish pointhead flounder; this allowed us to
331 record their acoustic characteristics for the first time, and thus, suggesting that acoustic
332 survey of pointhead flounder is possible. Further, we were able to discriminate between
333 juvenile walleye pollock and pointhead flounder around Funka Bay using the relative
334 frequency response of each species. The predation of pointhead flounder on juvenile walleye
335 pollock was successfully observed on echograms, and was further confirmed by analysing
336 stomach contents.

337 In this study, juvenile walleye pollock with a mean body length of 25.0 mm showed
338 stronger scatter at low frequency (Fig. 6). Although we conducted acoustic measurement on
339 small-sized juvenile walleye pollock, we found the same frequency characteristics on larger
340 walleye pollock specimens. Body length estimates obtained by Iida et al. (1999) for juvenile
341 and adult walleye pollock (46.9~293.8 mm) using the cage method showed lower mean TS at
342 a high frequency e.g., 50, 98, and 198 kHz. Moreover, the same trend was observed by

343 Gauthier and Horne (2004) using a theoretical model at 12, 38, 70, 120, and 200 kHz. Other
344 juvenile swim-bladder fishes, goldeye rockfish, purple puffer, and sea smelt (all samples
345 measured within 65 mm) showed the same frequency characteristics at 25, 50, 100, and 200
346 kHz (Iida et al., 1998). The decrease in TS of walleye pollock at higher frequencies is most
347 likely due to orientation dependence; specifically, the sharper the main lobe in the TS pattern
348 and the broader the orientation distribution, the smaller the TS (Kang et al., 2002).

349 The frequency characteristics of pointhead flounder showed the opposite pattern to that of
350 juvenile walleye pollock; specifically, the higher the frequency, the higher the S_v (Fig. 5).
351 The frequency characteristics of several swim-bladderless fish (arabesque greenling
352 (*Pleurogrammus azonus*), Takashima, 2006; sandeel (*Ammodytes marinus L.*), Matsukura et
353 al., 2013, Forland et al., 2014; butterfish (*Peprilus triacanthus*), Jech et al., 2017; skipjack
354 tuna (*Katsuwonus pelais*), Boyra et al., 2018) tend to show this profile. The most surveyed
355 fish species from this group is the Atlantic mackerel (*Scomber scombrus*) (Korneliussen and
356 Ona, 2002; Scoulding et al., 2017). In terms of measuring the relative frequency, the S_v of
357 Atlantic mackerel is four times higher at 200 kHz than at 38 kHz, which is also the case for
358 the pointhead flounder in this study. Thus, because flatfish, such as pointhead flounder, have
359 the same frequency characteristics as swim-bladderless fish, the scatter of swim-bladderless
360 fish might primarily depend on the bone at high frequency (70, 120, 200 kHz; Gorska, 2007).
361 Therefore, although pointhead flounder share the same frequency characteristics with other
362 swim-bladderless fish, due to the wide cross-section against the incident wave, they might
363 have stronger scatter than other spindle-shaped swim-bladderless fish. Therefore, it is
364 necessary to measure TS before acoustic quantitative surveys for pointhead flounder to be

365 considered reliable. Similarly, zooplankton also exhibit higher scatter at high frequencies.
366 However, zooplankton can be distinguished from pointhead flounder because they show an
367 $S_v(120) - S_v(38)$ over 10 dB (Kang et al., 2002; Murase et al., 2009).

368 In this study, we identified the frequency characteristics of the flatfish, pointhead flounder,
369 and demonstrated that the frequency response of pointhead flounder and juvenile walleye
370 pollock can be effectively used to discriminate one from the other. Furthermore, echoes from
371 the pointhead flounder showed a patch shape; therefore, we could roughly identify the echoes
372 from the pointhead flounder by echo shapes, in addition to frequency characteristics. The
373 results of this study will be useful for the acoustic surveying of juvenile walleye pollock in
374 the presence of pointhead flounder. In addition, our echograms allow the evaluation of
375 predation pressure on juvenile walleye pollock by pointhead flounder, reportedly the most
376 important predator of juvenile walleye pollock at water depths of up to 70 m around Funka
377 Bay (Japan); thus, likely, the two species strongly overlap at this depth. However, we carried
378 out our survey down to 100 m depth. Our results showed that the two species also overlap at
379 this depth. Therefore, 70 m is not the depth limit for discriminating between the two species.
380 We used the relative frequency response at 38 and 200 kHz, with higher frequencies causing
381 higher noise. Thus, it is necessary to survey at the depth limit, where the organism of interest
382 may be detected even at 200 kHz.

383 In the Northwest Atlantic, Atlantic mackerel (swim-bladderless) is one of the most
384 important predators of larval Atlantic cod (swim-bladder), and it is assumed that this
385 predator-prey relationship can influence abundance of prey species (Serchuk et al., 1994;
386 Rosel et al., 2002). This is a similar situation to what we observed in this research. Further, by

387 using our method to separate them, the acoustic abundance accuracy of two species can be
388 improved, and the predation pressure can also be investigated.

389 **5 Conclusions**

390 This is the first reported study using relative frequency responses to distinguish pointhead
391 flounder from juvenile walleye pollock. Using the difference in S_V at 38 and 200 kHz, which
392 was -8 to 0 dB for juvenile walleye pollock and 1-10 dB for pointhead flounder, we can
393 extract 92% scatter of juvenile walleye pollock and 83% echoes of pointhead flounder (Fig.
394 7). This is also the first study to succeed in observing flatfish using the acoustic method. The
395 abundance of pointhead flounder, a swimming flatfish, can be evaluated by acoustic surveys,
396 but they always swim in schools and therefore it is difficult to examine their single TS in
397 field. However, we can use a broadband echosounder or maintain the sample in a tank or cage
398 to obtain the single TS distribution to evaluate its abundance accurately. Therefore, the TS
399 will be examined in our next study to carry out acoustic abundance survey.

