In Situ Test for Fish Attraction Effect of Krill Extract by Using Quantitative Echo-Sounder [*1]

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Abstract

The effect of the krill extract (8.5mg/ml as leucine) and the arginine solution (8.5mg/ml) to attract fish was examined in the waters near the coastal line by using quantitative echo-sounder on an anchored boat. The *in situ* test consisted of the three phases conducted consecutively, *i. e.*, the first, dummy tube (upper)-krill extract (lower), the second, krill-dummy, and third, arginine-non.

A series of the volume back-scattering strength (SV) obtained by the 88 kHz quantitative echo-sounder Furuno FQ-60 was averaged in 30 sec intervals and 1 m thickness by FQ-510 integrator. Then, the averaged SV were converted into the mean weight density per volume (g/m^3) as a measure to examine the effect of test solution quantitatively.

The attraction effect was found not in the change in the vertical profile but in the increasing trend in the density in accordance with elapse of time, because the experiment was conducted at very shallow water. Namely, the weight density per surface area (y in g/m^2) was expressed by the following equation at 0.01 level of significance (elapse of time, x in min).

$$y = 0.245x - 0.05 \quad (r = 0.811, n = 41)$$

It was concluded that the krill extract and arginine are effective in making the fish attracted near it.

1. Introduction

In many researches on the attraction and/or the stimulation of a variety of feeds for fish so far especially those of krill *Euphausia superba* are extremely few (Harada, 1989a), in spite of the fact that the krill has been widely used as chum and important component of artificial fishing baits (Harada, 1989c). Among few studies, it has been found that the extract of the krill attracted strongly juvenile yellowtail *Seriola quinqueradiata* in a laboratory experiment (Harada, 1982). The artificial feed containing the krill meal was proved to be more effective than commercial eel feed (by an outdoor experiment but not field one) in respect of weight gain of red sea bream *Chrysophry major,* Japanese eel *Anguilla japonica*, and gray mullet

Mugil cephalus (Allahpichay and Simizu, 1984). This is one of the subsidiary evidence in support of the attraction and/or stimulation effect of the krill. The crustaceans like the krill have been reported to be considerably effective in attracting and/or stimulating variety of aquatic animals (Harada, 1989a; 1989b).

On the other hand, arginine, which attends at nearly 266mg/100g of muscle, is the most predominant component of free amino acids in krill extract (Suyama *et al.*, 1965). This amino acid was proved by laboratory experiment to be effective in attracting and/or stimulating certain seawater fishes cod *Gadus morhua* (Ellingsen and Doeving, 1986) and yellowtail (Harada, 1985a), brackish water fish Japanese eel (Hashimoto *et al.*, 1963), and freshwater fishes walleye *Stizostedion vitreum* (Rottiers and Lemm, 1985) and oriental weatherfish *Misgurnus anguillicaudatus* (Harada, 1985b).

All the above-mentioned facts were obtained

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mainly from laboratory experiments. Before apply-ing the krill extract or its derived products as the attractant in practical fishing, therefore, it is neces-sary to confirm their effectiveness in outdoor. This is the reason why the present *in situ* test was con-ducted.

2. Materials and Methods
Test Sample

Some weight of whole body of the defrosted krill was added the equivolume of water. The mixture was homogenized by Ultra-Turrax homogenizer. The homogenate was furthermore added one half volume of 25% trichloroacetic acid and stood for 30 min at room temperature. After centrifugation, the supernatant thus prepared was adjusted to pH 6.5 by the addition of 10 N sodium hydroxide solution. The supernatant adjusted was used as test sample of krill extract. The content of amino acid in the sample was 8.5mg/ml as leucine amount, on the basis of the determination by ninhydrin method. Likewise, the test sample of arginine (Ajinomoto Co., Ltd.) was also prepared to concentration of 8.5 mg/ml. A 300ml of the test solution was poured into a semi-dialysis tube, Meatlonn (folded diame-ter 13.5cm, Fujimori Ind. Co., Ltd.). And the tube was wrapped with a piece of blue nylon mono-filament net and used in the present *in situ* test.

