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Direct Observation of Fish Spleen by an Abdominal Window Method and Its Application to Exercised and Hypoxic Yellowtail

Ken-ichi Yamamoto, Yasuo Itazawa and Hiroshi Kobayashi

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Abstract The spleen of the yellowtail (*Seriola quinqueradiata*) was directly observed through a window opened at the abdomen during exercise experiments by continuous chasing and during hypoxia experiments by nitrogen bubbling. The spleen decreased above 30% in length, 60% or more in weight and 70% or more in hemoglobin content, either by exercise or by hypoxia.

The mammalian spleen has been known to contract and supply stored erythrocytes into the circulating blood during severe exercise (Barcroft et al., 1925; Barcroft and Florey, 1929) and hypoxia (Kramer and Luft, 1951). On the other hand, Osogoe (1954) considered that the fish spleen did not strongly contract either in elasmobranchs and teleosts because its outer membrane was very thin and very poor in smooth muscle fibres, and because no trabecular system was present. Opdyke and Opdyke (1971) reported that the spleen of the dogfish, *Squalus acanthias*, did not release normal erythrocytes in response to sympathetic stimulation by epinephrine. Stevens (1968) showed that the spleen of the rainbow trout, *Salmo gairdneri*, did not significantly change in weight while it decreased in blood content during severe exercise. Thus, contraction of the spleen during exercise and hypoxia were generally denied in fishes, although Hall (1928) found that the spleen of a puffer fish, *Spheroides maculatus*, decreased in weight during asphyxia.

The present authors have demonstrated contraction of the spleen and erythrocyte supply from the organ into the circulating blood in exercised and hypoxic fish, based on changes in weight and hemoglobin content of the organ (Yamamoto et al., 1980, 1983). But contraction of the spleen was not established with the same individual fish, because each determination of weight and hemoglobin content of the organ required sacrificing the fish. This paper presents a technique for an abdominal window to view the fish spleen, and results of direct observation of spleen contraction in the same individual fish in exercised and hypoxic yellowtail are reported.

Material and methods

The experiments were carried out on 33 individuals of the yellowtail, *Seriola quinqueradiata*, and 44 individuals of the fish for the experiments on the relationship between length and weight of the spleen. The fish were reared at a fish farm near Shimonoseki, transported to Shimonoseki University of Fisheries, and kept without feeding for one or two days before experimentation in a tank of 2 m×1 m×0.8 m (depth of water) irrigated with air-saturated water at a constant rate of about 40 l/min.

Operation for an abdominal window. A fish was anesthetized in 1: 10,000 quinaldine solution for one min, set on an operation table with the right side up, and then operated to open a window at the abdomen under branchial irrigation with 1: 50,000 quinaldine solution at a constant rate of about 0.5 l/min. A rectangular window of about 60 mm in length by about 13 mm in height was opened on the right side of the abdomen of fish of about 340 mm in fork length and about 630 g in body weight (Table 1). One third or less of the length of the spleen was not visible as a result of existence of the intestine, when the window was opened (Fig. 1). This covered part of the spleen was carefully drawn out over the intestine, and a whole lateral face of the organ became visible through the window. A polyethylene tube of 2 mm in outside diameter with an elliptic thin celluloid plate of 9 mm×6 mm in long and short axes was planted in the abdominal wall just over the

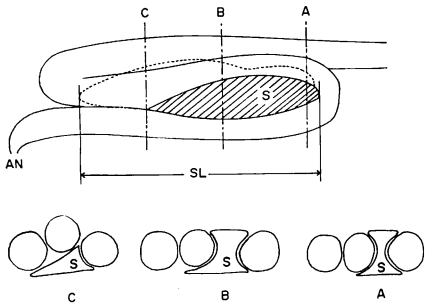

Fig. 1. Lateral view of the yellowtail spleen. The part shown by dotted line is hidden while the hatched part is visible, just when the abdominal window is opened. Lower three figures are cross sections at the planes, A, B and C. S, spleen; SL, spleen length; AN, anus.

window (Fig. 2). Lastly, the window was covered with a colorless, transparent polyethylene film of 0.8 mm in thickness sewn on the skin surrounding the window. The time needed for the whole procedure of operation was about 15 min.

