Journal: Fisheries Science

Title:

Comparison between surface-reading and cross-section methods using sagittal otolith for age determination of the marbled sole *Pseudopleuronectes yokohamae*

Authors:

Jeong-Hoon LEE[1,2*], Keita KODAMA[1], Gen KUME[3], Masaaki OYAMA[1], Satoshi KATAYAMA[4], Yuji TAKAO[5] AND Toshihiro HORIGUCHI[1]

Affiliations:

[1]Research Center for Environmental Risk, National Institute for Environmental Studies, Tsukuba, Ibaraki 305-8506, Japan; [2]Graduate School of Science and Technology, Nagasaki University, Nagasaki, Nagasaki 852-8521, Japan; [3]Landcare Research, Lincoln 7640, New Zealand; [4]Coastal Fisheries and Aquaculture Division, National Research Institute of Fisheries Science, Nagai, Yokosuka 238-0316, Japan; [5]Faculty of Environmental Studies, Nagasaki University, Nagasaki, Nagasaki 852-8521, Japan

---

[*] **Corresponding author**
Tel: +81-29-850-2852. Fax: +81-29-850-2870. E-mail: lee.jeong.hoon@nies.go.jp

ABSTRACT

To find an appropriate method for age determination in the marbled sole *Pseudopleuronectes yokohamae* in Tokyo Bay, Japan, sagittal otoliths of 1343 individuals were observed by surface-reading and cross-section methods and the results were compared. Opaque zones occurred once a year and were regarded as annuli in both methods. The surface-reading method sometimes provided a lower count of the number of annuli than the cross-section method, and the frequency of this discrepancy was highest in older fish (males above 5 years, females above 4 years). The oldest female fish was estimated to be age 10 years by the cross-section method but 8 years by the surface-reading method. The cross-section method could provide a more accurate estimate of age and is therefore likely to be indispensable to estimations of longevity. In contrast, the surface-reading method is superior in terms of cost and time efficiency but is likely to underestimate the ages of older fish. However, growth equations based on age estimated by the surface-reading method were sufficiently accurate if males ≥ 5 years and females ≥ 4 years were combined as specific, single age groups of 5+ and 4+, respectively.

KEY WORDS

Age determination, Cross-section method, Growth, Otolith, *Pseudopleuronectes yokohamae*, Surface-reading method, Tokyo Bay

INTRODUCTION

The marbled sole *Pseudopleuronectes yokohamae* distributed in Japan from southern Hokkaido to Oita Prefecture in Kyushu, as well as in the Yellow Sea, the Bohai Sea, and the northern part of the East China Sea [1]. In Tokyo Bay, Japan, *P. yokohamae* is the dominant species in the megabenthic assemblage and is a highly valued fish species exploited by commercial fishers in the bay [2, 3]. However, despite the importance of *P. yokohamae* as a commercial resource, the abundance of this species has markedly decreased since the late 1980s. According to statistics from the Shiba Branch of Yokohama City Fisheries Cooperative Association, the annual catch of *P. yokohamae* in Tokyo Bay exceeded approximately 500 t in the mid 1980s, but by the 2000s it had decreased substantially to around 50 t. Therefore, there is a need for elucidation of the cause of the decline of *P. yokohamae* for the recovery of the stock in the bay.

To understand mechanisms of the population dynamics of *P. yokohamae* in Tokyo Bay, we have to clarify the critical life-history stage (or stages) that determine the year-class strength, and identify factors that affect mortality during the critical life-history stage, as well as consideration of changes in life history traits (e.g., reproduction), which may contribute to the population decline. In studying these matters, age and growth is one of important life history traits [4, 5].

For the estimation of age and growth, establishment of an accurate aging procedure is indispensable. Age and growth of *P. yokohamae* have previously been investigated in several regions around Japan [3, 6-9]. These studies applied the surface-reading method for age determination, in which annuli visible on the surface of the sagittal otolith are examined. Recently, however, it has been reported that counting annuli on a cross-section of the otolith ("cross-section method") is more accurate than the surface-reading method for estimating the age of fish [10-17]. In *P. yokohamae*, determination of age by the cross-section method has not yet been reported. In this study, we compared the surface reading method and the cross-section method for age determination of *P. yokohamae* in Tokyo Bay in order to determine which method was more appropriate.