### Data acquisition systems and estimation of mean weight density

Two echo-sounders were used. The FQ-60 quanti-tative echo-sounder (88kHz, Furuno Electric Co., Ltd.) was used for collecting the echo signals as the basic data to estimate the density of target in re-spective depth layers at any moment. And Z-2A conventional echo-sounder (200kHz, Kaijyo Elec-tric Co., Ltd.) was used subsidiarily to get real time general information during experiment. The trans-ducer of FQ-60 was mounted on the iron rectangu-lar frame and hung overboard, and that of Z-2A was attached at the side of boat as illustrated in Fig. 1.

The calibration of FQ-60 was carried out by using the hydrophone (Johanneson and Mitson, 1985) just before the experiment. Key specifica-tions and the operating conditions of FQ-60 and echo-integrator FQ-510 were given in Table 1.

Acoustic signals received by FQ-60 were re-corded on cassette tapes by using TEAC, R-61. Re-corded signals were analyzed by using the echo-integrator (FQ-510) at laboratory. The cell size to measure the volume back-scattering strength SV (i,j) (Mitson, 1994) as the initial data was settled to be 30 sec × 1m thickness from 8m layer to sea-bed.

SV(i,j) to be used in the further analysis was es-timated from measured strength SV$_n(i,j)$ by using
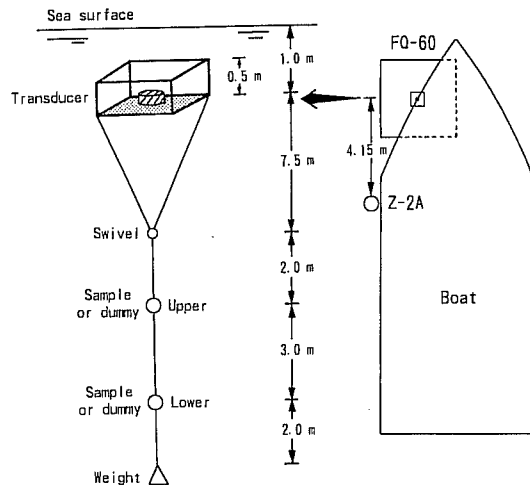
Fig. 1. Arrangement of experimental system and echo-sounders.

Table 1. Key specifications and operating conditions of the FQ-60 quantitative echo-sounder and FQ-510 integrator

Power (kW)	1
Frequency (kHz)	88
Pulse duration (ms)	0.6
Equivalent beam width (dB)	−17.2
Absorption coefficient (dB/km)	25.4
Gain constant (dB)	82.8
Time varied gain	$20 \log R$
Attenuator (dB)	10
Threshold (dB)	0
Sound velocity (m/s)	1500
Integration interval (sec)	30

the following correction equation (Hamano and Uchida, 1992).

$$SV(i,j) = 10 \log(10^{\frac{SV_n(i,j)}{10}} - 10^{\frac{SV_o(i,j)}{10}})$$

where the definitions of $SV(i,j)$, $SV_n(i,j)$ and $SV_o(i,j)$ are follows:

$SV(i,j)$: the corrected SV for the j-th depth layer in the i-th integration section (the i-j cell)

$SV_n(i,j)$: SV for the i-j cell actually measured

$SV_o(i,j)$: SV to be excluded. $SV(h,j)$ in the 20 cells of the same j were chosen, after exclusion of the cells with fish echoes and making the difference of h from i as small as possible. And their average was used. Practically, this value for other layers than those with either swivel or wrapped tube were negligible. $SV_o(i,j)$ from about 8.5m layer were from swivel, and those at 10.5 or 13.5m layers were from the wrapped tubes (in the phase Ⅲ, 11.5 and 14.5m layers, respectively)

To express the attraction effect either by the mean weight density per volume (g/m³) in respective depth layer or by the mean weight density per surface area (g/m²) both in a given time sections, the following equations were used.