The experimental fish was set in a fish chamber (respiration chamber) of 55 cm×10 cm×11 cm (height), and the window was instilled with physiological saline at a rate of 4 ml/min through the polyethylene tubing to keep the window clear during the experiment (Fig. 2). After setting the fish in the chamber, a small amount of air and clot left inside the window was sucked off through another polyethylene tubing inserted from the suture of the window film.

Some of the fish, after the operation, were cannulated to the dorsal aorta with polyethylene tubing of 1 mm in outside diameter following the method of Smith and Bell (1964), and then set in the fish chamber. The dorsal aortic cannula was used to sample blood for hematocrit determination by centrifugation in a capillary tube at 11,000 rpm for 5 min.

Determination of size and hemoglobin content of the spleen. The length of the spleen in mm was measured from a photograph taken through both the abdominal window and a window of the fish chamber. The actual length of spleen in mm was converted to that in mm per kg·body (SL), because discussions are given later based on body weight in kg following the methods used in the previous papers (Yamamoto et al., 1980, 1983). Fork length of a fish in mm (FL) was convered to body weight in g (BW) using the formula, $\log BW = 0.00445\ FL + 1.23$ ($n = 163$, $r = 0.987$). The weight of the spleen in g per kg·body (SW) was estimated from the spleen length in mm per kg·body (SL) using the formula, $\log SW = 0.020\ SL - 0.589$ ($n = 44$, $r = 0.930$) obtained in resting, exercised, hypoxic and recovery conditions (Fig. 3). The hemoglobin content of the spleen in g per kg·body (SHb) was calculated by the formula, $SHb = 0.232\ SW - 0.158$ ($n = 117$, $r = 0.954$) (Yamamoto et al., 1980).

Exercise experiment. The experiments were carried out, in water of 20.4±3.7°C (SD), on 20 individuals of yellowtail of 709±98 g in body weight and 358±34 mm in fork length. Experimental fish were at 4 to 6 h after the operation, transferred from the fish chamber to a tank of 62 cm×48 cm×43 cm (depth of water) irrigated with air-saturated water at 10 l/min and forced to violently swim by continuous chasing for 5 min, and then returned to the fish chamber. Photographs of the spleen were taken in resting condition (5 min before the exercise), exercised condition (just after the exericse) and recovering condition (every 5 or

Table 1. Measurements of yellowtail (n=38) used for exercise and hypoxia experiments and their abdominal window.

		$\bar{X}$±SD
Fork length	(mm)	342±29
Body depth	(mm)	78± 8
Body weight of the fish	(g)	631±78
Length of the upper edge of the window	(mm)	61± 8
Length of the lower edge of the window	(mm)	59± 7
Length of the anterior edge of the window	(mm)	14± 3
Length of the posterior edge of the window	(mm)	12± 3

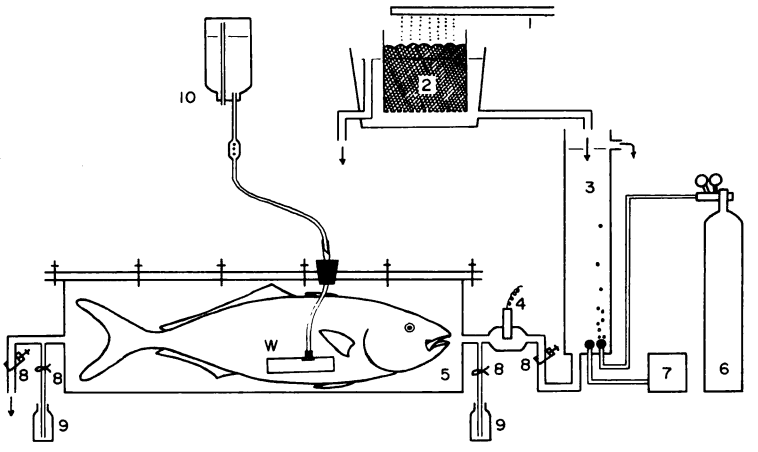

Fig. 2. The diagram of experimental arrangements. 1, supply of sea water; 2, filter; 3, equilibration column; 4, P_{O_2} electrode (only for monitoring); 5, fish chamber; 6, N_2 bottle; 7, aeration pump; 8, screw- or pinch-cock; 9, Winkler bottle for oxygen determination; 10, instillation bottle; W, abdominal window.

