Daily Ration of Hatchery-Reared Japanese Flounder *Paralichthys olivaceus* as an Indicator of Release Place, Time and Fry Quality. *In situ* Direct Estimation and Possibility of New Methods by Stable Isotope

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Abstract

In order to examine the availability of daily ration as an indicator of release place, time and fry quality, mass releases of hatchery-reared Japanese flounder *Paralichthys olivaceus* were conducted under various experiment conditions in Wakasa Bay, the Sea of Japan in 1997 and 1998. Daily ration was estimated in terms of percent of body weight by Elliott and Persson's method in the field. Daily ration was between 1.5% BW/day and 22.2% BW/day and closely related to the food availability, especially mysids biomass. Daily ration of wild flounder was 3 times as much as that of released fish in July 1997. Judging from these results, daily ration is thought to reflect food availability in the release area and seedling quality, and be used as an indicator to evaluate the quality of the release technique.

Elliott and Persson's method does not give the daily ration of individual fish, and provides food consumption during only one limited day. It is more important to know the accumulated food consumption after release and the ration on an individual basis. We tried to estimate the accumulated food consumption from the temporal change in carbon stable isotope ratio ($\delta^{13}C$) in dorsal muscle when the diet of juveniles was switched from the formula feed to live mysids (different $\delta^{13}C$ from the formula feed). The average $\delta^{13}C$ of experiment group fed mysids rapidly increased from -20.70‰ to -19.19‰ during the experiment (14 days). Our results suggested that the stable isotope could be a useful tool to estimate feeding condition after release.

Japanese flounder *Paralichthys olivaceus* is one of the most important target species of stock enhancement in Japan. The release size of hatchery-reared flounder is an important factor affecting survival after release, (Fujita *et al.,* 1993; Yamashita *et al.*, 1994; Tominaga and Watanabe, 1998). In order to improve the stocking effect of hatchery-reared juveniles, it is necessary to determine the optimum release place and time. Food availability for stocked juveniles is a key factor to evaluate the quality of release tactics. However, it is not easy to estimate the biomass of food organisms in the release area, because of the difficulty in quantitative sampling. Feeding intensity of released juveniles is affected by not only healthy condition of fish (vitality and nutritional status), but also physical, biological, and environmental conditions in the release area. Therefore, daily ration of fish after release becomes the useful tool to evaluate the release place, time and fry quality.

The quantity of food consumed by fish is commonly estimated on a daily basis. The daily ration model of Elliott and Persson's method (Elliott and Persson, 1978) is widely accepted as the most theoretically rigorous (Cochran, 1979; Eggers, 1979; Elliott, 1979). This method does not require a special instrument and is easy to apply for field investigations. However, it does not give the daily ration of individual fish, and provides food consumption during only one limited day. It is more important to know the accumulated food consumption after release and the ration on an individual basis.

Stable carbon and nitrogen isotope analysis is being used in the ecological study of energy flow because stable isotopic compositions of consumer tissues can often be related predictably to stable isotopic compositions of diet (DeNiro and Epstein, 1978). Hesslein *et al.* (1993) investigated the response of the isotopic compositions of broad whitefish *Coregonus nasus* to change in the isotopic compositions in their diet. They concluded that in the rapid growth fish, the changes of the stable isotope ratio of sulfur, carbon and nitrogen may be high and the rate of change would directly reflect growth ratio.

Japanese flounder juveniles grow fast and the diet of released fish drastically changes from artificial pellets to live organisms, mainly mysids and larval and juvenile fish. We speculated that the stable isotope ratio of carbon in the tissue of the released juveniles was approaching those of prey organisms in the release area and the more the released fish consumed prey, the faster the ratio of stable isotope change. We have no data available to determine how long it would take for the dorsal muscle to respond isotopically to the new food source.

In the present study, mass releases of hatchery-reared Japanese flounder were conducted under various experimental conditions in Wakasa Bay, the Sea of Japan, from 1997 to 1998. Daily ration of a week after release was estimated in terms of percent of body weight (BW) by Elliott and Persson's method in the field. We investigated the preliminary experiment for temporal change in the stable isotope ratio of $\delta^{13}C$ in the dorsal muscle when the diet of juveniles was switched from the artificial pellet to live mysids.

Materials and Methods

Field Experiment

Mass releases were conducted at 1-2 m depth in Wada beach, Wakasa Bay mid coastal area of the Sea of Japan in 1997 and 1998 (Fig. 1, Table 1). In 1997, forty thousand flounder juveniles were released on 29 May (early group) and 2 July (late group), respectively (80,000 in total). Average total length (TL) of the early and late group was 53.5 mm and 51.8 mm, respectively.