MATERIALS AND METHODS

For the comparison of aging methods, 1022 specimens (453 males, 569 females) were obtained from fishermen's unions at Yokosuka (gill net fishery) and Shiba and Koitogawa (bottom trawl fisheries; Fig. 1). In addition, 321 specimens (155 males, 166 females) were collected from Tokyo Bay by bottom trawl surveys in February, May, August, and October in 2003; February, May, August, and November in 2006 and 2007; and February 2008 (Fig. 1). Detailed methods of the bottom trawl surveys have been described by Kodama *et al.* [2]. The specimens were brought back to the laboratory without any fixation. Standard length (SL) was measured to the nearest 1 mm. Sex was determined by external observation and histological examination of the gonad. Histological examination was conducted according to the procedure given by Kume *et al.* [3]. Sagittal otoliths were dissected out, cleaned with ethanol (70%), and stored dry in well plates for later processing.

The opaque and translucent zones of the sagittal otoliths were observed by using two age determination methods (surface-reading method and cross-section method). For the surface-reading method, blind-side otoliths were used for observation from the following reasons; firstly, the annuli on the blind side otoliths are more visible along various direction of the axis compared with those on the ocular side otoliths of *P. yokohamae*, as reported in other flatfish species [18, 19]. This would facilitate reading annuli on the otolith by checking growth zones in various directions, which contributes increasing accuracy of the reading. Secondly, crystallization, by which annuli become invisible, has sometimes been observed in ocular side otoliths of *P. yokohamae* in Tokyo Bay as observed in Pacific halibut *Hippoglossus stenolepis* [20]. Meanwhile, no crystallization has occurred in blind side otoliths in the present study. The otoliths were immersed in 50% glycerol solution and observed under reflected light against a dark background using a stereomicroscope at 16× magnification (SZX-ILLB100, Olympus Optical Co., Ltd, Japan). After completing the examination of all otoliths by the surface-reading method, the same otoliths were examined by the cross-section method. In the observation by the surface-reading method, the surfaces of some blind-side otoliths (n = 30) were ground with sandpaper until the opaque and transparent zones were clearly visible. Because these blind-side otoliths were not useable for the cross-section method, we used the ocular-side otoliths instead. There was no difference in the number of opaque rings between these blind- and ocular-side otoliths (n = 30), therefore, we assumed both otoliths have identical number of annulus. Otoliths were placed on clay and embedded in polyester resin, and each resin block was sectioned transversely through the core at a thickness of 300 μm by using a saw microtome (SP1600, Leica Microsystems, Heerbrugg, Switzerland). The sectioned otoliths were then mounted on glass slides using sticky wax and ground almost to the core with lapping film sheets (60, 30 and 12 μm, 3M, Tokyo, Japan). To enhance the contrast between the opaque and translucent regions, sectioned otoliths were etched with 0.1 N

HCl solution for 30 s, and then covered with transparent enamel. The sectioned otoliths were observed through a microscope at 40× magnification under transmitted light (BX40 F-3, Olympus Optical Co., Ltd, Japan).

All otoliths were examined twice each by two readers who had no knowledge of each fish's SL or sex. To examine the degree of reproducibility of the aging results, the percent agreement (PA) and average percent error (APE) were calculated for both methods. PA was the ratio of the number of agreements between results to the total number of readings made by two readers. APE, which was used to assess the consistency of the results of the two counts by each reader, was calculated by using the equation given by Beamish and Fournier [21]:

$$\text{APE} = \frac{1}{N}\sum_{j=1}^{N}\left[\frac{1}{R}\sum_{i=1}^{R}\frac{|\ X_{ij} - X_j\ |}{X_j}\right] \times 100$$

where N is number of fish examined, R is number of age counts for the jth fish (here $R = 2$), and X_{ij} and X_j are the ith age determination of the jth fish and mean age of the jth fish, respectively. Haas and Recksiek [12] and Powers [22] suggest that APE values below 10% are within an acceptable level of reproducibility for the results of an aging method used in stock assessment.