400

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407 **Declarations of Interest**

408 None.

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576

577 Table 1. Echo sounder (EK60) parameter setting

| Year | 2015 | | | 2016 | | | 2017 | | | 2018 | | |
|-----------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Frequency[kHz] | 38 | 120 | 200 | 38 | 120 | 200 | 38 | 120 | 200 | 38 | 120 | 200 |
| Gain[dB] | 24.25 | 26.56 | 26.63 | 24.01 | 25.74 | 25.18 | 23.81 | 26.54 | 24.6 | 23.8 | 26.26 | 26.12 |
| Sa corr[dB] | -0.72 | -0.73 | -0.40 | -0.68 | -0.61 | -1.25 | -0.70 | -0.70 | -0.35 | -0.76 | -0.64 | -0.44 |
| 3-dB Beam width, Along.[deg] | 6.99 | 6.59 | 6.45 | 6.94 | 6.41 | 6.35 | 7.10 | 6.56 | 7.05 | 7.30 | 6.54 | 6.48 |
| 3-dB Beam width, Athwart.[deg] | 7.00 | 6.56 | 6.67 | 7.02 | 6.41 | 6.39 | 6.91 | 6.50 | 6.94 | 7.22 | 6.54 | 6.45 |

578

579

580 **Fig. 1.** Study areas. Marks denote rod fishing spots and lines denote the towing lines of frame-type midwater
581 trawl net (FMT) lines. Only one species' collection area was considered as the target spot for surveying.
582 Grey lines are isobaths at an interval is 50 m.

583

584 **Fig. 2.** Total length distribution of juvenile walleye pollock caught by FMT.

585

586 **Fig. 3.** Total length distribution of pointhead flounder fishing by rod.

587

588 **Fig. 4.** Echograms of the volume backscattering strength (S_V ; dB) at the FMT towing line for the juvenile
589 walleye pollock during nighttime at 38, 120, and 200 kHz, from 20:25 to 20:35, 14th May 2016. The yellow
590 broken line denotes the depth of the FMT (13 m).

591

592 **Fig. 5.** Echograms of the volume backscattering strength (S_V ; dB) for the pointhead flounder at the rod
593 fishing spots at 38, 120, and 200 kHz, from 08:10 to 08:30, 10th May 2016. Arrows denote the pointhead
594 flounder schools.

595

596 **Fig. 6.** Frequency characteristics of the two species (data from 2015 to 2017). Upper: juveniles; lower:
597 pointhead flounder. The panels from left to right show the relationship between the $S_V(38)$ and $S_V(120)$,
598 $S_V(120)$ and $S_V(200)$, and $S_V(38)$ and $S_V(200)$. The black line represents the same S_V at two frequencies and
599 the dotted line represents the mean S_V .

600

601 **Fig. 7.** Histogram of $S_V(200) - S_V(38)$ for echoes identified as juvenile walleye pollock (black) and pointhead
602 flounder (white) for the data from 2015 to 2017.

603

604 **Fig. 8.** Example of implementation using the difference of S_V at 38 and 200 kHz to identify the juvenile
605 walleye pollock. (a) and (b) are the original echograms of S_V at 38 and 200 kHz of S_V , respectively (c)
606 presents the echograms $S_V(200) - S_V(38)$, (d) is the 38 kHz echogram that conforms to $-8 \text{ dB} < S_V(200) -$
607 $S_V(38) < 0 \text{ dB}$ used to identify juvenile walleye pollock, (e) is the 38 kHz echogram that conforms to 1 dB
608 $< S_V(200) - S_V(38) < 10 \text{ dB}$ used to identify pointhead flounder. Color scales for (a) (b) (d) (e) and (c) are
609 shown, and black horizontal lines demarcate 20 m depth intervals.

610

611 **Fig. 9.** Example of implementation using the difference of S_V at 38 and 200 kHz to identify the pointhead
612 flounder. (a) and (b) are the original echograms of S_V at 38 and 200 kHz of S_V , respectively (c) presents the
613 echograms $S_V(200) - S_V(38)$, (d) is the 38 kHz echogram that conforms to $-8 \text{ dB} < S_V(200) - S_V(38) < 0 \text{ dB}$
614 used to identify juvenile walleye pollock, (e) is the 38 kHz echogram that conforms to $1 \text{ dB} < S_V(200) -$
615 $S_V(38) < 10 \text{ dB}$ used to identify pointhead flounder. Color scales for (a) (b) (d) (e) and (c) are shown, and
616 black horizontal lines demarcate 20 m depth intervals.

617

618 **Fig. 10.** Example of implementation using the difference of S_V at 38 and 200 kHz to identify the juvenile
619 walleye pollock at 15:00 to 15:20, 19th April 2018. (a) and (b) are the original echograms of S_V at 38 and
620 200 kHz of S_V , respectively (c) presents the echograms $S_V(200) - S_V(38)$, (d) is the 38 kHz echogram that
621 conforms to $-8 \text{ dB} < S_V(200) - S_V(38) < 0 \text{ dB}$ used to identify juvenile walleye pollock, (e) is the 38 kHz
622 echogram that conforms to $1 \text{ dB} < S_V(200) - S_V(38) < 10 \text{ dB}$ used to identify pointhead flounder. Color
623 scales for (a) (b) (d) (e) and (c) are shown, and black horizontal lines demarcate 20 m depth intervals.

624

625 **Fig. 11.** Soft X-ray photo of the pointhead flounder (preyed on two juvenile walleye pollocks, at 0 hour).