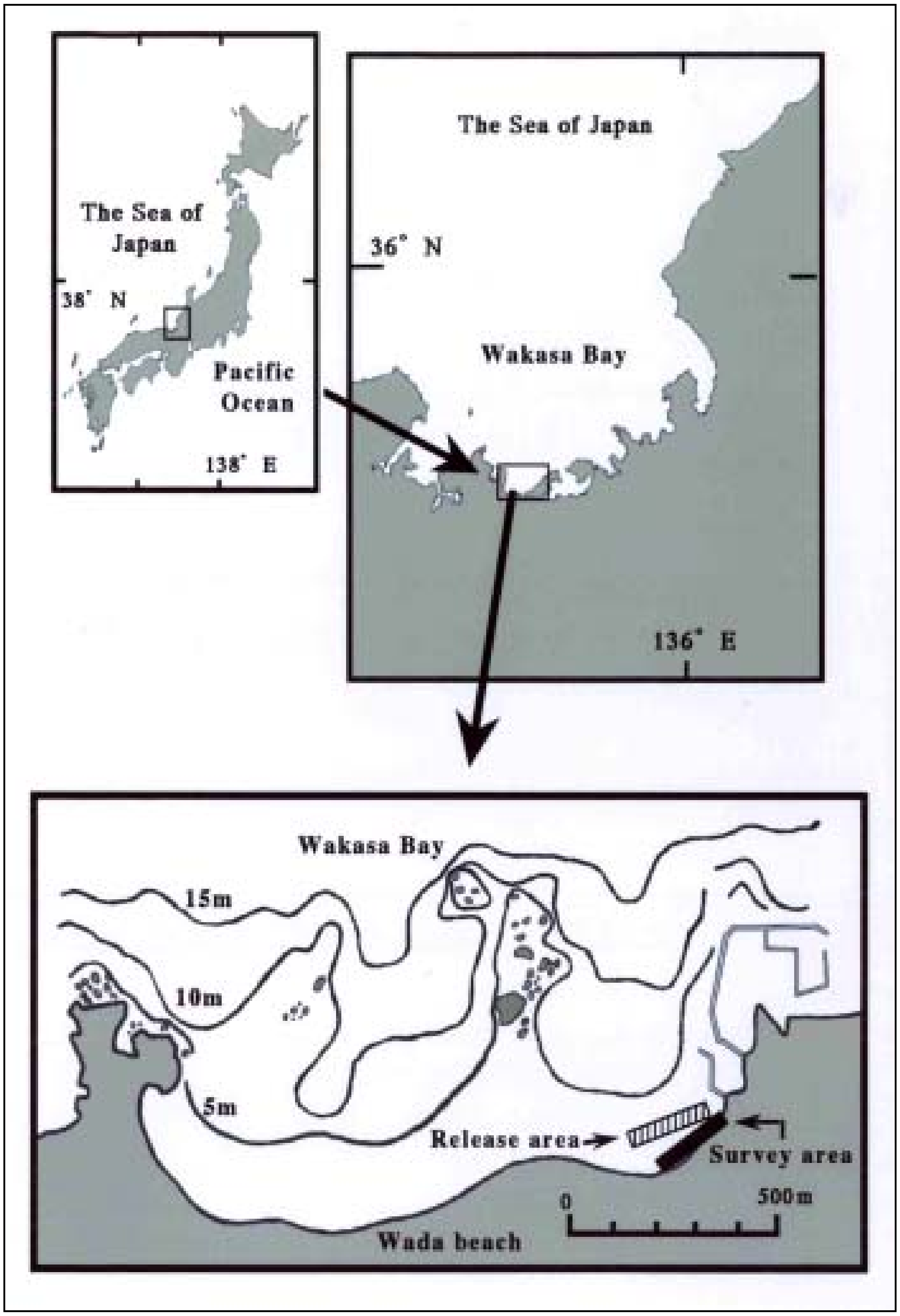

Figure 1. Map of Wada beach, Wakasa Bay, the Sea of Japan, showing locations of flounder release and 24-hour surveys.

Table 1. Date, size, number of individual, environmental conditions of releases for hatchery-reared Japanese flounder Paralicthys olivaceus juveniles conducted in 1997 and 1998

	1997		1998	
	Early group	Late group	Large Group	Small group
Date of release	29.May	2.Jul	21.May	
Release size	53.5mmTL	51.8mmTL	60.7mmTL	37.1mmTL
No. of fish released	40,000	40,000	50,000	50,000
Release area				
Water depth	1.5m	2m	2m	
Water temperature	19℃	24℃	21℃	

In 1998, two different size groups of flounder of which the mean total length was 60.7 mm TL (large group) and 37.1 mm TL (small group) were stocked on 21 May. The number of individuals released was 50,000 each. Hatchery-reared fish were marked on the otoliths by alizarin complexone (ALC). The large and small groups were given single and double rings of ALC, respectively. Sampling surveys were carried out in the surf zone where released fish were densely distributed on 7 or 8 days (d) after release (Fig. 1). Japanese flounder juveniles were caught by beam trawl (1.5 m x 0.5 m) at 3 hour intervals over a 24 hour period. The beam trawl was towed by manpower. All fish were frozen in the field by using dry ice. Food organisms of juvenile flounder were also collected simultaneously by small beam trawl (0.5 m x 0.4 m).

Total length (mm), body weight (0.01 g) and body weight excluding the viscera (0.01 g) were measured. Stomachs were excised and preserved in 5% formalin for later analysis. The total stomach contents were weighed to the nearest milligram after blotting with filter paper and prey items were identified to the lowest possible taxonomic level and counted under microscope. Wet weights of the prey items were recorded to the nearest milligram. Food organisms collected by small beam trawl were also analyzed as the stomach contents.

Estimation of Daily Ration

Daily ration was estimated in terms of percent of BW from the model of Elliott and Persson (1978):

$$Ct = (St - S0 \ EXP(-R \times t)) \times R \times t / (1 - R \times t), \qquad (1)$$

where Ct is the consumption of food during the time interval (t), S0 and St are the average stomach contents index (SCI : stomach contents weight x 100/BW) at time 0 and t , respectively, and R is the instantaneous evacuation rate. The estimates of Ct calculated for each time interval are then summed to give the total daily ration. Feeding is assumed constant within each time interval.

In the laboratory experiment under natural photoperiod condition, even though Japanese flounder juveniles can feed on live mysids ad libitum, the average SCI of flounder clearly decreased during nighttime (Tominaga and Kawai, unpublished data). Assuming no feeding between sunset and sunrise, the instantaneous evacuation rates were estimated from the depletion of stomach content index during night (including empty stomachs). Evacuation rate is therefore given by:

$$R = (1 / t') \, Ln(Smax / Smin), \qquad (2)$$

where the instantaneous evacuation rate is calculated from the maximum (Smax) and minimum (Smin) average SCI of the sample collected during night and the time interval between tmax and tmin (t').

Daily ration of the wild flounder was estimated only in July 1997, because the size of wild fish collected in the field is much smaller than that of hatchery-reared fish collected before July.