To determine the age of *P. yokohamae*, we assumed that the age of all individuals increased by 1 year on 1 January, because the spawning season of *P. yokohamae* in Tokyo Bay lasts from late November to February [3]. The age of each individual was calculated by the following equation:

$$t = O + (M - 1)/12$$

where t, O, and M are the age (years), number of opaque zones on the otolith, and month of sampling, respectively. The innermost opaque region was excluded from the counting of opaque zones. When there was a difference in the age estimated by the two readers, those data were excluded from the growth analysis.

To estimate the growth of *P. yokohamae* in Tokyo Bay, the von Bertalanffy growth equation was fitted to mean SL at each age separating by the two aging methods:

$$SL_t = SL_\infty\{1 - \exp[-K(t - t_0)]\}$$

where SL_t is standard length at age t, and SL_∞, K, and t_0 are the asymptotic length, the growth coefficient, and the hypothetical age when standard length would be zero, respectively. We did not use back-calculated SL for the growth equation. Parameters of the von Bertalanffy growth equation were estimated by using the Solver on MS-Excel (Microsoft, Redmond, WA, USA), which implements a quasi-Newton method for nonlinear least-squares parameter estimation [23, 24]. To examine differences in the growth equations between the sexes or between different age-determination methods, we conducted an *F*-test in accordance with the method of Akamine [25].

RESULTS

Opaque and translucent zones are clearly visible on the blind-side sagitta observed by both the surface-reading and the cross-section methods (Fig. 2a, b). When viewed from the external or internal side of the fish, the otolith grows almost elliptically with faster increment toward the antero-posterior direction than toward the dorso-ventral direction, and the focus remains in the central region (Fig. 2a). On the other hand, observation from the anterior or posterior side of the fish showed that otolith grows faster toward the internal side than the external side (Fig. 2b). As the otolith grows, the width of the growth band (i.e. a pair of adjacent opaque and translucent zones) becomes narrower (Fig. 2a, b), and the direction of the growth along the dorso-ventral axis curves to the internal side of the fish (Fig. 2b).

Opaque and translucent regions for age estimation were observable in 99.8% of samples by both the surface-reading and the cross-section methods. PA by the two readers was recorded as 99.6% for the surface-reading method and 99.7% for the cross-section method. For APE, the values for the surface-reading and cross-section methods by reader 1 were 0.4% and 0.2%, and by reader 2 were 0.2% and 0.1%, respectively.

We determined the monthly changes in the percentage occurrence of otoliths with opaque zones at their margins (Fig. 3). Data for both sexes were combined, because no significant differences in the percentage occurrence of individuals with opaque zones at the outer edge of the otolith were found between males and females by either aging method (*G*-test: surface-reading method, $G = 0.41$, $P = 0.52$; cross-section method, $G = 0.36$, $P = 0.55$). Also, there were no significant differences between the surface-reading method and cross-section method in terms of opaque edge in sex (*G*-test: male, $G = 0.001$, $P = 0.98$; female, $G = 0.02$, $P = 0.89$). The opaque zone at the outer edge of the otolith appeared from January to July. The peak percentage occurrence of the opaque zone was observed in April. No individuals with opaque zones at their otolith edges were found between August and December. These results suggest that the opaque zone is formed only once a year. Therefore, we regarded the opaque zone as a suitable annulus for age determination. We assumed the region from the innermost opaque zone to the outer margin of the first translucent zone as indicating 0 years of age, and the region from the inner margin of the second opaque zone to the outer margin of the second translucent zone as 1 year of age, and so on.