$$\overline{SV}(j) = 10 \log((\sum_{i=1}^{k} m \, sv(i,j))/k)$$

$$\overline{SV}(i) = 10 \log((\sum_{j=8}^{n} m \, sv(i,j))/n - 7)$$

where $sv(i,j) = 10^{\frac{SV(i,j)}{10}}$

$\overline{SV}(j)$: the mean of SV for the j-th depth layer

$\overline{SV}(i)$: that for the i-th interval

　k　: number of integration intervals

　n　: that of layers

Here, in order to distinguish the cells showing SV of school from those showing the values derived from other targets, 1 was given to m of the cells with echoes from fish, and 0 to that of the others (Hamano, 1993).

The mean weight density per volume $\rho_v(j)$ (g/m³) for the depth layer j is expressed as follows:

$$\rho_v(j) = 10^{\frac{\overline{SV}(j) - TS}{10}} \times w$$

where TS is target strength and w weight of target species.

The mean weight density per surface area $\rho_s(i)$ (g/m²) for the integration interval i is shown as follows:

$$\rho_s(i) = 10^{\frac{\overline{SV}(i) - TS}{10}} \times w$$

In Situ Test

The *in situ* test was carried out 12:10–15:56 on November 14, 1990 off Hotokebana, Shimonoseki, at 16.5m in depth and 26.2℃ in surface temperature.

As shown in Fig. 1, two small loops were tied on a nylon snood at 9.5m (upper, *i. e.* 10.5m deep) and 12.5m (lower, *i. e.* 13.5m deep) below the transducer. The experiments consisted of the three phases of different arrangement of the wrapped tubes with and without test solution (the latter tube was hereafter expressed simply as dummy), as indicated in Figs. 2 to 4.

As the basic data to evaluate the effect, $SV(i, 8)$ to $SV(i, 15)$ were used, for the purpose of escaping from the acoustic near-field phenomenon.

And fly fishing was done in 30 min during the phase Ⅱ, to confirm the objects responsible for echoes.

3. Results

The examples of echogram during the three phases were shown in Fig. 2. The successive dotty echo traces in 8–9m, 10–11m and 13–14m layers were from the swivel of snood and the wrapped tubes.

In the phase I (12:10 – 13:35) consisting with dummy at the upper loop and krill extract at lower one, the successive tiny echo traces were found on the echogram just above sea-bed during 12:10–12:18 just after start of test (phase I -a). From about 12:41 (phase I -b), the dense echo traces increased gradually with elapse of time in the depth interval between the lower loop (krill extract) and sea-bed,
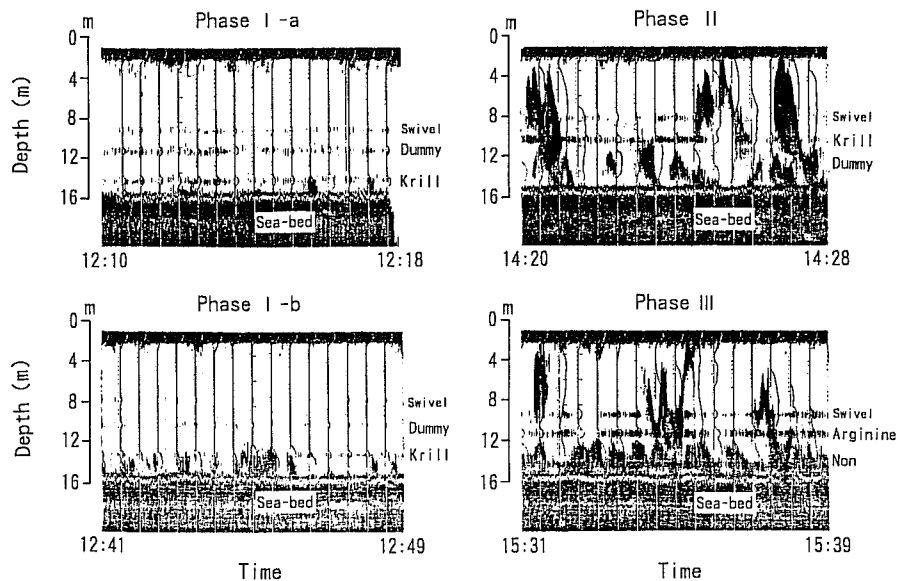
Fig. 2. Examples of echogram in the three phases (by the FQ-60 quantitative echo-sounder).