<u>Laboratory Experiment of Stable Isotope</u>

The rearing experiment was conducted at the Ocean Research Center of Fukui Prefectural University in May 1999. A total of 228 juveniles (average TL of 47.5 mm) to which formula feed had been fed for two weeks were divided into two groups on 16 May 1999. Seventy-eight individuals were transferred to 30-L polycarbonate tank and reared by the same formula feed (control group). The remaining 150 individuals were reared by live mysids (*Archaeomysis* sp.) in 200-L polyethylene tank for 2 weeks (experiment group). Mysids were caught in Obase Beach Wakasa Bay twice a week and preserved in a 500-L polycarbonate tank. Enough live mysids were added to the tank every day as the juveniles could prey on them ad libitum. Five individuals from the 30-L tank were sampled at 6 and 13 d after the beginning of the experiments and 5 individuals from the 200-L tank were randomly collected every two days during the experiment.

The fish were kept frozen until analyzing. Total length and body weight of individual fish were measured. The dorsal muscle on the eye side was excised, dried, ground to a powder and preserved in a chloroform-methanol solution to remove lipids. The powdered sample (0.5-1.0 mg) was put in a tin container. The carbon isotope ratio ($\delta^{13}C$) was analyzed by a Mat Delta S staple isotope ratio mass spectrometer (Finnigan MAT) equipped with an EA-1108 elemental analyzer (Carlo Erba) at the Center for Ecological Research, Kyoto University.

Since the variation of stable isotope ratios is so small, the isotopic compositions are described by a per mil (‰) deviation from each international standard as defined by the following equation:

$$\delta^{13}C = (Rsample / Rstandard - 1) \times 1000, \qquad (3)$$

where R denotes $^{13}C/^{12}C$. A fossil calcium carbonate was used as the standard. Then, DL-Alanine was used as a laboratory secondary standard to insure instrument accuracy and precision. Instrumental precision was 0.1 ‰.

We fitted our data to Hesslein's model (Hesslein *et al.,* 1993):

$$\delta^{13}C = A + (B - A) \times exp(-C \times t), \qquad (4)$$

where *A* and *B* are parameters determined by asymptotic and initial conditions, respectively, *C* is the rate of change in $\delta^{13}C$ related to growth rate and turnover rate of carbon in the tissue and t is time (day) since the diet switch. Each parameter was estimated by the maximum-likelihood method with STATISTICA (THREE'S COMPANY, INC.).

Results

Biomass of Food Organisms

An average biomass of both total food oraganisms and mysids collected with the small beam trawl net was highest in May 1997 and lowest in July 1997, (Table 2). The biomass of mysids in May, which was a main diet of Japanese flounder juveniles, was 11 times that in July. The biomass was an intermediate value in May 1998. The dominant genera of mysids were *Arcaheomysis* and *Nipponomysis* in all surveys.

Table 2. Average biomass of total food organisms and mysids sampled at 24-hour surveys in Wada beach

	1997		1998
	5.Jun	9.Jul	29.May
Average biomass g/m^2			
Total food organisms	0.36	0.08	0.17
Mysids	0.22	0.02	0.07

Stomach Contents

Mysids were the most important prey item in all surveys. Mysids were especially dominant in stomachs in June 1997 when mysids were the most abundant in the environment (Fig. 2). In May 1998, stomach contents of both large and small groups were similar and mainly composed of mysids, but also amphipods (Fig. 3). Stomach content composition of the late group in 1997 was similar to that of wild juveniles collected together (Fig. 2). Japanese anchovy, amphipods and maclura were also important food items.

