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Age Determination of Pond Smelt Using Otolith Phase*[1]

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Summary

As a part of study to clarify the population structure of the pond smelt, *Hypomesus transpacificus nippoensis* McAllister, the validity of method using the otolith phase for age determination of the fish is examined in this study. Opaque zones and translucent zones of the otolith are formed sequentially. In Lake Ogawara and off Hachinohe, the opaque zones are formed in December-July, the translucent zones are formed in August-Novemver. Eggs of the fish in the lake usually hatch out around early May. Therefore the fish that have formed a second translucent zone of their otolith are estimated to be 1 year old. Moreover, this method can be applied to fish from other lakes, and would in general be useful for the fish inhabiting various geographical conditions. The relationship between otolith phase and life record is examined. The length of the opaque core, after complete formation, is proportional to the body length from August to October and is considered to correspond to the early stage growth. The otolith phase would be useful for analyzing the difference in past growth of subpopulation of the fish.

In the ecological study of fish populations, establishing a precise technique for age determination is a fundamental problem in analyzing characteristics of each species population. There are various methods for determining the age of fish, such as tagging-releasing and successive sampling, which have been used by many researchers. In addition, other methods using hard tissue, e.g. scales, otolith, vertebrae and operclaria, are known. However, not only preciseness but also handiness is required in every method. Furthermore, availability of a method is different according to the biological character and developmental stage of each species. Therefore, it is nessesary to examine the preciseness and handiness of a method for every developmental stage when determining the age of fish.

For the pond smelt, *Hypomesus transpacificus nippoensis* McAllister, the age

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in years has been estimated by using their scales as reported by Amamiya (1) and Hamada (2), or by analyzing the body length distribution.

However, these methods have some drawbacks. For example, it is often difficult to get suitable scales for age determination because they are easily deciduous, spawning marks on the scales are often unidentifiable and the scale structure varies from place to place (2,3). The method of analyzing body length distridution is insufficient in preciseness because its distribution of each age-group is not sometimes completely separate.

On the other hand, recently it has been found that the age in days of pond smelt can be determined using the daily rings formed on their otoliths (sagitta (4), lapillus (5)). Although this techniqus is precise, it seems to be unsuitable for age determination of the fish because it can not be applied to the fish older than 150 days.

This study is carried out as a part of a study to clarify the population structure of the fish inhabiting Lake Ogawara and off Hachinohe, in northernmost Honshu, Japan. In this study, we examine the validity of the method using the otolith phase for age determination of pond smelt over their developmental stages. The reason for using the otolith is that it is stable in structure and often has the trace showing the individual life record relatively well. Still more, the generality of using the otolith phase as a method for age determination is examined by applying this method to the fish inhabiting other lakes in which the environment for the fish is different from the two areas mentioned above. Concerning the life history of the fish, we examine the life record, which is shown in the otolth phase, by investigating the relationship between the body length and the opaque core length of the otolith.

Materials and Methods

Sampling

In order to prove the validity of using the otolith phase for age determination, it is necessary to clarify the periodical correlation between the life cycle in terms of years and the formation of the otolith rings. Pond smelt, 21 samples / 1688 individuals (Table 1), in the young-mature stage were caught from January to December , 1991 in Lake Ogawara and from June 1990 to May 1991 off Hachinhe. The samples from Lake Ogawara were caught by a Danish seine fishery, off Hachinohe by angling. Also, immature or mature fish were also caught in lakes located from Hokkaido to the Chugoku region, such as Lakes Abashiri, Kamafusa, Kasumigaura, Suwa Kawaguchi, Yamanaka and Shinji (Table 1, Fig. 1).

Measurement and observation

All of the samples were frozen for preservation in order to observe the otolith

TABLE 1. *Showing Data of Sampling ; Locality, Date, Gear Used and Sample Size*

Locality	Date	Gear used	Sample size
Lake Ogawara	Jan. 19, 1991	Danish seine	120
	Feb. 28, 1991	Set net	120
	Apr. 10, 1991	Set net	120
	May 2, 1991	Danish seine	120
	June 1, 1991	Danish seine	120
	July 10, 1991	Danish seine	23
	Aug. 8, 1991	Danish seine	120
	Sep. 4, 1991	Danish seine	120
	Oct. 2, 1991	Danish seine	120
	Nov. 5, 1991	Danish seine	37
	Dec. 26, 1991	Danish seine	120
Off Hachinohe	June 19, 1990	Pole and line	96
	July 16, 1990	Pole and line	35
	Sep. 27, 1990	Pole and line	71
	Oct. 22, 1990	Pole and line	18
	Nov. 15, 1990	Pole and line	10
	Nov. 24, 1990	Pole and line	20
	Dec. 1, 1990	Pole and line	66
	Jan. 31, 1991	Pole and line	120
	Feb. 1, 1991	Pole and line	84
	Mar. 7, 1991	Pole and line	28
Lake Abashiri	Jan. 21, 1992	Danish seine	117
Lake Kamafusa	Jan. 29, 1992	Pole and line	101
Lake Kasumigaura	Jan. 10, 1992	Set net	120
Lake Suwa	Dec. 23, 1992	Pole and line	20
Lake Kawaguchi	Sep. 5, 1990	Cast net	50
Lake Yamanaka	Sep. 27, 1990	Cast net	47
Lake shinji	Oct. 30, 1991	Set net	30

phase. The standard body length (BL), from the tip of the upper jaw to the end of the caudal skeleton, was measured. Then the standard body length distribution was examined. Otoliths were taken out of the all fish if its sample size was 35 or less. If the sample size was greater than 35, they were taken out of 35 or more individuals were re-sampled, which covered the overall extent of the their body length distribution (Table 2). Among the three types of otolith, sagittae, lapilli and asterisks, sagittae were used to determine age because they are easier to take out and the rings on them are clearer than on the others.