626 Arrows indicate the swim-bladders of the juvenile walleye pollocks.

627

628

629

Fig. 1

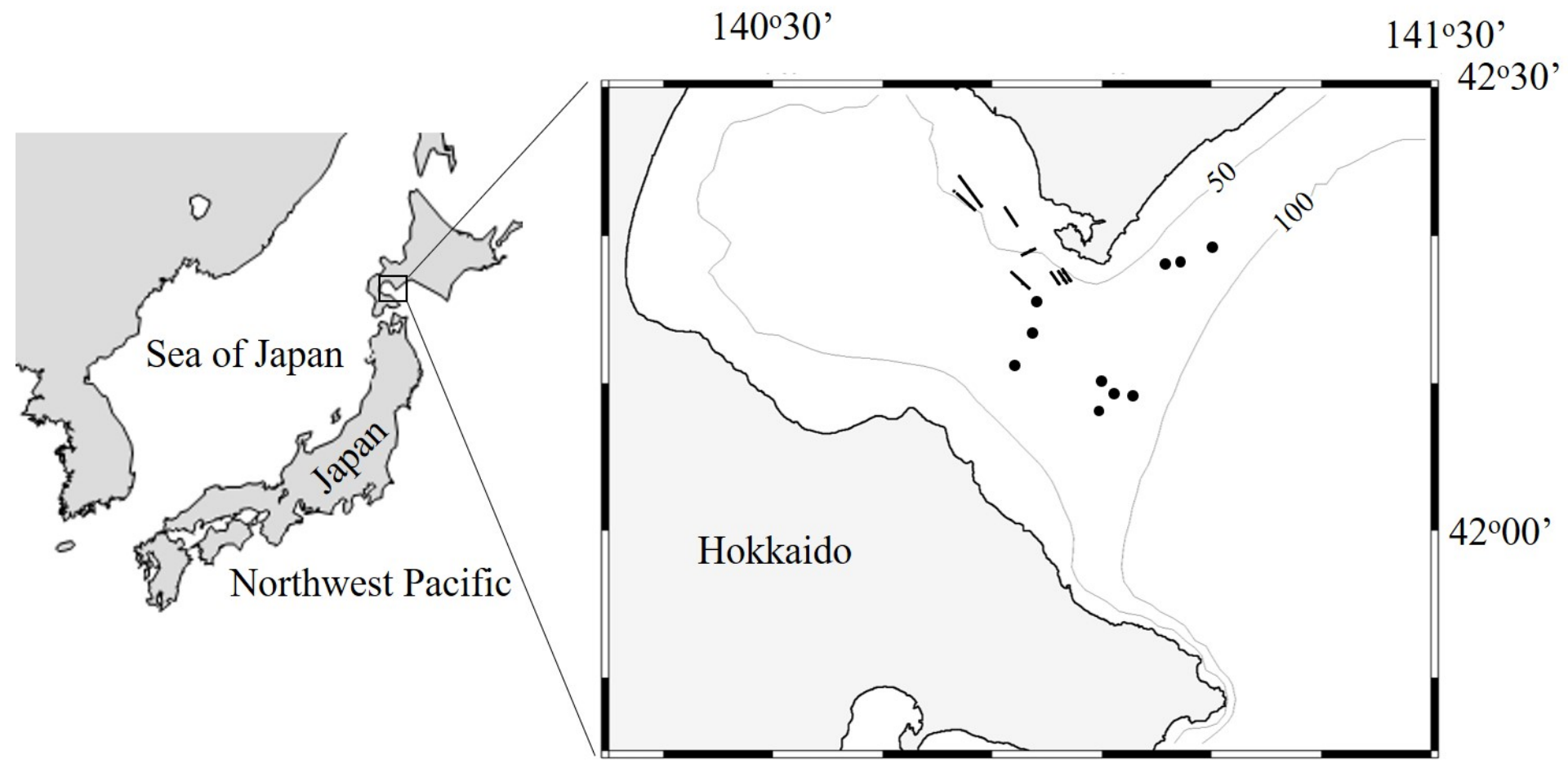


Fig. 2

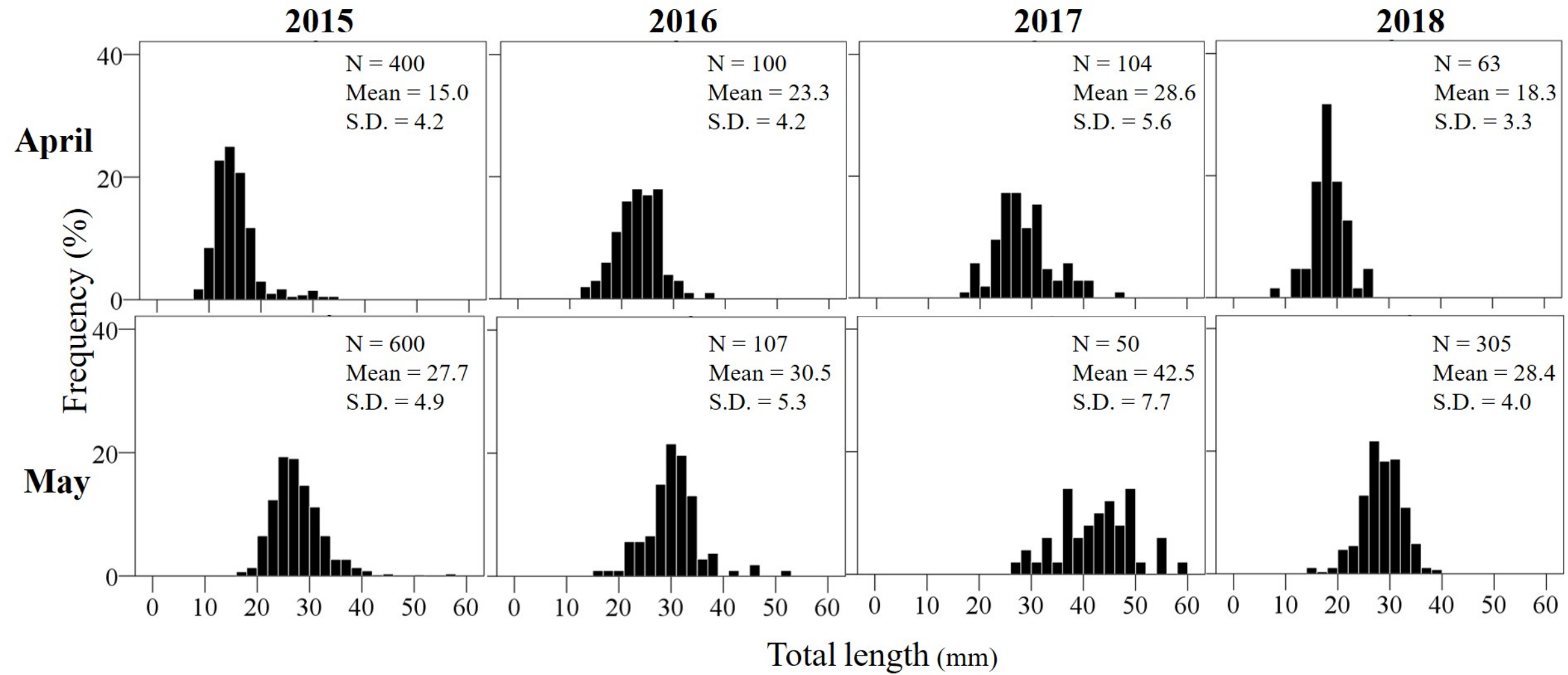


Fig. 3