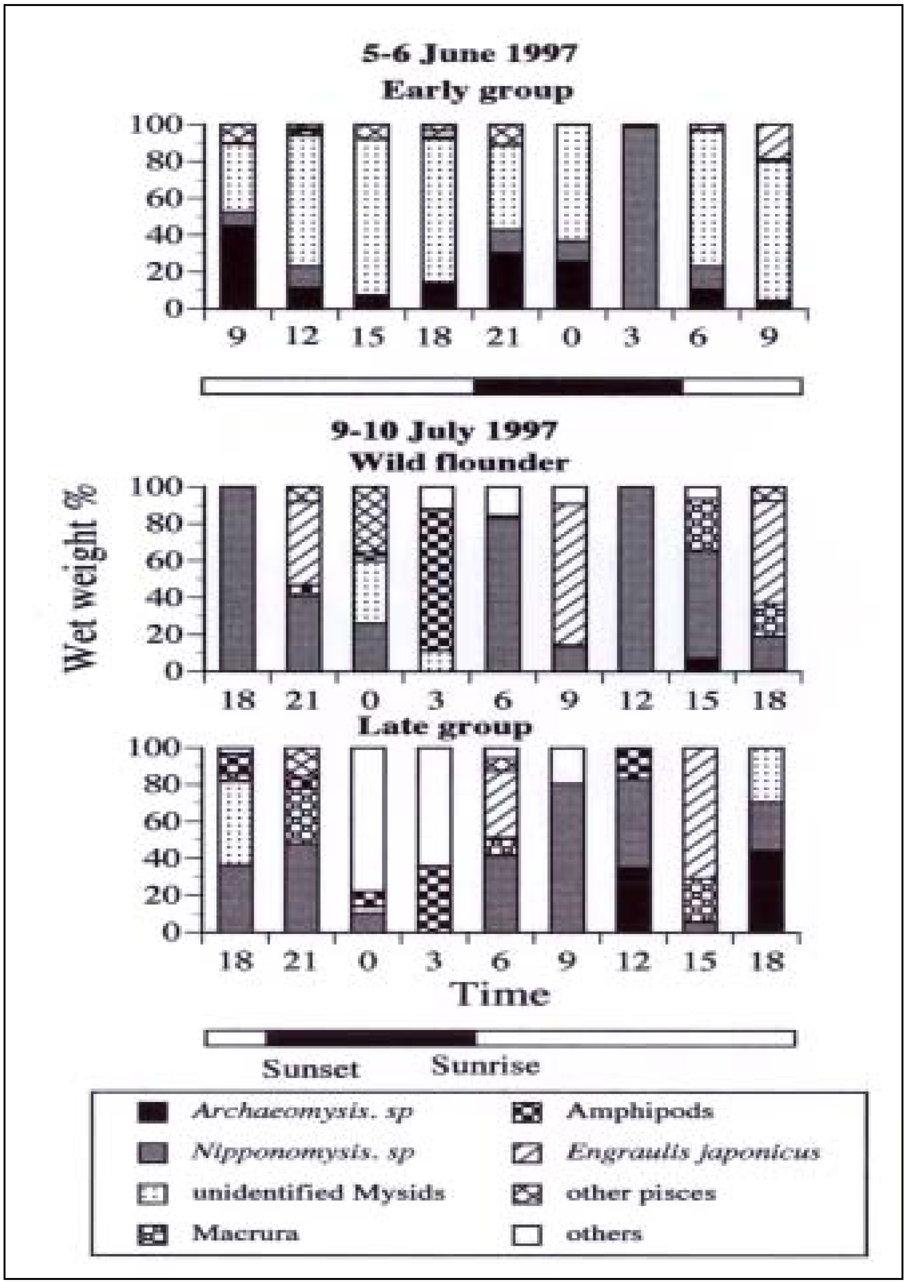

Figure 2. Diel changes in diet composition (wet weight %) in stomachs of hatchery-reared and wild *Paralichthys olivaceus* juveniles in June and July 1997. Open and closed bars indicate daytime and nighttime, respectively.

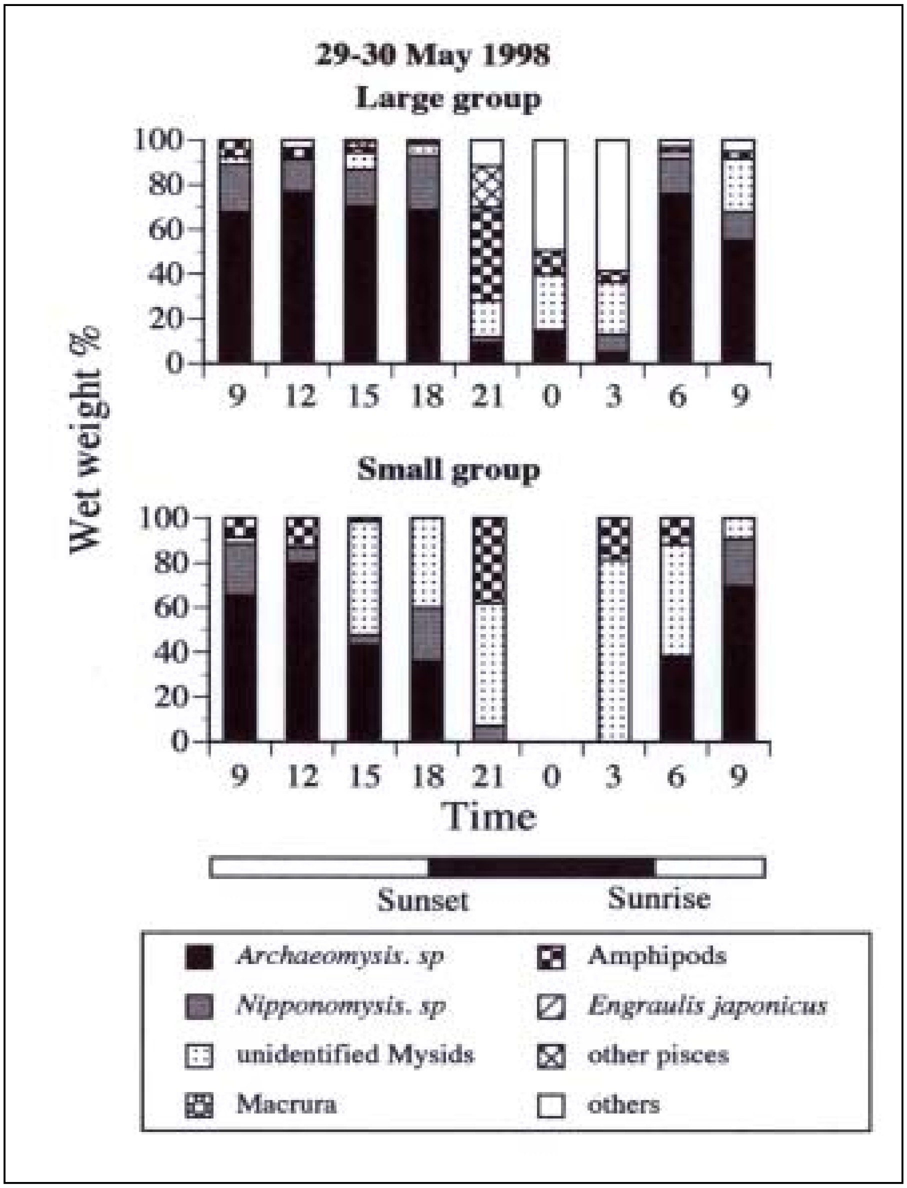

Figure 3. Diel changes in diet composition (wet weight %) in stomachs of hatchery-reared *Paralichthys olivaceus* juveniles in May 1998. Open and closed bars indicate daytime and nighttime, respectively.

<u>Feeding Periodicity and Daily Ration</u>

The average SCIs of flounder juveniles collected in 24 hour surveys were high during day time (Fig. 4). The clear peak of SCI was usually found around dusk and/or dawn, except for the late group in 1997 (Fig. 5). The average SCI of fish in the late group was constantly low throughout the day. However, it was common that the depletion of SCI was seen during nighttime. In 1998 the SCIs of wild flounder juveniles were constantly higher than those of the late group during the day (Fig. 4).