The otoliths were cleaned with 30% sodium hypochlorite solution to remove any substances stuck to them, and cleaned again with desalted water. The

Table 2 *Standard Body Length Composition of each Sample of the Pond Smelt. Number in the paretheses indicates the numbers of the fish*

Locality and sampling date	Standard								
	20-	25-	30-	35-	40-	45-	50-	55-	60-
Lake Ogawara									
Jan. 19, 1991						2 (1)	16 (4)	31 (16)	43 (15)
Feb. 28, 1991						5 (2)	11 (4)	21 (4)	42 (15)
Apr. 10, 1991						7 (3)	18 (8)	9 (3)	14 (7)
May 2, 1991						18 (3)	29 (7)	36 (12)	22 (7)
June 1, 1991						1	26 (7)	54 (14)	33 (12)
July 10, 1991	5 (5)	3 (3)	1 (1)	1 (1)					
Aug. 8, 1991	1	14 (2)	63 (16)	26 (9)	10 (4)	3 (2)			2 (2)
Sep. 4, 1991			3 (2)	35 (11)	67 (19)	9 (1)	4 (2)	1	1 (1)
Oct. 2, 1991				12 (1)	71 (21)	27 (9)	8 (3)	2 (2)	
Nov. 5, 1991					2 (2)	7 (7)	4 (4)	3 (3)	1 (1)
Dec. 26, 1991				1 (1)	8 (5)	31 (20)	38 (14)	28 (4)	11 (3)
Off Hachinohe									
June 19, 1990									
July 16, 1990				9 (9)	5 (5)	1 (1)	2 (2)		
Sep. 27, 1990							2 (2)	3 (3)	12 (9)
Oct. 22, 1990							2 (2)		
Nov. 15, 1990									1 (1)
Nov. 24, 1990									2 (1)
Jan. 31, 1991									5 (1)
Feb. 1, 1991									5 (4)
Mar. 7, 1991						1 (1)	7 (7)	2 (2)	2 (2)
Lake Abashiri Jan. 21, 1992								2	4 (2)
Lake Kamafusa Jan. 29, 1992								3 (2)	9 (6)
Lake Kasumigaura Jan. 10, 1992								3	4 (1)
Lake Suwa Dec. 23, 1992							4 (4)	9 (9)	1 (1)
Lake Kawagachi Sep. 5, 1990						2 (2)	8 (8)	29 (29)	10 (10)
Lake Yamanaka Sep. 27, 1990			7 (7)	11 (11)	3 (3)	3 (3)	5 (5)	8 (8)	6 (6)
Lake Shinji Oct. 30, 1991									

using for age determination by otolith.

body length (mm)										
65-	70-	75-	80-	85-	90-	95-	100-	105-	110-	115-
29 (11)	23 (16)	16 (9)	6 (4)	1 (1)	1 (1)	1 (1)	1 (1)			
45 (15)	34 (14)	14 (4)	3 (2)	1						
11 (5)	13 (3)	7 (1)	9 (4)	3 (1)	12 (6)	9 (4)	7 (3)	1		
7 (1)	2 (2)	4 (2)	2 (2)							
5 (2)	1 (1)									
2 (2)	1 (1)	1 (1)	1 (1)	1 (1)	2 (2)	1 (1)		2 (2)		
		1 (1)								
3 (1)										
	1 (1)	9 (5)	19 (8)	20 (12)	17 (10)	11 (5)	9 (4)	2 (2)	5 (1)	3
			1 (1)	2 (2)	5 (5)	3 (3)	7 (7)			
8 (6)	5 (4)	3 (0)	11 (6)	11 (6)	9 (5)		2 (2)	2 (2)	2 (2)	1 (1)
3 (3)	2 (2)	1 (1)	1 (1)							
3 (3)	3 (3)	3 (3)								
18 (9)	26 (13)	15 (9)	3 (2)	1 (1)	1 (1)					
25 (4)	49 (17)	19 (8)	13 (2)	6 (3)	2				1 (1)	
16 (12)	27 (14)	19 (14)	7 (7)	6 (5)	3 (3)	1 (1)				
3 (3)	3 (3)	3 (3)	2 (2)	5 (5)						
9 (3)	24 (14)	45 (12)	25 (12)	6 (4)	2 (1)					
49 (21)	20 (9)	14 (8)	3 (2)	3 (1)						
8 (3)	12 (5)	13 (6)	9 (4)	14 (5)	17 (7)	10 (5)	13 (5)	5 (3)	10 (4)	2
	1 (1)	5 (5)								
1 (1)										
1 (1)		1 (1)	1 (1)	1 (1)						
			2 (2)	7 (7)	12 (12)	7 (7)	1 (1)	1 (1)		