The surface method sometimes provided fewer counts in the number of annuli than did the cross-section method. The oldest fish in this study was estimated to be 10 years old by the cross-section method, whereas it was 8 years old by the surface-reading method using the same otolith (Fig. 2a, b). In the present study, we evaluated the degrees of discrepancy in ages determined by the surface-reading method and the cross-section method (Fig. 4). For males, the frequency of 1-year age discrepancies between the two methods ranged from 0% to 7.5% in individuals aged 0 to 4 years, whereas it was 100% in 5- and 6-year-olds, although only one individual was examined in each of the latter age groups. In females, the frequency of 1-years age discrepancies was 0% to 28.6% in individuals aged 0 to 5 years, whereas it was 67% in 6-year-old females. A 2-year age discrepancy was found in one 10-year-old female.

Parameters of von Bertalanffy growth equations estimated for males and females for the two aging methods are shown in Table 1. Significant differences in growth equations were found between males and females within each aging method (F-test: surface-reading method, F (degree of freedom; 3, 88) = 18.84, $P < 0.001$; cross-section method, F (3, 91) = 19.78, $P < 0.001$). Therefore, the von Bertalanffy growth curves were separated by sex. In contrast, the growth equations for males and females did not differ significantly between the two aging methods (F-test: males, F (3,

88) = 0.09, $P > 0.05$; females, F (3, 91) = 0.41, $P > 0.05$).

Since proportion of discrepancy in estimated age between the surface-reading and the cross-section methods increased in older fish (Fig. 4), we combined males aged 5-years and older and females age 4-aged and older into single age categories of 5+ and 4+, respectively, in the surface-reading method, and tried to make new growth equations. The new equations derived using ages based on the surface-reading method and including these age categories (male: $L_\infty = 301.5$ mm, $K = 0.545$ per year, $t_0 = -0.407$ year; female: $L_\infty = 349.7$ mm, $K = 0.497$ per year, $t_0 = -0.300$ year) are also not significantly different from those derived using ages based on the cross-section method (male: F (3, 89) = 0.04, $P > 0.05$; female: F (3, 83) = 0.37, $P > 0.05$).

Growth for both males and females was similar up to age 2 years; SL on the growth curves at age 2 years was about 230 mm for males and 240 mm for females (Fig. 5). However, females aged 3 years or older attained a larger SL than males of the same age. The growth rates of both sexes decelerated as fish aged. The maximum SL we recorded for *P. yokohamae* males was 314 mm, and for females, 400 mm.

DISCUSSION

We conducted age determinations by two different methods, a surface-reading method and a cross-section method. The PAs for the number of annuli counted by two readers were high. In addition, the APEs for the two readers for the two methods were low (less than 1%)—well below the threshold value (10%) for consistency of the results [12, 22]. Therefore, these results suggest that both methods provide consistent counts of the annuli of *P. yokohamae* among readers.

We observed discrepancies in age between the two aging methods. The discrepancies were found in individuals aged 3 years or more, and the proportion of discrepancies increased with age, especially from 5 years for males and 4 years for females. Underestimations of age by the surface-reading method compared with the cross-section method have been reported in many species [10-17]. For example, the maximum discrepancy in age reached 4 years for *Pseudopleuronectes americanus* [12], 9 years for *Parapristipoma trilineatum* [17] and 23 years for *Sebastes vulpes* [15]. Underestimations in the number of annuli by the surface-reading method were probably related to the developmental patterns of the otolith. In general, there is a positive relationship between somatic growth rate and otolith increments [6, 7]. However, Beamish [10] reported that otoliths of older individuals of *Merluccius productus* appeared to increase in the thickness, but not in the height or length. Similar result has been reported for *Paralichthys olivaceus* [16]. From the observation of the transverse section of sagittal otolith of *P. yokohamae*, we also found that the otolith develops in a curve toward the internal (proximal) side of the fish with age (Fig. 2). Provided that the direction of the growth in other part of the otolith (e.g., the anterior and posterior region) is the same, two or more annuli could seem to overlap each other when observed by the surface-reading method. These characteristics might have caused the underestimation in the count of annuli by the surface-reading method. Therefore, the use of the cross-section method would be indispensable in estimating the age of older individuals or the longevity in *P. yokohamae*. In the present study, we observed only dorso-ventral section of the otoliths in the cross-section method. The most appropriate direction of the sectioning needs to be clarified in future.