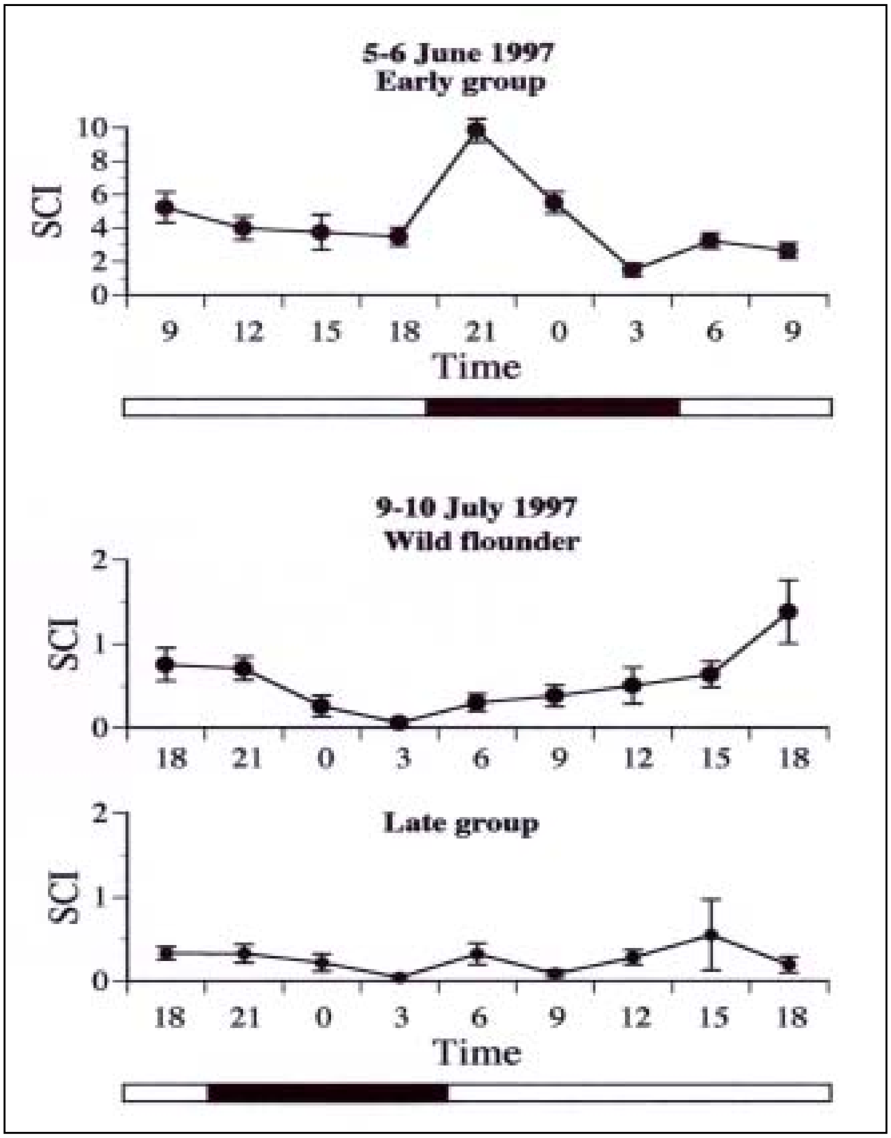

Figure 4. Diel changes in mean stomach contents index (stomach contents weight x 100/body weight) of hatchery-reared and wild *Paralichthys olivaceus* juveniles in June and July 1997. Vertical lines indicate standard error. Open and closed bars indicate daytime and nighttime, respectively.

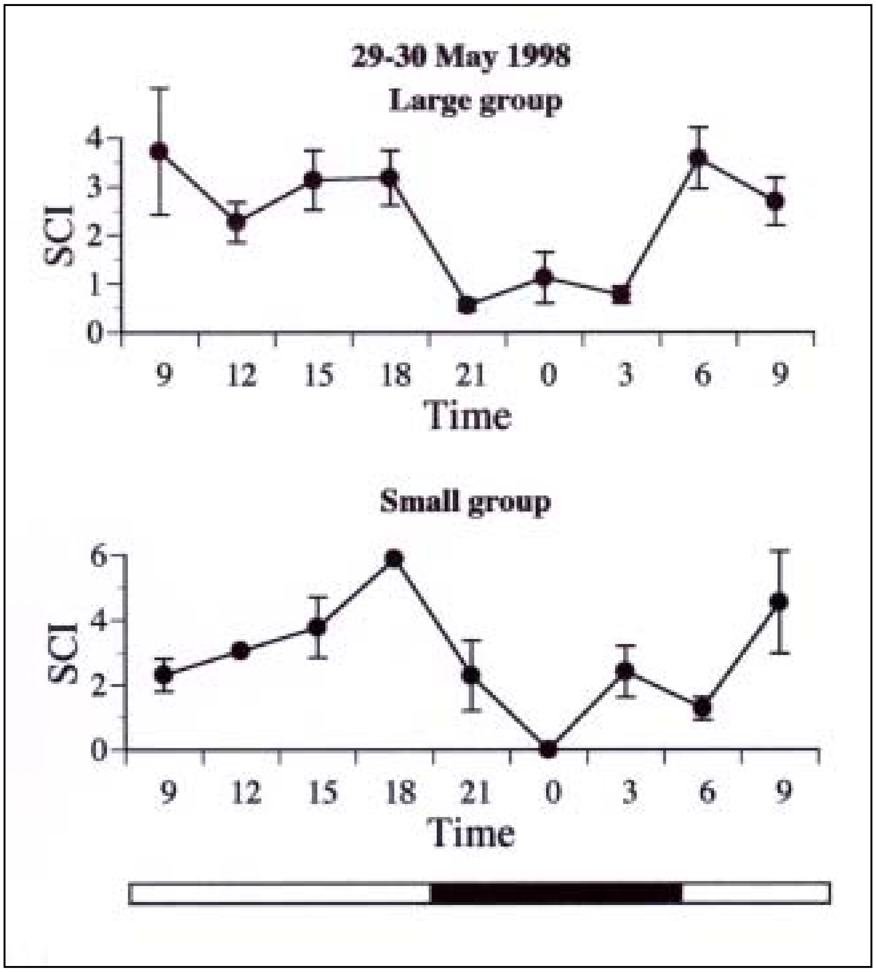

Figure 5. Diel changes in stomach contents index (stomach contents weight x 100/body weight) of hatchery-reared *Paralichthys olivaceus* juveniles in May 1998. Vertical lines indicate standard error. Open and closed bars indicate daytime and nighttime, respectively.