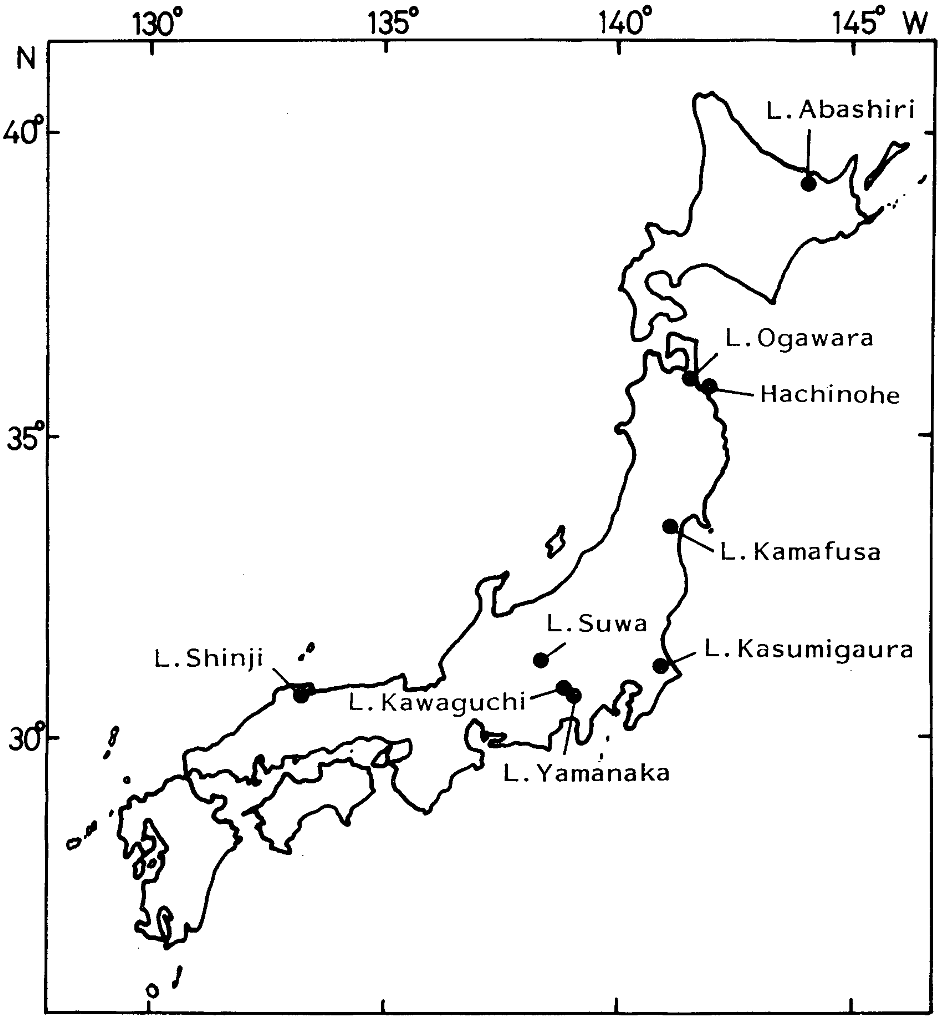

FIG. 1. Map showing sampling locations.

observation and transcription of the otolith phase, immersed in water, were carried out using a stereoscopic microscope with reflceted light. After distinguishing the opaque and translucent parts, the border lines and outer line of the otolith were transcribed with transcription equipment attached to the microscope. The length of the otolith and otolith rings are commonly represented by their radii. In this case, since the core of the otolith was not easily recognizable under this condition, the maximum length of an otolith was defined as the otolith length instead of the radius. This definition was also applied to the length of the rings (Fig. 2). The length of the otolith and rings of the transcribed figure were measured with a ruler.

Estimation of the formation period of the otolith rings

In order to estimate the period of formation of the otolith rings, it is necessary to examine what kind of ring is being formed at the edge of the otolith and how much the ring is growing, after making the relationship between body length and

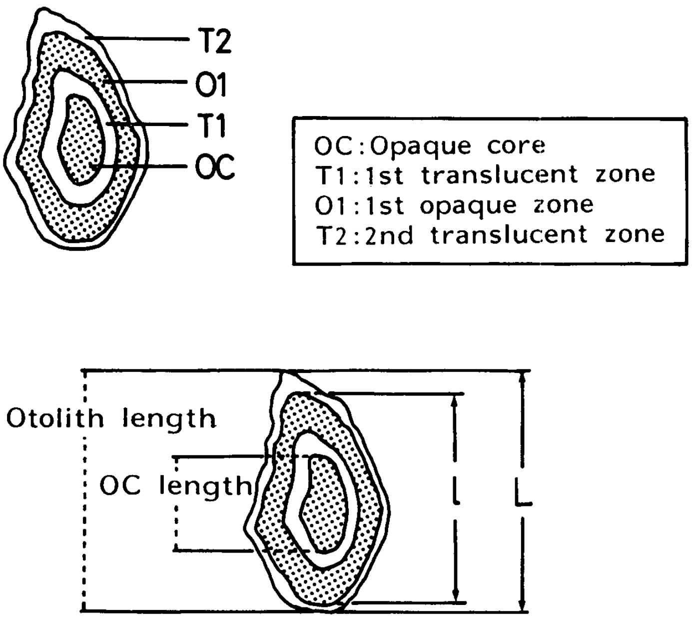

FIG. 2. Definition of opaque core, translucent and opaque zones and otloith and opaque core length in the otolith of pond smelt.
Marginal growth rate = (L − l)/l
L: Otolith length l: ring length

otolith length clear.