30 min after being returned to the fish chamber). The rate of oxygen consumption in ml/min, kg·body ($\dot{V}_{O_2}$) was determined by the constant flow method based on the difference of oxygen concentration between the water inflowing and outflowing to the chamber and the flow rate of the water.

Hypoxia experiment. The experiments were carried out, in water of 23.9±1.8°C, on 18 individuals of yellowtail of 496±89 g in body weight and 325±17 mm in fork length. Experimental fish were kept for 4 to 6 h before experimentation in the fish chamber irrigated with well-aerated water. The level of oxygen dissolved in the irrigating water was changed from 4.83±0.43 ml/l (normoxic condition, oxygen saturation 96%, estimated P_{O_2} 149 mmHg) to 1.16±0.08 ml/l (hypoxic condition, 23%, 36 mmHg) within 15 min by changing the gas introduced into the equilibration column from air to nitrogen. The hypoxic condition was kept until the fish fell into asphyxiation which was defined by cessation of respiratory movement. When the fish did not fall into asphyxiation within 45 min, the ambient level of oxygen was further diminished to zero. Immediately after cessation of respiratory movement, the level of ambient oxygen was rapidly raised to the normoxic condition within 1 min by rechanging the gas from nitrogen to air. The condition after the raising oxygen level is called

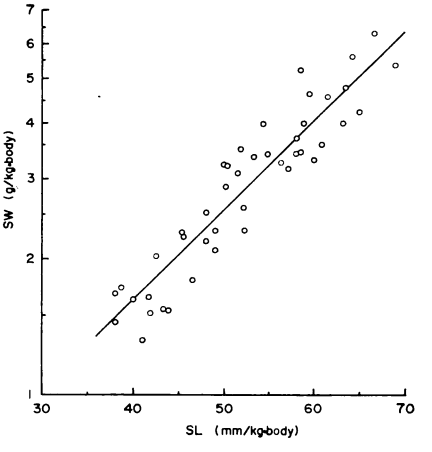

Fig. 3. The relationship between spleen weight (SW) and spleen length (SL) in yellowtail.

"recovering condition" in this paper. Photographs of the spleen were taken in normoxic condition, every 5 min in hypoxic condition (every few or 1 min just before asphyxiation), and every 5 min in recovering condition (every 10 min after the first 60 min in the condition). The weight and the hemoglobin content of the spleen were estimated from the length of the organ in the photographs as described before.

Results

External appearance of the spleen. Immediately after the window was opened at the abdo-