Instantaneous evacuation rate (R) was estimated by using equation (2). The evacuation rate estimate of hatchery-reared fish was similar (0.28-0.31) in all surveys (Table 3). However, R (0.4) of wild flounder was much higher than that of hatchery-reared fish.

The average SCI, which included fish with empty stomachs, was used to estimate the average food consumption for each time interval (Ct) with equation (1). Food consumption per unit time (1 hour) presented negative, as well as positive, values. Daily ration was obtained by summing the amount of food consumption during each interval, including negative values.

Diel change in food consumption per unit time was similar to that of the SCI. The feeding intensity of released flounder in May 1997 and wild flounder in July 1997 gradually increased after sunrise and peaked around dusk (Fig. 6). However, the average food consumption per unit time of fish released in July 1997 was constantly low for 24 hours and clear feeding periodicity was not found. There were two peaks of feeding around dusk and dawn in 1998 (Fig.7).

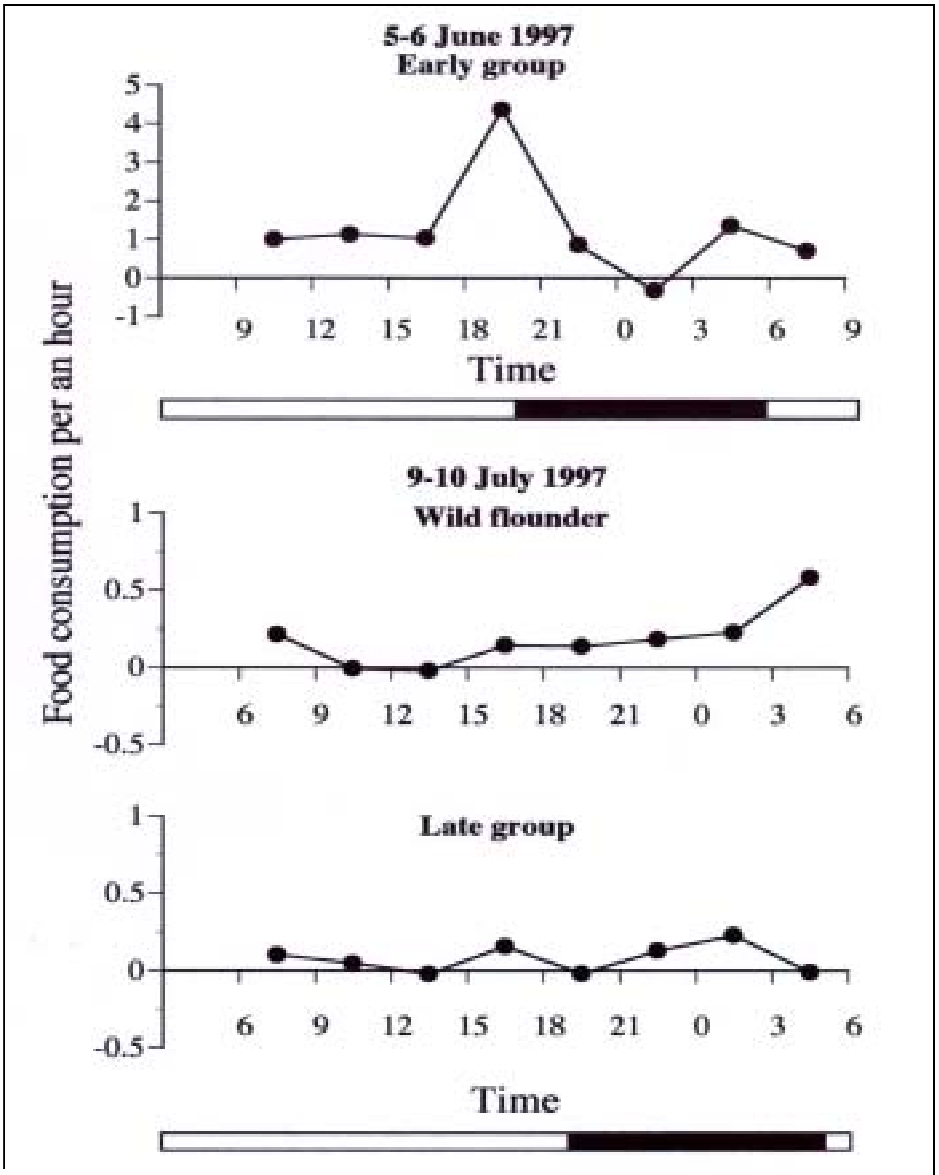

Figure 6. Diel changes in mean food consumption per an hour of hatchery-reared and wild *Paralichthys olivaceus* juveniles in June and July 1997. Open and closed bars indicate daytime and nighttime, respectively.