First, the regression of the otolith length to the standard body length of pond smelt was examined. Then the seasonally changes in the composition of rings, opaque core or zone and translucent zone, and the marginal growth rate of each ring of the fish in Lake Ogawara and off Hachinohe were examined. When the relative frequency of the number of the otolith with the specific zone in the samples is over 50%, the time is defined as the start of the zone formation. The marginal growth rate was obtained using the formula as below.

$$\text{Marginal growth rate} = (L - l)/l$$

L: Otolith length l: Ring length

Examination of the generality of the method using otolith for age determination

The method mentioned above was applied to the fish taken from other lakes. The locality of the otolith phase and the age determined using otolith phase were examined.

Relationship between otolith phase and life record

Concerning the fish life history, fish live in various environmental conditions according to ontogenotic development and growth. The otolith phase is considered to show the accumulated result of the fish existing in various conditions throughout its life. If it is so, the difference of the hatching period and/or environment would be reflected on the phase on the otolith.

We took notice of the opaque core formed in early life in order to clarify the effect of the difference of life record on the otolith phase. So the average and standard deviation of the opaque core length, after complete formation, by body lenghth class in Lake Ogawara and off Hachinohe were examined. To compare the otolith phase of the fish in Lake Ogawara and off Hachinohe, which would have grown in different environment, the average of the opaque core length of both areas was caliculated.

Results

Growth of the body and the otolith

Viewing the otolith of large enough pond smelt, its shape is like an arrowhead as shown in Fig. 2. The otolith phase of the fish is as below ; the opaque core (OC) is at the central part, and its outer side is surrounded by a narrow translucent zone, the first translucent zone (T1). The wide opaque zone, the first opaque zone (01), is formed around T1. The outer side of 01 is surrounded by a narrow translucent zone, the second translucent zone (T2).

The regressive relationship of otolith length to standard body lenght is shown in Fig. 3. From the results of the least square approach, there is an inflection

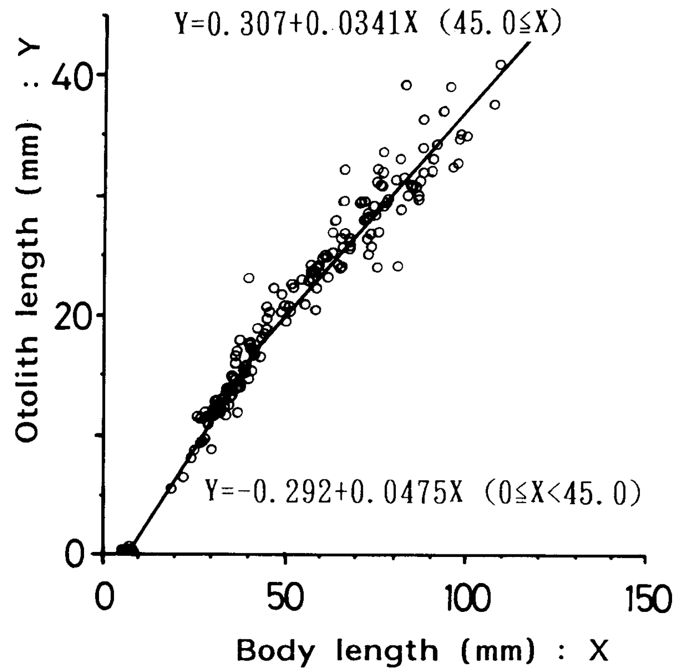

FIG. 3. Relationship between body length and otolith length.
The circles and line in figure show the data and the regression line, respectively.