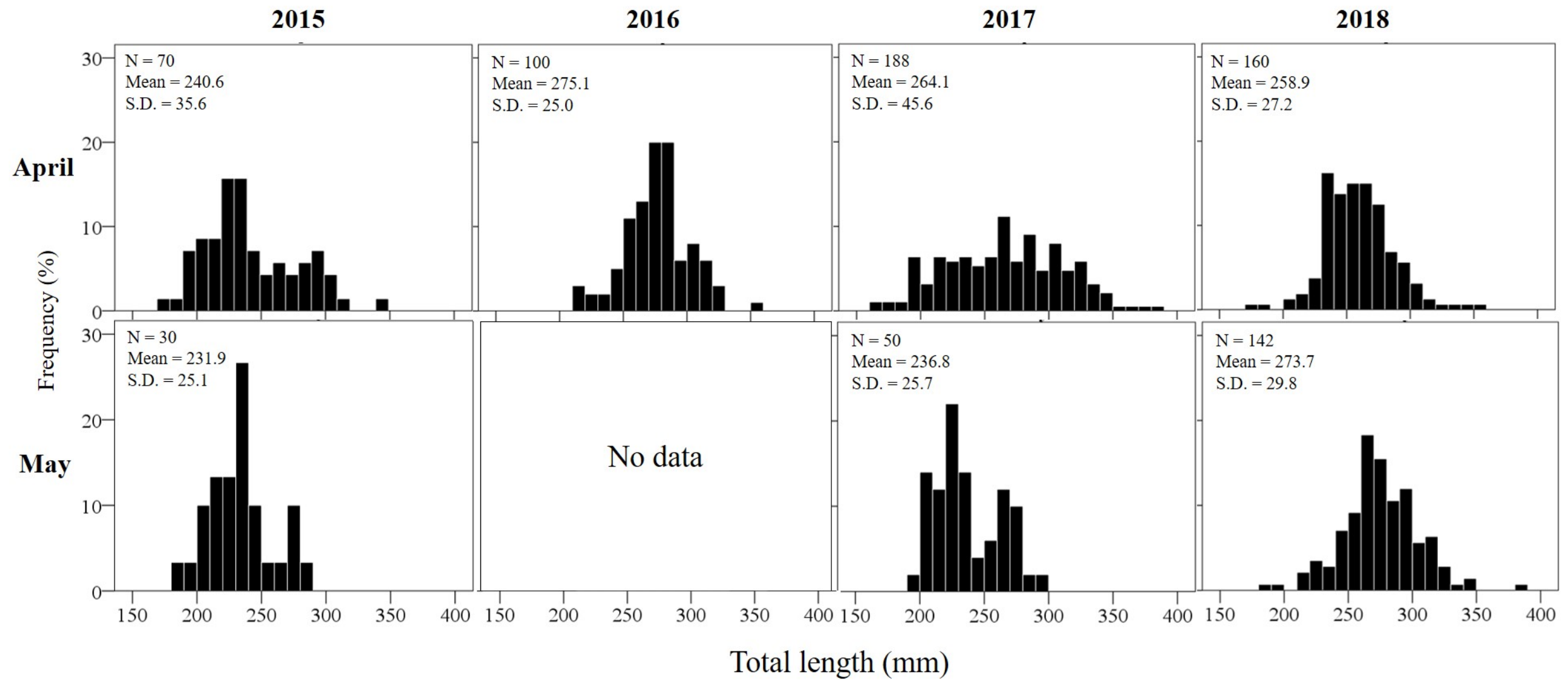


Fig. 4

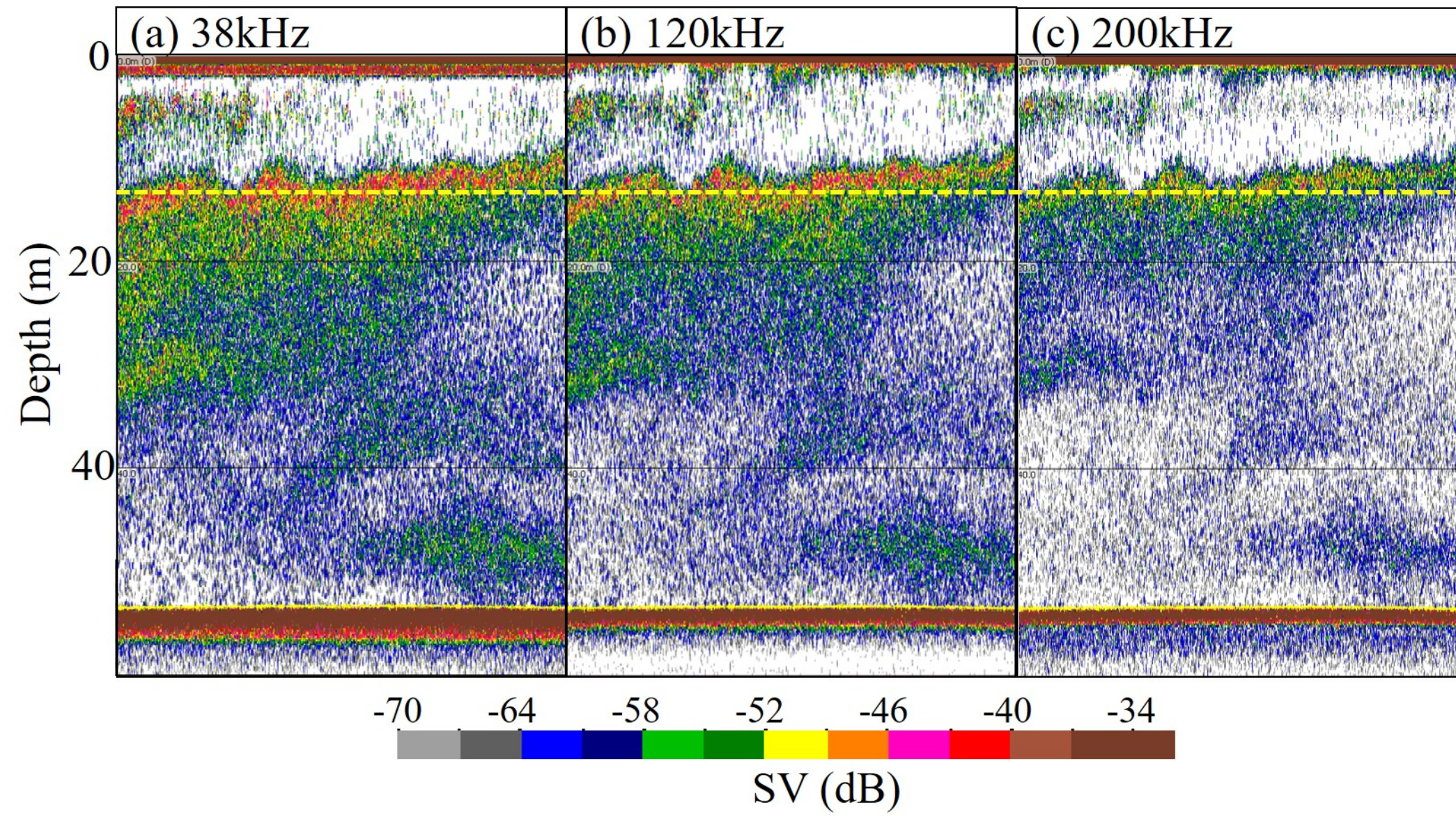


Fig. 5