Table 2. Species and descriptions of fishes hooked during test fly fishing in the phase II

Species*	Descriptions	
	Body length	Body weight
	(cm)	(g)
Jack mackerel- "Maaji" -*Trachurus japonicus*	14.5	49.0
〃	12.4	33.0
Multicolorfin rainbowfish- "kyusen" -*Halichoeres poecilpterus*	13.5	32.0
〃	11.9	20.0
〃	9.8	15.0
Bambooleaf wrasse- "Sasanohabera" -*Pseudolabrus japonicus*	12.9	60.0
〃	12.9	40.0
〃	12.0	50.0
Coral fish- "Suzumedai" -*Chromis notata*	9.0	25.0
〃	8.1	20.0
Slim wrasse- "Itobera" -*Pseudolabrus gracilis*	9.5	21.0
Japanese stingfish- "Kasago" -*Sebasticus marmoratus*	9.8	30.0

* Common name- "Japanese name" -Scientific name.

while the echo traces were scarcely found in the layers from surface to the lower loop. In the phase II (13:44–14:43) consisting with krill extract at the upper and dummy at the lower loop, the echo traces probably from school were found frequently.

In the phase III (14:57 – 15:56) consisting with arginine tube at the upper loop and no dummy at the lower one, the echo traces of fish school appeared also more frequently near or over the sea-bed. As shown in Table 2, the fish caught were
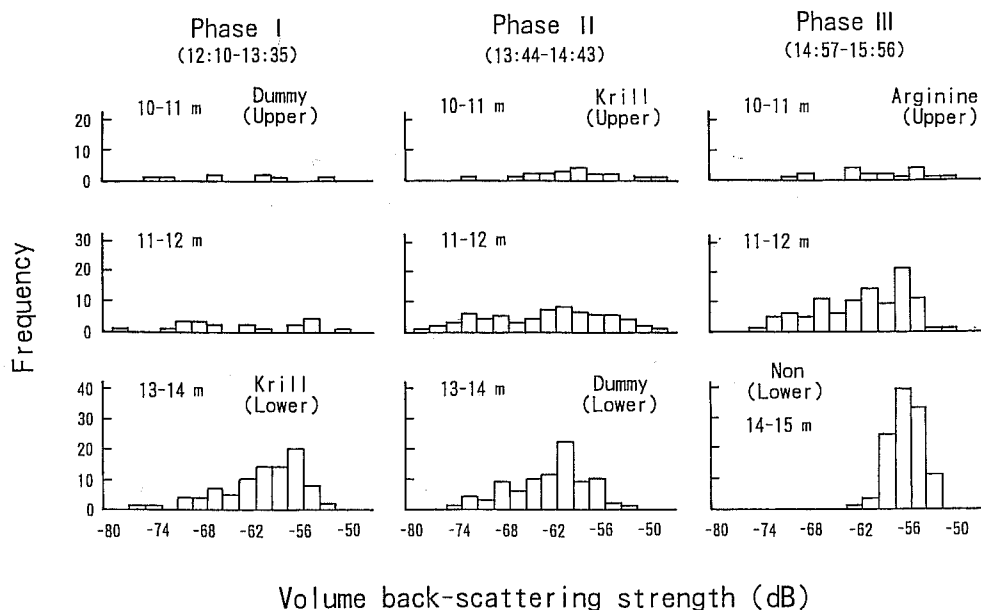
Fig. 3. Frequency distribution of the volume back-scattering strength in the three depth layers.

mainly the reef inhabitants and partly juvenile jack mackerel.

The frequency distributions of the volume back-scattering strength (SV) in the depth layers of the upper and lower loops and a layer between them in each of these three phases were shown in Fig. 3.