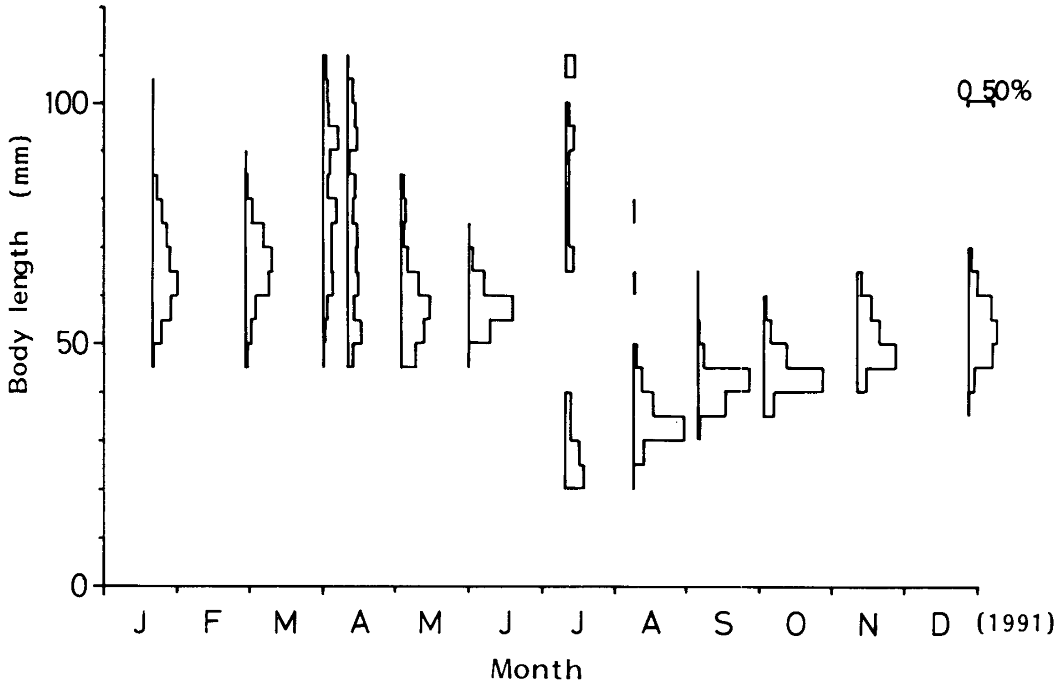

FIG. 4. Seasonal change in the body length distribution of the pond smelt in Lake Ogawara.

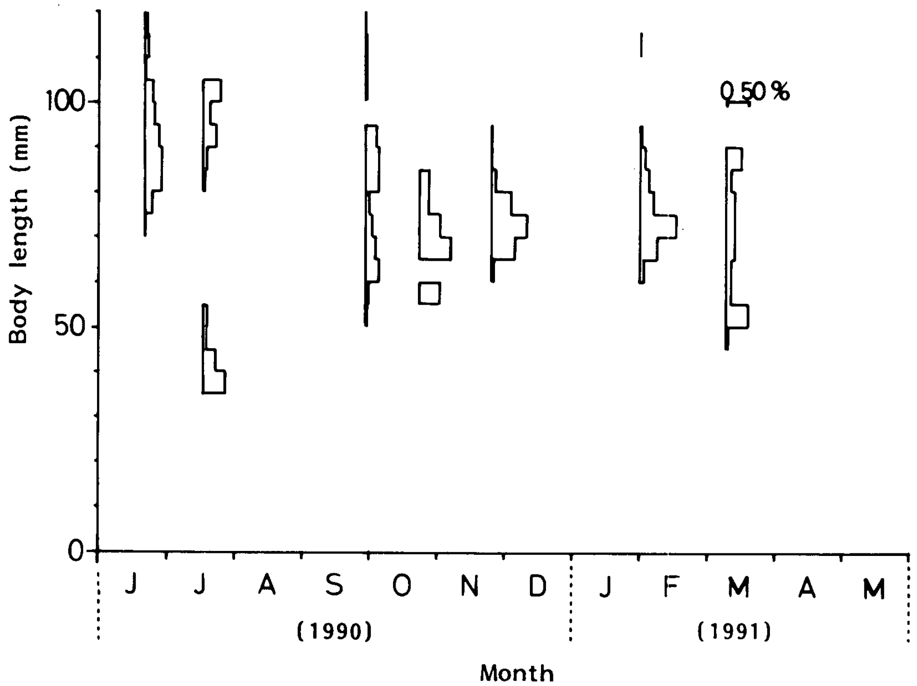

FIG. 5. Seasonal change in the body length distribution of the pond smelt off Hachinohe

point at 45.0 mm BL. The otolith length Y (mm) is presented by the following linear function of the BL X (mm) for each range with 5% statistical significance:

$$Y = -0.292 + 0.0475\ X\ (0 \leqq X < 45.0)$$
$$Y = 0.307 + 0.0341\ X\ (45.0 \leqq X)$$

Figs. 4 and 5 show seasonal chasges in the standard body length distribution of fish sampled in Lake Ogawara and off Hachionhe, respectively. In both areas, after recruitment in July, the fish grow rapidly until September. From October to March they grow slowly.

Season of otolith ring formation in Lake Ogawara and off Hachinohe

The compostion of the rings formed at the edge of the otolith samples are shown in Table 3. Based on them, seasonal change in their frequency percent in Lake Ogawara and off Hachinohe are schematized as Figs. 6 and 7, respectively. Otolith, rings, OC, T1, O1 and T2 are formed in turn. From the definition, in Lake Ogawara, T1 formation begins in August, and O1 in December. T2 forma-

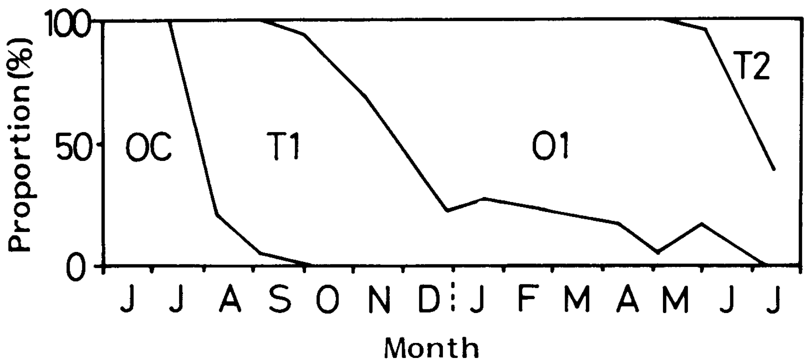

Fig. 6. Seasonal change in the composition of zones at margin of the otloith of pond smelt in Lake Ogawara.