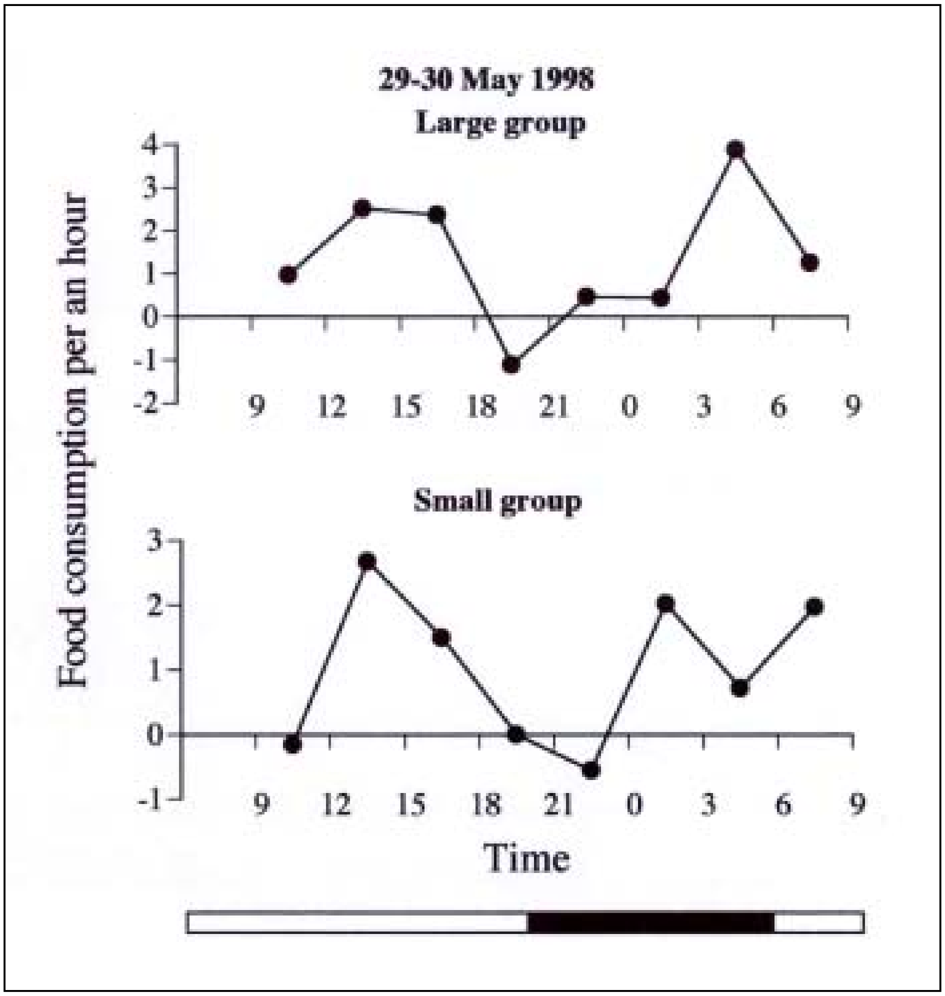

Figure 7. Diel changes in mean food consumption per hour of hatchery-reared *Paralichthys olivaceus* juvenile in May 1998. Open and closed bars indicate daytime and nighttime, respectively.

The estimated daily ration was the highest in May 1997 (22.2 %/BW/d, Table 3). In 1998, daily ration of the large and small groups was 10.8%/BW and 8.2%/BW, respectively. The minimum value (1.5%/BW) of daily ration was found in July 1997. Daily ration (4.8%/BW) of wild flounder collected in July 1997 was 3 times that of hatchery-reared fish (Table 3).

Table 3. Instantaneous evacuation rate (R) and daily ration of released and wild Japanese flounder *Paralichthys olivaceus,* and data about 24-hour field surveys in 1997 and 1998.

	1997	1997		1998	
	Early group	Late group	Wild fish	Large Group	Small group
Date of survey	5.Jun	9.Jul		29.May	
Average TL collected	46.6mmTL	50.7mmTL	55.9mmTL	53.3mmTL	38.4mmTL
Average No. of fish collected during night time (No. /5 min tow)	49.7	10.3	30.9	19.1	3.8
R-value	0.29	0.31	0.40	0.29	0.28
Daily ration %BW/day	22.2	1.5	4.8	10.8	8.2
Water temperature	20℃	24℃		21℃	

The Experiment of Stable Isotope

The initial average body weight of fish used in this experiment was 1.06 g (0.46 SD). The body weight at the end of the experiment was 3.80 g (0.69 SD) for the experiment group and 2.95 g (1.37 SD) for control group. The daily growth rate (g/day) of the experimental group and control groups was 0.20 and 0.15, respectively.

The average $\delta^{13}C$ for the formula feed was -21.09‰ and -19.03‰ for the mysids, a difference of 2.06‰ (Fig. 8). The average $\delta^{13}C$ in the dorsal muscle was not significantly different among control groups sampled at 0, 6, and 13 d after the beginning of experiment (ANOVA). The average $\delta^{13}C$ of the control group was 20.67‰ and slightly more than the formula feed. However, the average $\delta^{13}C$ of the experimental group gradually increased from -20.70‰ to -19.19‰ (Fig. 8). The average $\delta^{13}C$ at the end of the experiment was slightly smaller than the mysids. A significant difference was found between the initial $\delta^{13}C$ and experimental group collected on 2 d (Tukey HSD test $p = 0.013$).

Our data were fitted to equation (4): $\delta^{13}C = -18.97 - 1.73\ exp\ (-0.14 \times t)$.