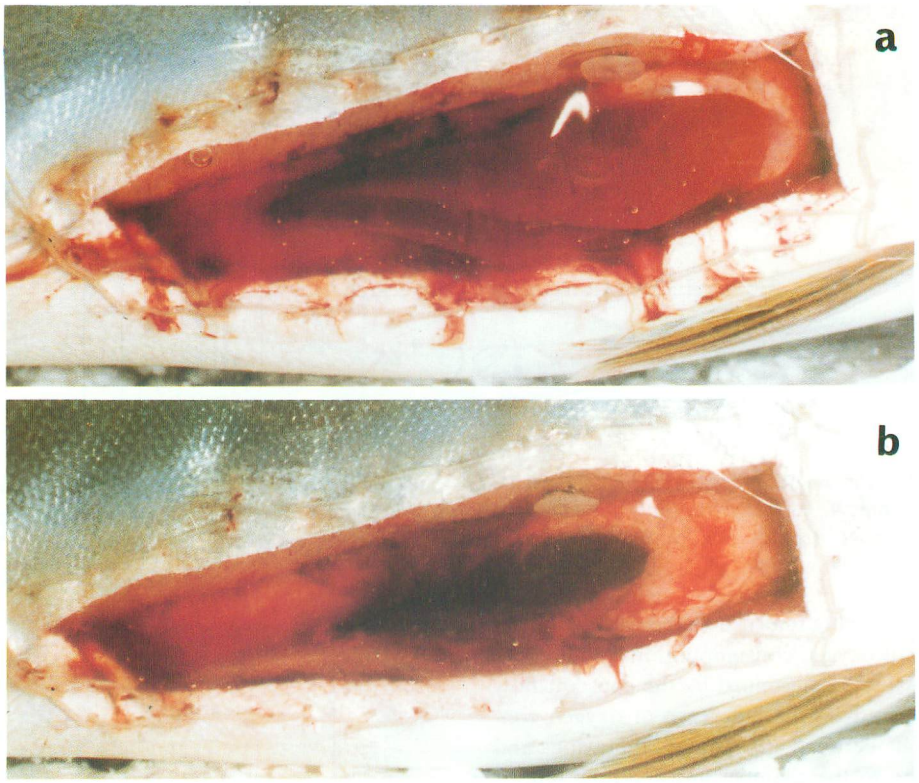

Fig. 4. External appearance of the yellowtail spleen. a (upper), a normal spleen in a fish resting in well-oxygenated water; b (lower), a contracted spleen in a fish just after severe exercise forced by continuous chasing for 5 min.

men, the external feature of the spleen varied in appearance, i.e., already contracted and dark red in color, not contracted but soon contracting after change in color from fresh arterial blood to dark red, or not contracted through the period of operation. After the experimented fish was set in the fish chamber, the spleen gradually enlarged (recovery of length) and became the color of fresh arterial blood within 3 h as shown in Fig. 4a. After 15 h, the polyethylene film covering the window became soiled due to coagulation of tissue fluid, and the spleen became difficult to observe. The fish survived more than 14 days at 14°C or more than 3 days at 26°C in the chamber after the operation.

Response of the spleen to severe exercise. The experimental fish showed the same behavior as control fish during the severe exercise experiment. The spleen contracted strongly and changed in color from fresh arterial blood to dark red within 2 min (Fig. 4b). The length of the organ showed 32% decrease from the resting level of 61.09±2.33 mm/kg·body to the minimum level of 41.35±3.35 mm/kg·body after severe exercise for 5 min. The organ maintained the contracted state for 25 min after the exercise, and then gradually recovered the length (Fig. 5). The weight of the spleen showed 59% decrease from the resting level of 4.23±0.47 g/kg·body to the minimum level of 1.72±0.28 g/kg·body, and the hemoglobin content of the organ showed 71% decrease from the resting level of 0.82±0.11 g/kg·body to the minimum level of 0.24±0.06 g/kg·body.

The rate of oxygen consumption rose drastically to 5.75±0.54 ml/min, kg·body due to the severe exercise from the resting level of 1.93±0.42 ml/min, kg·body, and then gradually lowered (Fig. 6). The response of oxygen consumption in the experimental fish was similar to that in control fish, although the

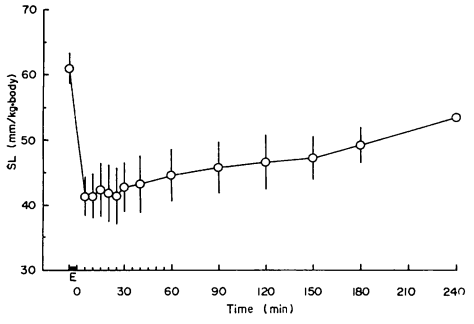

Fig. 5. Change in spleen length (SL) of yellowtail, in resting, just after severe exercise for 5 min, and in recovering conditions. The results are shown in the mean values (open circles) and the standard deviations (vertical bars). 'E' shows the period of severe exercise forced by continuous chasing for 5 min. The same as to Fig. 6.

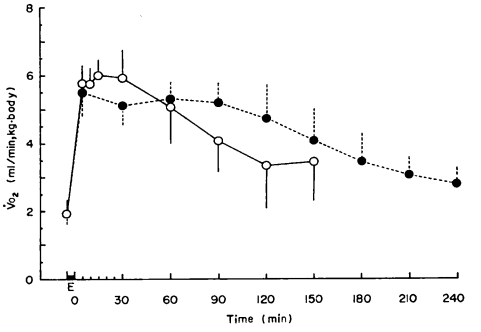

Fig. 6. Change in the rate of oxygen consumption ($\dot{V}_{O_2}$) of yellowtail, in resting, just after severe exercise, and in recovering conditions. Open circles are the results in fish with the abdominal window, and solid ones those in non-operated fish.

change was more rapid in the former (Fig. 6).