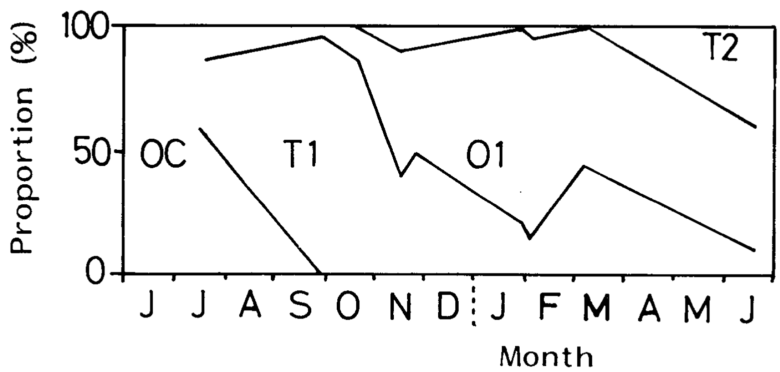

Fig. 7. Seasonal change in the composition of zones at margin of the otolith of pond smelt off Hachinohe

TABLE 3. *Showing Frequency of each Zone at the Margin of Otolith for each Sample. It can be seen what zones is formed at each date by the locality.*

Locality and sampling date		Frequency of zones				
		OC	T1	O1	T2	O2
Lake Ogawara						
Jan. 19,	1991		11	27	11	
Feb. 28,	1991		11	34	6	1
Apr. 10,	1991		3	26		1
May 2,	1991		2	34		
June 1,	1991		9	24	2	1
July 10,	1991	9		6	7	
Aug. 8,	1991	6	24			1
Sep. 4,	1991	2	32			
Oct. 2,	1991		33	2		
Nov. 5,	1991		14	6		
Dec. 26,	1991		11	35		
Off Hachinohe						
June 19,	1990		3	16	11	
July 16,	1990	9	4	3	8	1
Sep. 27,	1990		30	6	5	
Oct. 22,	1990		14	2		
Nov. 15,	1990		4	4	1	
Nov. 24,	1990		18	15	3	
Dec. 1,	1990		15	16	2	
Jan. 31,	1991		8	28		
Feb. 1,	1991		5	43	3	
Mar. 7,	1991		11	16		
Lake Abashiri						
Jan. 21,	1992			15		
Lake Kamafusa						
Jan. 29,	1992		38	3	7	
Lake Kasumigaura						
Jan. 10,	1992		3	45		
Lake Suwa						
Dec. 23,	1992		6	8	6	
Lake Kawaguchi						
Sep. 5,	1990		17	2		
Lake Yamanaka						
Sep. 27,	1990		9	1		
Lake shinji						
Oct. 30,	1991		18	12		

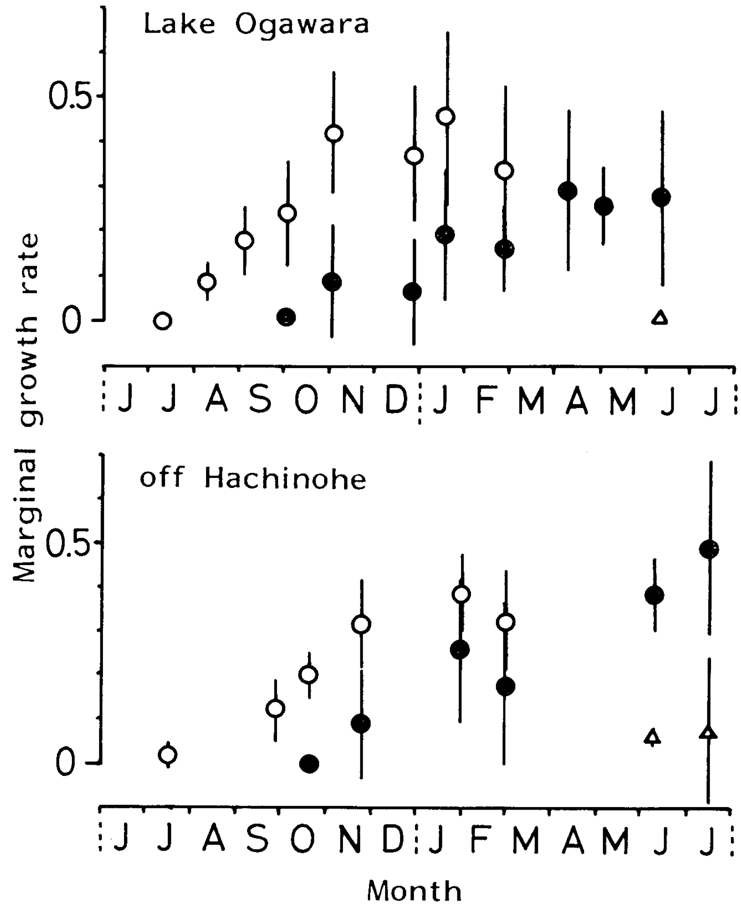

FIG. 8. Seasonal change in means and standard deviations of the marginal growth rates of each zone.
The open and solid circle and open triangle in figures indicate the means of the first translucent and opaque zones and second translucent zone, respectively. The all bars in figures show their standard deviations.

tion is isitiated the next July. Off Hachiohe, the formation season of each ring is similar to the lake, that T1 formation begins in August, and O1 in November.