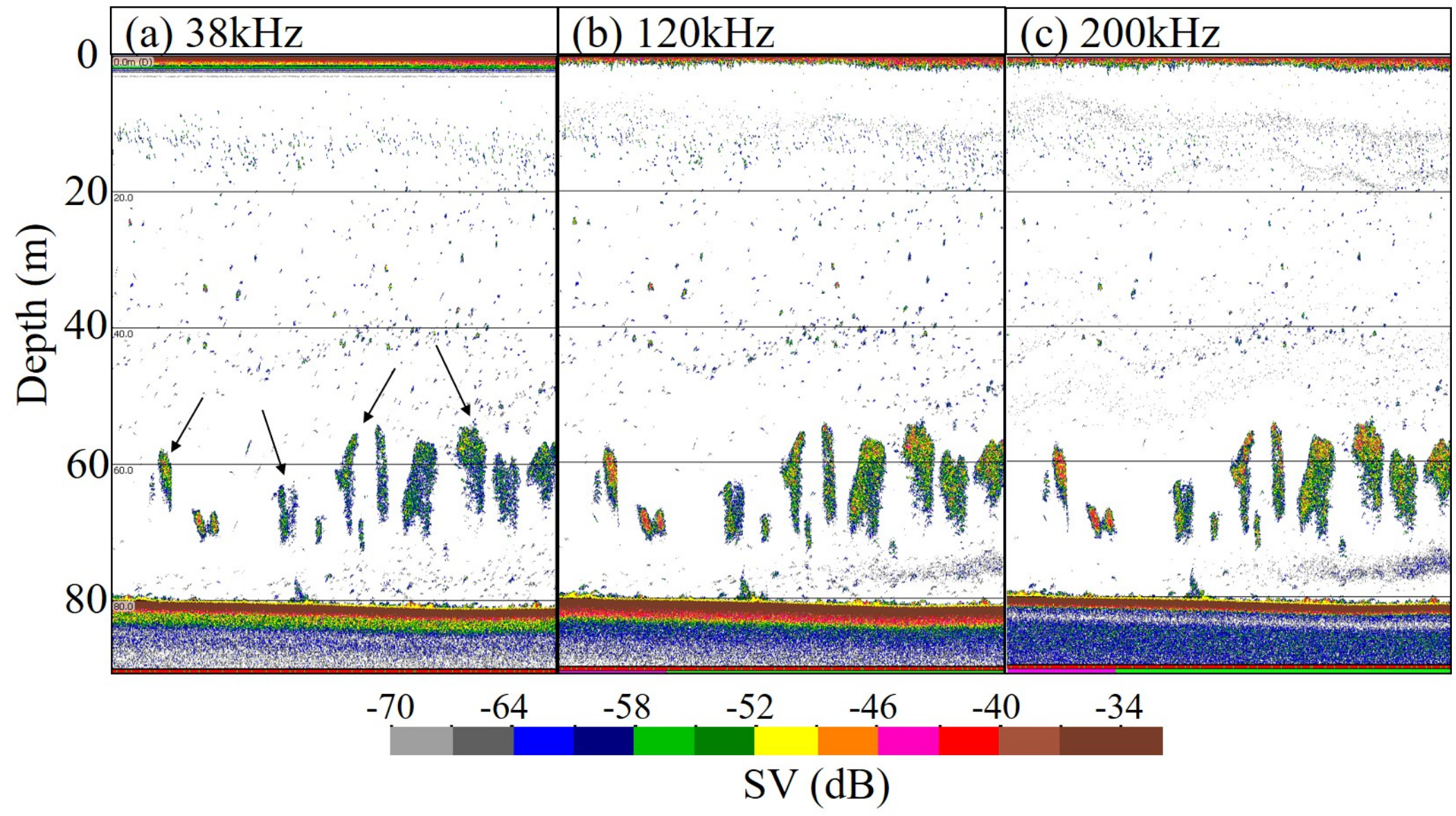


Fig. 6

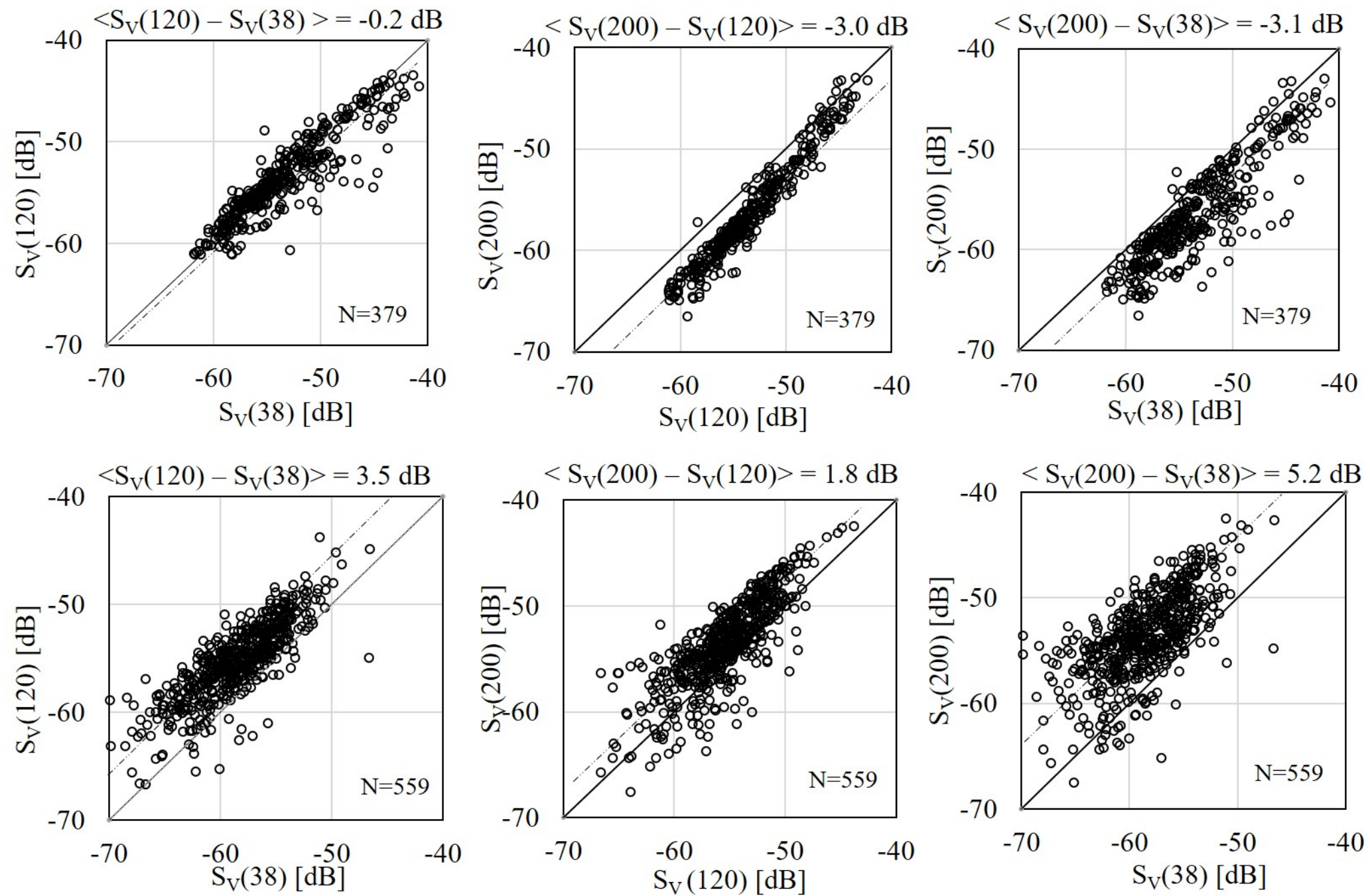


Fig. 7