Response of the spleen to severe hypoxia. The spleen gradually changed in color from fresh arterial blood to dark red during the hypoxic period, and rapidly reduced in length for the last 10 min of the hypoxic period. The organ maintained the contracted state for 40 to 50 min after the hypoxic period and then gradually regained its original length (Fig. 7). Fig. 7 is a typical example to indicate the change in spleen length in response to the change in oxygen level of the ambient water. In this case the fish fell into asphyxiation by a hypoxic condition of 40 min.

The length of the spleen showed 37.3% decrease from the resting level of 60.9±2.0 mm/kg·body to the miniumm level of 38.2±2.8 mm/kg·body at the maximum contraction. The maximum contraction or the minimum length of the organ was observed at 2 to 3 min after asphyxiation or at the first 2 to 3 min of the recovering period. The time for 25% contraction was 32±5 min, that for 25% to 50% contraction 6±2 min, that for 50% to 75% contraction 3±2 min, and that for 75% to the entire contraction 4±2 min. The weight of the spleen showed 65% decrease from the resting level of 4.20±0.40 g/kg·body to the minimum level of 1.48±0.39 g/kg·body, and the hemoglobin content of the organ showed 77% decrease from the resting level of 0.82±0.09 g/kg·body

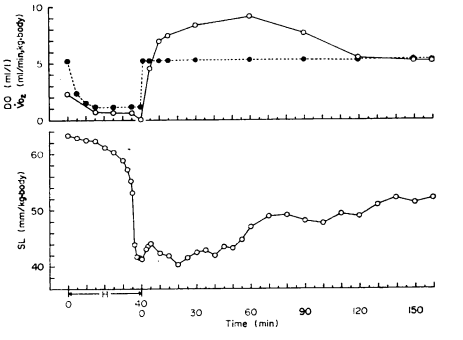

Fig. 7. Changes in spleen length (SL) of yellowtail with the abdominal window in normoxic, hypoxic and recovery conditions (the lower figure), and the rate of oxygen consumption ($\dot{V}_{O_2}$) (open circles in the upper figure) as well as oxygen concentration in the ambient water (DO) (solid circles in the upper figure).

to the minimum level of 0.19±0.09 g/kg·body. The rate of oxygen consumption showed a prominent increase at the beginning of the recovering period reaching the maximum level at 60 min of recovering followed by a gradual decrease implying oxygen debt during the hypoxic period.

Discussion

Effects of the operation for an abdominal window on the spleen and the blood. The operation for an abdominal window seems to give no remarkable effect on the spleen and the blood of the yellowtail, as shown in Table 2, although

all of weight and hemoglobin content of the spleen, contractility of the organ in severe exercise and severe hypoxia, and hematocrit value of the arterial blood showed a slightly smaller figures in operated fish than in normal ones. The abdominal window method is, therefore, considered to be useful for direct observation of the spleen and estimation of response of the organ to exercise and hypoxia. This method is also thought to be applicable for direct observation of other organs in the abdominal cavity of fish.

Erythrocyte supply from the spleen during severe exercise and severe hypoxia. The amount of hemoglobin released from the spleen into the circulating blood is estimated to be 0.82–0.24= 0.58 g/kg·body in severely exercised yellowtail and 0.82–0.19=0.63 g/kg·body in severely hypoxic fish. These values of hemoglobin correspond to the amounts of erythrocytes, 0.58× 3.15=1.83 ml/kg·body and 0.63×3.15=1.98 ml/kg·body, respectively, calculated based on a formula, Ht=3.15 Hb (Yamamoto et al., 1980), where Ht is hematocrit value in % and Hb hemoglobin content of the blood in g/dl.

If the hemoglobin in the erythrocytes released from the spleen is fully oxygenated, the amount of oxygen supplied from the organ could be calculated to be 1.83×0.436=0.80 ml/kg·body and 1.98×0.436=0.86 ml/kg·body, based on a formula, O_2 cap.=0.436 Ht−0.683 (Yamamoto et al., 1981), where O_2 cap. is the oxygen capacity of blood in ml/dl. These amounts of oxygen correspond to the oxygen demand for about 25 sec in resting yellowtail and that for about 9 sec in violently swimming fish, estimated from the rate of oxygen consumption, 1.93 ml/min, kg·body in resting yellowtail and 5.50 ml/min, kg·body in violently swimming fish (Fig. 6). The oxygen supplied from the spleen, therefore, contributes little to the oxygen demand of the fish. But the increase in oxygen capacity of the blood by the supply of hemoglobin from the spleen is considered to largely contribute to oxygen uptake during severe exercise and severe hypoxia.