Next, to examine the growth of each ring, seasonal changes in the marginal growth rate of each zone of the fish in Lake Ogawara and off Hachinohe are shown in Fig. 8. There seems to be regularity that T1 begins to grow gradually in July-August, and finishes growing in November, followed by the start of O1 formation. Formation of T2 is initiated the next June. Therefore, fish with the second translucent zone (T2) on their otoliths are considered to be one year old, supposing that the spawning season of the fish is March-April.

Based on the results mentioned above, the age determination method using otolith phase was applied to the fish sampled from other lakes. The similarity of the otolith phase that opaque and translucent rings are formed sequentially was confirmed in other lakes also, though clear rings can not be observed for about 50 percent of the fish in Lake Abashiri. So standard body length distributions and ages estimated by using otolith phase of samples from other lakes and part of the samples from Lake Ogawara and off Hachinohe were examined (Fig. 9).

Although all fish in Lakes Abashiri, Kasumigaura, Yamanaka, Kawaguchi and Shinji do not have the second translucent zone (T2) on their otoliths, about 15% of the fish in Lake Kamafusa and 30% of the fish in Lake Suwa have T2. They are estimated as age one. In Lake Suwa, there are two modes in the body length distributions, the group with mode at 75-80 mm BL is estimated at one year, while another one with mode at 55-60 mm BL is estimated at under one year. Since its distribution of each age in completely separate, the age composition corresponds well to the body length composition.

Relationship between opaque core length and body length

To clarify the relationship between the body length and the length of OC with T1 outside, body length distributions and the means and standard deviations by body length class for the samples of Lake Ogawara on September 4 and October 2 are shown in Fig. 10. The mean lengths of OC range from about 1.1 to 1.7 mm. The size of OC, after complete formation, was proportional to the body length for

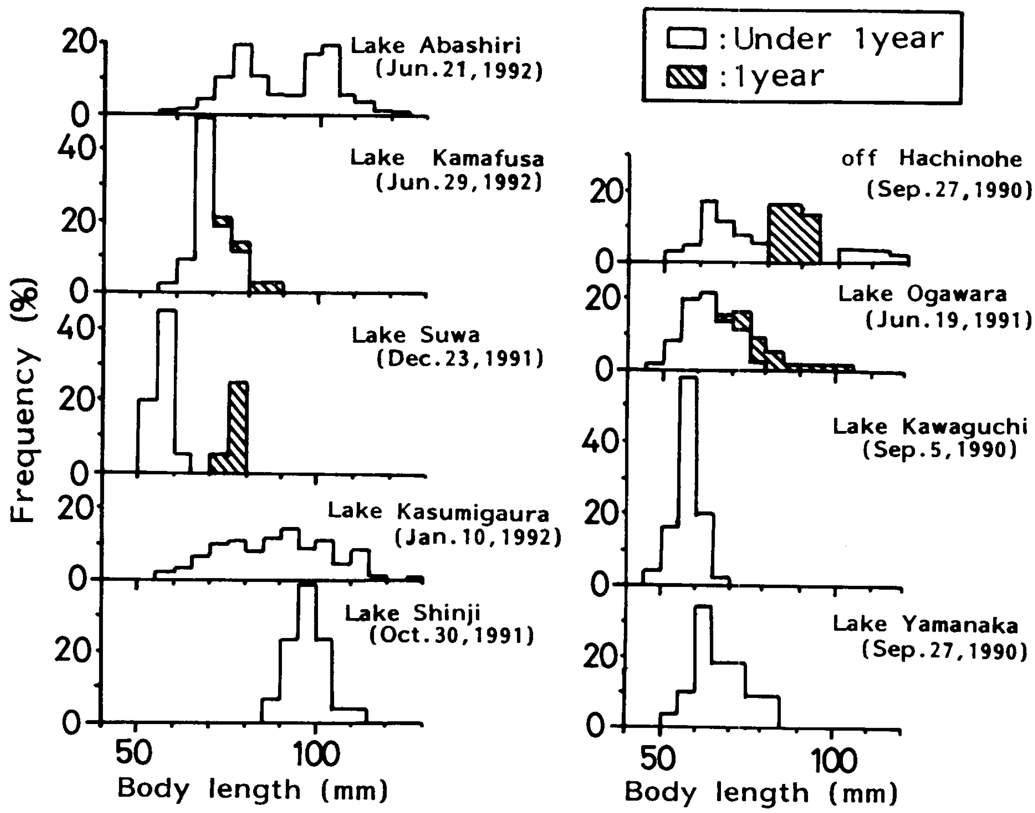

FIG. 9. Body length distribution of each age estimated by otolith phase in various areas.