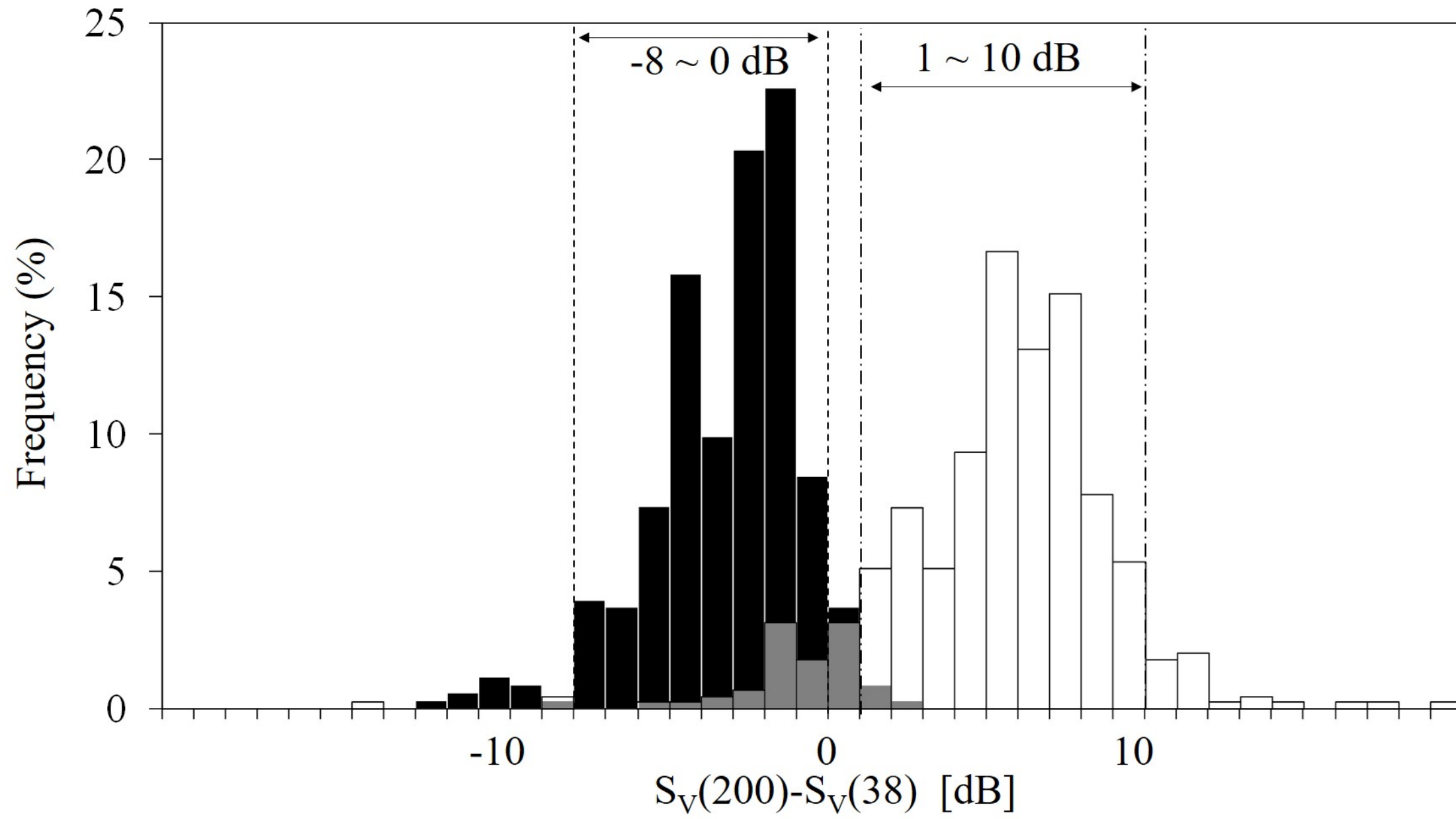


Fig. 8

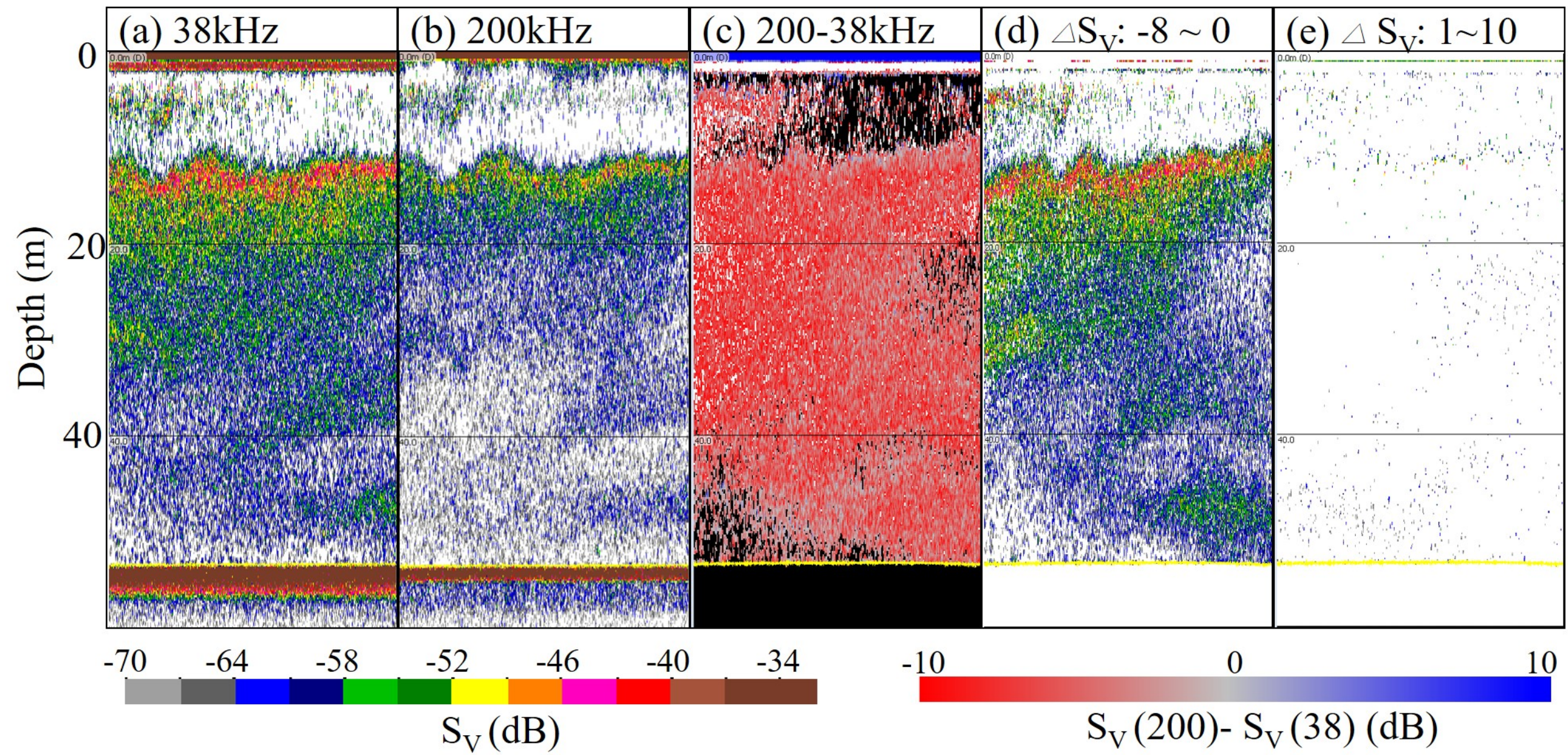


Fig. 9