Here, SV below -80 dB were regarded as the ambient noise and excluded. This figure showed the following trends : In the phase I, SV showed wide variation, and the frequency increased with depth. This was clear in large SV. In the phase II, wide variation was the clearest trend of SV in the middle layer. The same trend could also be found in the upper layer. SV in the lower layer showed less severer variation with the mode at the -60 dB class. In the phase III, the distribution of SV was similar to that in the phase II, except the clear concentration in the classes of strong SV in the lower layer.

The weight density was converted from SV under the following three assumptions, paying attention to choose TS as standard so as to avoid from over-estimation of the effect : (1) The SV of the main target on the sound axis may be -56 dB as shown in Fig. 3. (2) The average body length of

target is settled to be about 12cm, basing on the results of flying fishing. And (3) among the species caught and those in the list of TS (Nat. Res. Inst. Fish. Eng., 1984), the jack mackerel is chosen as the tentative standard of conversion. A value of -46.0 dB was used as the target strength of the dorsal aspect of this species (12.4cm in body length; 33.0g in body weight). Even though the standard in conversion were not adequate, its influence appears only in the scale of Figs. 4 and 5, but the trends of change with depth and time are free from it.

As shown in Fig. 4, $\rho_v(j)$ increased with depth, regardless of the arrangement of krill or arginine solution and dummy one, indicating that the effect of krill or arginine solution was not strong enough to change the original pattern of vertical distribution of density of fish, but was found in the density as its increasing trend with elapse of time.

To illustrate the effect in this form more clearly, 5 minute average of $\rho_s(i)$ (g/m^2) was estimated, summing up from SV(i, 8) to SV(i, 15), and plotted against elapse of time from start of experiment. And the effect could be extracted as the highly significant following regression line of $\rho_s(x)$ (g/m^2)
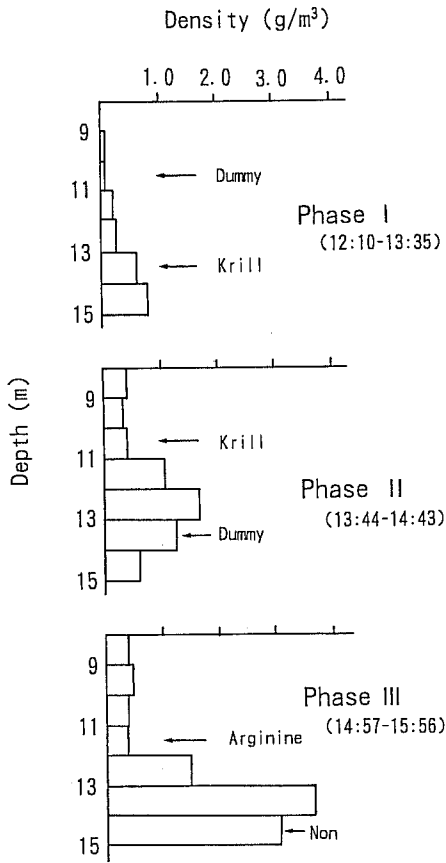
Density (g/m³)

Fig. 4. Vertical distribution of mean weight density per volume (g/m³) in respective phases of test.

Fig. 5. Relationship between elapse of time and 5 minute mean weight density per surface area (g/m²) (the data in the three phases pooled).

on the elapse of time (x in min):

$$\rho_s(x) = 0.245x - 0.05$$
$$(r = 0.811, n = 41, \text{significant at 0.01 level})$$