Comparison with mammals. The contraction rate of the spleen in severely exercised yellowtail was 59% (the present study) and 71% (Yamamoto et al., 1980) in weight. These figures are almost the same as those in dogs (70%, Barcroft et al., 1925; 60% Barcroft and Florey, 1929), cats (50–60%, Barcroft et al., 1925) and rabbits (40%, Barcroft et al., 1925). The time needed for the entire contraction of the spleen was about 2 min in severely exercised yellowtail, while the time was only 4 sec in exercised dogs (Barcroft and Florey, 1929).

Table 2. Effects of the operation for an abdominal window on spleen and blood.

		Operated	Unoperated
In resting condition:			
Spleen weight	(g/kg·body)	4.23±0.47	4.81±0.10*
		4.20±0.40	5.72±0.20*
			4.35±0.70**
Hemoglobin content of the spleen	(g/kg·body)	0.82±0.11	1.09±0.08*
		0.82±0.09	0.87±0.16**
Ht value of the arterial blood	(%)	27.8 ±2.6	26.1 ±1.5*
			28.0 ±0.4*
			27.5 ±1.9**
After 5 min severe exercise:			
Decrease in spleen weight	(%)	59	71*
Decrease in spleen hemoglobin	(%)	71	84*
After severe hypoxia:			
Decrease in spleen weight	(%)	65	80**
Decrease in spleen hemoglobin	(%)	77	91**

* Yamamoto et al. (1980). ** Yamamoto et al. (1983).

Different values for the same items show the results of different series of experiments. Values are shown in the mean and standard deviations.

The spleen of the dog was reported to commence its contraction when the oxygen level of the arterial blood was lowered to less than 25% saturation, and then contract strongly within several min. The situations in hypoxic yellowtail were quite similar to those in the dog, although the yellowtail spleen required about 13 min from 25% contraction to the entire contraction.

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(KY: Department of Fisheries, Fakulty of Agriculture, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812, Japan; Present address; Shimonoseki University of Fisheries, Nagatohonmachi, Yoshimi, Shimonoseki 759–65, Japan; YI: Department of Fisheries, Faculty of Agriculture, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812, Japan; HK: Shimonoseki University of Fisheries, Nagatohonmachi, Yoshimi, Shimonoseki 759–65, Japan; Present address; Department of Fisheries, Faculty of Agriculture, Kinki University 3–4–1, Kowakae, Higashiosaka, Osaka 577, Japan)

魚類脾臓の腹窓法による直接観察およびその運動時ならびに酸素欠乏時のブリへの適用

山元憲一・板沢靖男・小林　博

ブリ（尾叉長約 34 cm，体重約 630 g）の右側体壁の脾臓と対応する部位に窓（約 6 cm×約 1.3 cm）を開き，脾臓の全側面が見えるように腸管に被われている部分を引き出し，無色透明のポリエチレン・フィルム（厚さ 0.8 mm）を体壁に縫合して脾臓観察用の腹窓を作った．魚を呼吸実験に常用される水槽に収容し，4～6 時間後より実験に供した．魚はこの状態で水温 14°C で 14 日以上，26°C では 3 日以上生存した．脾臓長は腹窓を通して撮影した写真を基に測定した．

脾臓は正常状態では鮮紅色で，長さ約 6 cm/kg であったが，激しい遊泳運動および著しい酸素欠乏下では暗赤色で，約 4 cm/kg に収縮した．脾臓長のこの収縮は，脾臓重量および脾臓中ヘモグロビン量のそれぞれ 60% 以上および 70% 以上の減少に相当し，脾臓中の貯蔵赤血球がそれだけ循環血液中に供給されて血液の酸素摂取能力が著しく高められたことを示している．

(山元：812 福岡市東区箱崎　九州大学農学部水産学科；現住所，759-65 下関市吉見永田本町　水産大学校増殖学科；板沢：812 福岡市東区箱崎　九州大学農学部水産学科；小林：759-65 下関市吉見永田本町　水産大学校増殖学科；現住所，577 東大阪市小若江 3-4-1 近畿大学農学部水産学科)