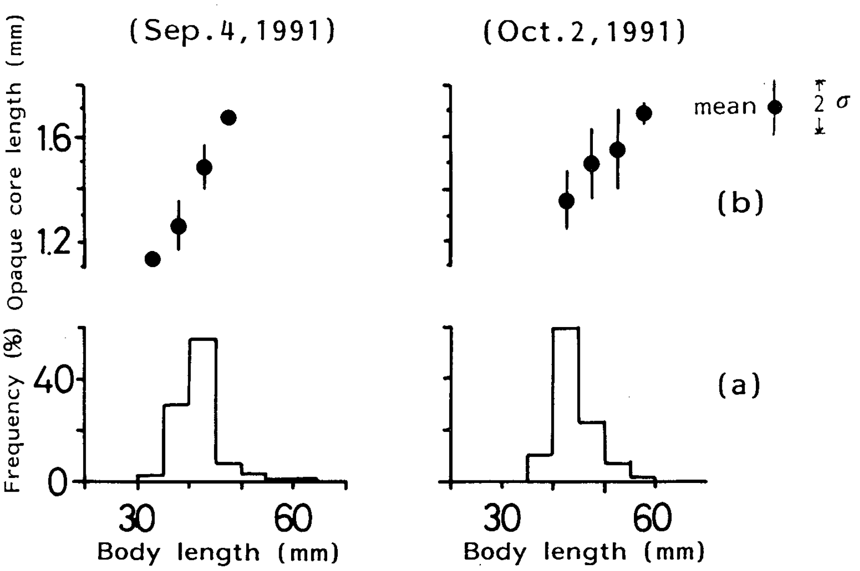

FIG. 10. Body length distribution (a) and corresponding means of opaque core length (b) for the sample of Lake Ogawara.
The all bars in figures show their standard deviations.

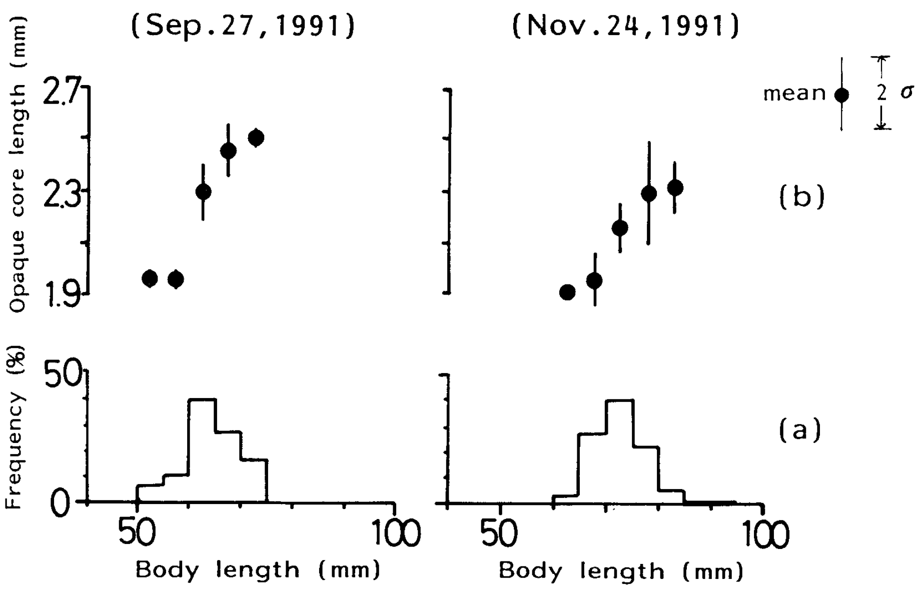

FIG. 11. Body length distribution (a) and corresponding means of opaque core length (b) for the sample from off Hachinohe.
The all bars in figures show their standard deviations.

the two months after September. Applying this analysis to samples from off Hachinohe, body length distributions and the means and standard deviations by body length class for the samples from off Hachinohe on September 27 and November 15 are shown in Fig. 11. The mean lengths of OC range from 1.9 to 2.5 mm. A similar trend with the fish in the lake, in which the size of OC was proportional to the body length, is observed.

Then, comparing the averages of the samples from Lake Ogawara and off Hachinohe, while it is 1.42 mm in the lake, off Hachinohe it in 2.31 mm is Septemper and 2.16 in November, so there is a difference of 0.7-0.9 mm between them. On the other hand, comparing the mode of the body length distribution of samples from these areas caught in almost the same period, while it is 40-45 mm BL on October 2 in the lake, it is 60-65 mm BL on September 27 off Hachionohe. The fish off Hachinohe are larger than those in Lake Ogawara by about 20 mm in the season. This difference is considered to reflect the growth in different environments for the fish, supposing that their hatching period should be almost the same.

Discussion

Property of age determination using the otolith phase

In this study we have found that the otolith phase of pond smelt is useful for age determination. Specifically, we judged that fish with the 2nd translucent zone on their otoliths are one year old, in Lake Ogawara and off Hachinohe. Moreover, this method can be applied to fish from other lakes, would in general be useful for fish inhabiting various geographical conditions.

In many teleost fishes the translucent zone is known to be formed in the growth-stagnant season and/or in the spawning season. However, regarding the pond smelt in Lake Ogawara and off Hachinohe, growth is not stagnant from August to November when the translucent zones are being formed. In addition, the spawning season in usually March-April (6-8). Accordingly, in order to clarify this subject, it seems necessary to examine the specific uniqueness of the mechanism of otolith ring formation in the growth process, relating environmental conditions such as water temperature and light intensity.

Relationship between otolith phase and life record

We have shown that the opaque core length, after complete formation, is almost proportional to the body length from August to October. Since the opaque core has already been formed in July-August, the OC length would correspond to the growth until July-August, after hatching. Therefore it shows the growth difference of the fish caused by environmental differences, that the average of the opaque core length of the fish from off Hachiohe is 0.7-0.9 mm larger than those in Lake Ogawara. It would be useful for analyzing the difference in past growth of subpopulations, by using the regularity of that the opaque core length's correspondence to the growth.

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