4. Discussion

Preceding to the present study, preliminary test was conducted in field by using stainless cage for suspending and protecting the semi-dialysis tube. The back-scattering strength of the cage was acoustically very strong, which made it difficult to distinguish the fish echoes from those of suspension and protection system. The echograms, however, suggested that there remained some possibility to apply the acoustic method in the present purpose, if less sound reflective system can be used in suspending the test solution in tube at an
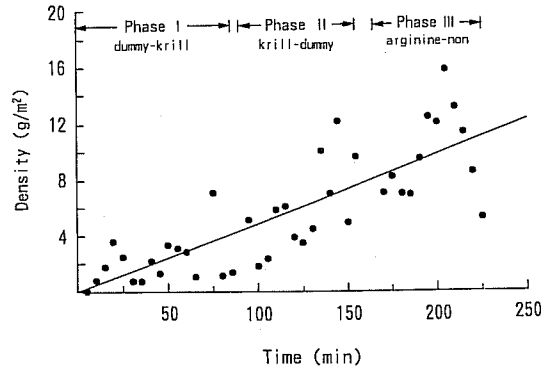
intended depth layer. After several trials, it was found out that wrapping by a piece of available nylon mono-filament net was one of the applicable methods to collect the record for the present purpose.

The clearest symptom of krill extract and arginine solution in making the fish attracted could be found as the increasing trend of echo strength with elapse of time, but not in the vertical profile of the density. The probable reasons responsible for this pattern may be : (1) the experiment was done at very shallow water and it was difficult to separate clearly the depth to hang of test solution from dummy tube when compared with the effective range of test solution continuously diffusing into waters and (2) the same fact also resulted in making it difficult to be free from the effect of sea-bed and made the reef inhabitants occupying the unnegligible part of fish attracted as supported by the results of test fly fishing.

Judging from the results of the experiment, it was ascertained that the fish echo traces increased with the elapse of time during the experiment. This supports strongly the possibility of the krill extract and arginine to attract fish.

The above-mentioned conclusion was deduced from vertical profile of $\rho_v(j)$, which are the values calculated from SV(i, j) for the same j. $\rho_v(j)$ depends on the average of them but has no relation to variation. SV(i, j) differed according to the depth

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layer and to the phase not only in its average but also in its variation. Accordingly, it is necessary to give some consideration on the variation of SV. The tailing toward low SV was found in the lower layer of the phase I and II and in the middle layer of the phase III. The short periodic appearance and disappearance of echo traces was found in the echogram for the phase II and III as shown in Fig. 2. This was clear in shallow layer. The same fact was found in the echogram of other sounder of 200kHz. One of the possibilities of causing these phenomena may be as follows : if the attracted fish swim actively in the sphere or within an area, and radius is larger than the area covered by the sound corn, the rate of area covered by sound corn increase with depth, which makes the variation decrease with increase in depth. The apexes of comet traces were found mainly in 4 to 6m and 12m deep.

These facts indicated that attracted fish keep their swimming depth, which may explain the high density near sea-bed regardless of the arrangement of test solution and dummy tube. The sharp comet form in the echo trace in the shallow layer suggested active swim of attracted fish while continuously appeared dull comet traces near the sea-bed suggested that the fish attracted near sea-bed staying within the area covered by sound corn, although the depth depending difference in the area covered by sound corn could not be neglected, especially in the area near transducers. This depth depending difference in the swim activity in relation to the area covered by sound corn may suggest the mechanism of the above-mentioned variation in SV.

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オキアミ抽出物の魚類誘引効果判定に対する計量魚探機の応用

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　自然環境下における魚類に対するオキアミの誘引効果を，計量魚群探知機（FQ-60，88kHz）によって調べた。実験は1990年11月14日（12:10-15:56），山口県響灘水深16.5m の沿岸域において，錨泊させた小型艇より垂下した送受波器の直下（9.5m，12.5m）に誘引物質とダミーを3m離して吊り下げ，それらの位置を交替させておこなった。計量魚探機により計測された音響信号からエコーインテグレータ（FQ-510）により，30秒×深度1mごとの平均体積散乱強度を求めた。この値を単位体積当りの重量密度（g/m^3）と単位海表面当りの重量密度（g/m^2）に変換し，重量密度の垂直的断面および時間経過の両面から魚群の蝟集状況を検討した。その結果，オキアミ抽出物の誘引効果は浅海では魚類の重量密度の垂直分布でなく，時間経過として定量的に把握できた。