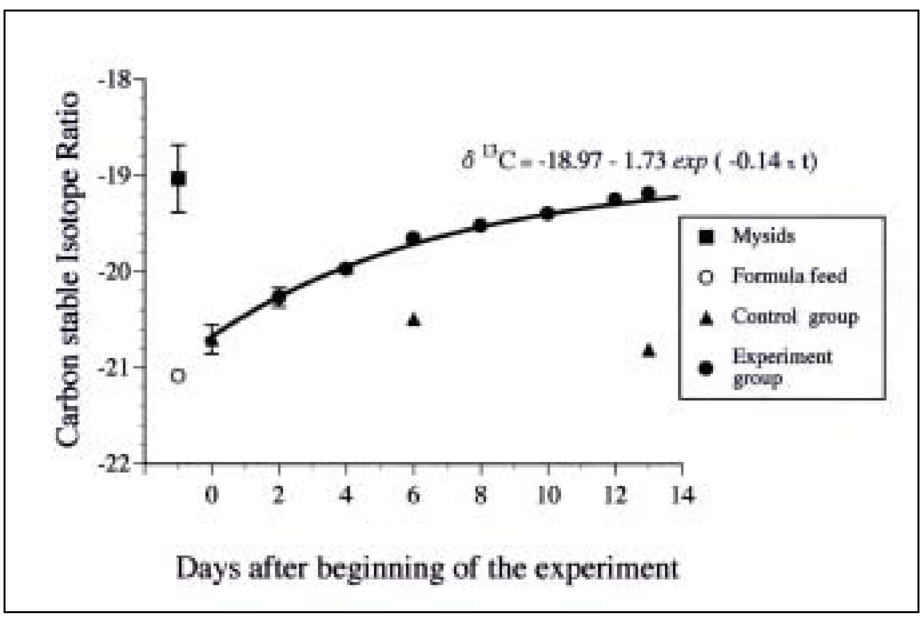

Figure 8. Changes in mean carbon stable isotope ($\delta^{13}C$) in the dorsal muscle of the control group fed formula feed and the experimental group fed live mysids. The $\delta^{13}C$ of mysids collected in Wakasa Bay and formula feed are plotted. Hesslein's model, $\delta^{13}C = -18.97 - 1.73\ exp\ (-0.14xt)$, was fitted to the data of the experimental group. Vertical lines indicate standard error.

Discussion

Daily Ration as an Indicator Evaluating Release Tactics

Japanese flounder juveniles prey mainly on mysids in the nursery area (Yasunaga and Koshiishi, 1981; Kato, 1987; Furuta *et al.,* 1997; Tanaka *et al.,* 1999). In the present study, mysids were the major food item for the released flounder. However, stomach contents composition fluctuated according to mysid biomass. Although mysids composed more than 90% of the diet in May 1997 when the mysids were the most abundant, the diversity of diet composition in the stomach increased as the mysid biomass decreased. Tanaka *et al.* (1999) pointed out that the early shift in diet from mysids to fish was found in this study area comparing to other areas (Tane, 1992; Fujii and Noguchi, 1996) and suggested that mysid availability was relatively low. The biomass of mysids fluctuates seasonally and yearly at Wada beach and the variation of stomach contents composition seems to reflect the food availability for released fish.

Daily ration of released fish was also closely related to the food availability, especially mysid biomass. Daily ration of the early group in 1997 was 14.8 times that of the late group. The catch rate of released flounder in fishing surveys decreased rapidly during the first several days (Tominaga, 1991; Furuta *et al.,* 1997) and predation is thought to be a major cause of early mortality (Sudo *et al.,* 1992; Yamashita *et al.,* 1993; Furuta *et al.,* 1998). However, Furuta (1998) reported that a short-term starvation (3 to 7 days) changed feeding behavior of flounder juveniles and resulted in the vulnerability to predation. It is reasonable to think that feeding conditions of released fish indirectly affect early mortality after release. An average number of early group fish caught during nighttime (49.7 individuals/5 minute tow) was about 5 times as much as that of late group (10.3 inds/5min. tow). A large reduction of released flounder (including an emigration from release area) was found in the late group, where feeding intensity was low.

Food intake of released flounder was lower and incidence of empty stomachs were higher than wild flounder in Tottori (Furuta *et al.,* 1997). In the present study, the daily ration of wild flounder in July 1997 was 3 times that of the late group. These results show that hatchery-reared fish are inferior predators. Predation ability is necessary for hatchery-reared fish seedling quality and survival.

It is possible to compare daily ration of fish collected in different places and/or at different times. Daily ration is thought to reflect food availability in the release area and seedling quality, and be used as an indicator to evaluate the quality of the release technique. However, to evaluate the actual effect of release, sampling surveys of commercial landings are indispensable.

Possibility of New Methods by Stable Isotope

Carbon has stable isotope, ^{12}C and ^{13}C. Every biogenic substance and living organism in an ecosystem consists of these isotopes (Wada *et al.,* 1998). Stable isotopic compositions of consumer tissues can often be related to stable-isotopic compositions of diet (DeNiro and Epstein, 1978). Therefore, stable isotope (especially carbon and nitrogen) has been used in the ecological study of energy flow (e.g. Hobson and Welch, 1995; Wainwright *et al.,* 1993).

In the present study we tried to apply the carbon stable isotope to the quantitative study of diet consumed by hatchery-reared flounder. When the diet was switched from formula feed to live mysid, $\delta^{13}C$ in the dorsal muscle of the experimental group began to approach $\delta^{13}C$ of mysids. In the Hesslein's model, coefficient "*C*" indicates the magnitude of the rate of change in $\delta^{13}C$. This value is associated with carbon turnover rate and growth rate of fish. The growth rate of juveniles used in this experiment is extremely high. Average BW changed from 1.06 g in the beginning to 3.80 g at the end of the experiment (14 d). There are few studies on turnover rate of carbon isotope in fish tissue (Matsubara, 1997). Hesslein *et al.* (1993) examined the change in the isotope composition of carbon in broad whitefish (*Coregonus nasus*) tissues in response to a change in the isotope composition of their food and showed that turnover rate of carbon isotope was very small.

In the present experiment, it is thought that change in $\delta^{13}C$ is mainly attributed to fish growth rate. In Japanese flounder juveniles, the rate of change in $\delta^{13}C$ would directly reflect the growth rate. Therefore it would be possible to estimate the growth rate and consequently the cumulative food consumption after release by the rate of change in $\delta^{13}C$. However, experiments of different amounts of food and mixtures of diet with different stable isotope ratios must be conducted.

Acknowledgments

Professor E. Wada, Dr. A. Sugimoto and Ms. F. Kimura of CER, Kyoto University allowed us to use IRMS and helped us to analyze staple isotopes. Prof. H. Fushimi of Fukuyama University helped with field sampling. Hatchery-reared juveniles were provided by Japan Sea Farming Association (JASFA). We thank Messrs. S. Kunitoh, T. Hamaguchi and R. Fujio for their assistance. We are grateful to the staffs of JASFA Miyazu and Obama stations, students of Kyoto University and Fukui Prefectural University for their helpful assistance in producing and releasing juveniles, and field sampling. We also thank the staffs of Wakasa Wada fisheries cooperative and Takahama Fisheries cooperative for their cooperation.

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