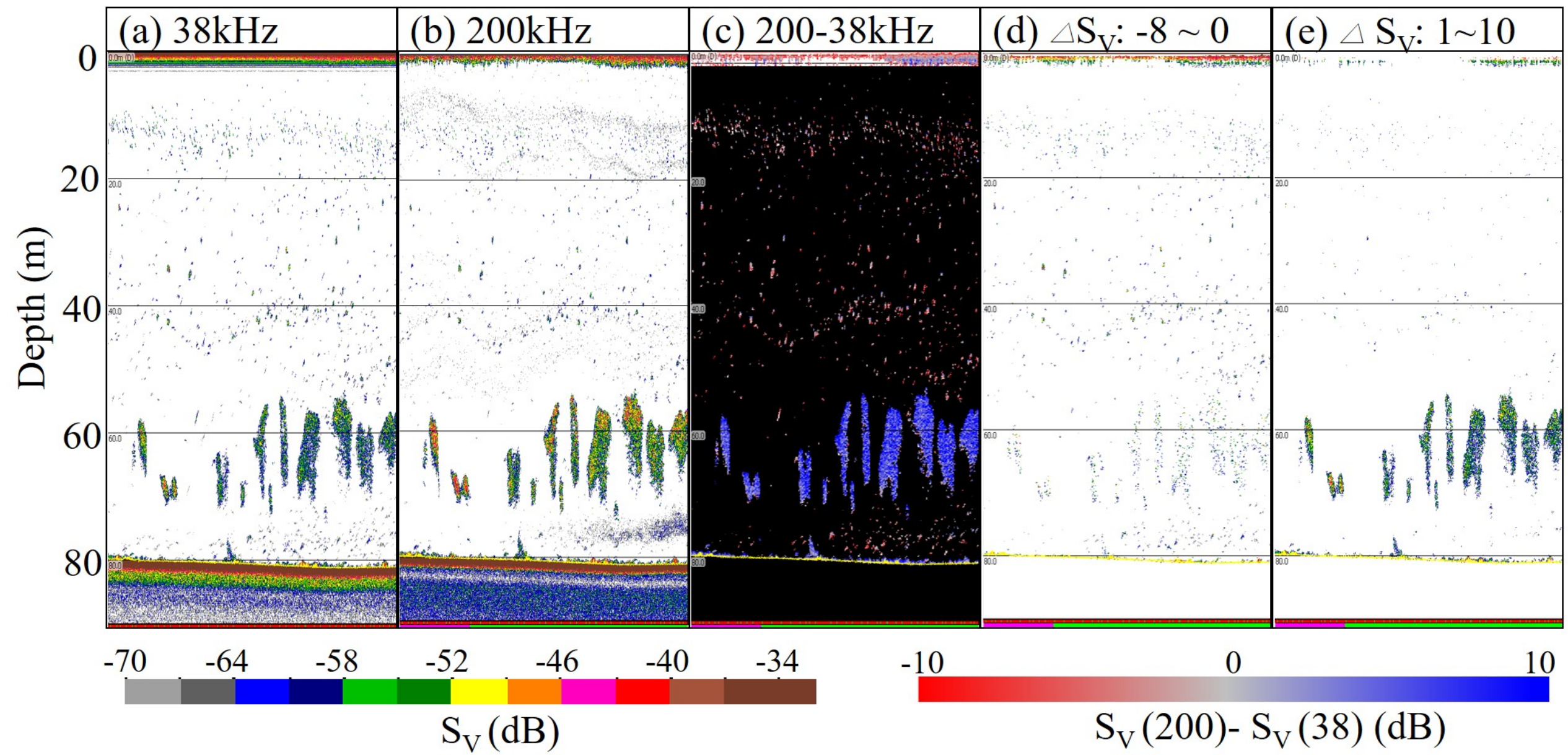


Fig. 10

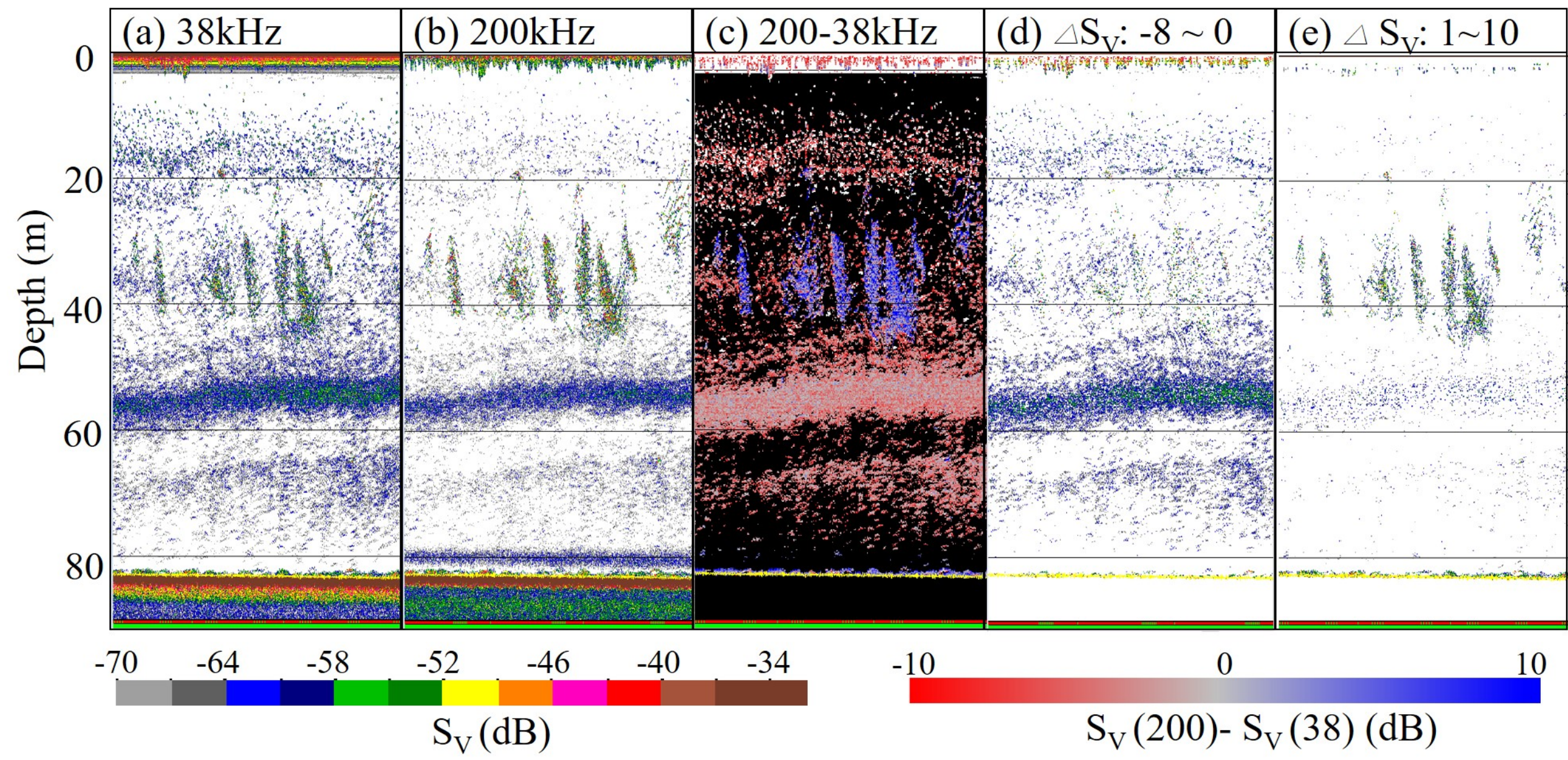


Fig. 11