The surface-reading method could have advantages over the cross-section method in terms of efficiency in time and cost, although it sometimes leads to age underestimation in older fish. However, the surface-reading method could be still applicable to analysis of the growth of *P. yokohamae*, because there were no significant difference in the growth curves between the two different aging methods even in the case where combined age groups were applied in the surface-reading method (5+ and 4+ for males and females, respectively, in which proportion of discrepancies in estimated age between the two aging methods increased). However, the number of specimens of older fish showing poor accuracy of the age estimation by the surface-reading method was very small in the present study. Therefore, we need to reconsider the validity of the surface-reading method in the case where the number of older fish specimens is increased.

In present study, we could not obtain large number of specimens for 5 years of age and older, resulting in some uncertainty in the reliability of the results on age determination for old individuals. The present conclusion regarding the superiority of the cross-section method, however, might be probable because (1) the direction of the growth of the otolith curves as the fish become older, resulting in difficulties in reading annuli on the periphery of the otolith by the surface-reading method as described earlier, and (2) when the discrepancies in ages were found, the numbers of annuli by the surface-reading method were never higher than those by the cross-section method. To improve the reliability of results in the present study, we need to collect more specimens of old age fish, although it might be difficult to collect sufficient number of old individuals from Tokyo Bay under the current low stock-size condition.

ACKNOWLEDGMENTS

We are grateful to Mr. Tatsuya Isshiki of the Kanagawa Prefectural Fisheries Technology Center for providing some of the samples. We would also like to thank the commercial fishermen of Yokosuka, Shiba, and Koitogawa for their help in sampling.

REFERENCES

1. Sakamoto K (1984) Pleuronectidae righteye flounders. In: Masuda H, Amoaoka K, Araga C, Ueno T, Yoshino T (eds) The Fishes of the Japanese Archipelago, Tokai University Press, Tokyo
2. Kodama K, Aoki I, Shimizu M, Taniuchi T (2002) Long-term changes in the assemblage of demersal fishes and invertebrates in relation to environmental variations in Tokyo Bay, Japan. Fish Manag Ecol. 9:303−313
3. Kume G, Horiguchi T, Goto A, Shiraishi H, Shibata Y, Morita M, Shimizu M (2006) Seasonal distribution, age, growth, and reproductive biology of marbled sole *Pleuronectes yokohamae* in Tokyo Bay, Japan. Fish Sci. 72:289−298
4. Beamish RJ, McFarlane GA (1983) The forgotten requirement for age validation in fisheries biology. Trans Am Fish Soc. 112:735−743
5. Campana SE (2001) Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J Fish Biol. 59:197−242
6. Solomon G, Sano M, Shimizu M, Nose Y (1987) Age and growth of the pleuronectid flounder *Limanda yokohamae* in Tokyo Bay, Japan. Nippon Suisan Gakkaishi. 53:711−716
7. Park JS (1988) A study on the pleuronectid flounder *Limanda yokohamae* population in Tokyo Bay. PhD Dissertation, University of Tokyo, Tokyo (in Japanese)
8. Shafieipour MM, Takahashi T, Ishino K, Takatsu T, Nakatani T (1999) Age and growth of marbled sole *Pleuronectes yokohamae* in Kikonai Bay, Hokkaido, Japan.

Iran J Fish Sci. 1:5−21

9. Tanda M, Gorie S, Nakamura Y, Okamoto S (2008) Age and growth of marbled sole *Pleuronectes yokohamae* in Harima Nada and Osaka Bay, the Seto Inland Sea, Japan. Nippon Suisan Gakkaishi. 74:1−7 (in Japanese with English abstract)
10. Beamish RJ (1979) Differences in the age of Pacific hake (*Merluccius productus*) using whole otoliths and sections of otoliths. J Fish Res Board Can. 36:141−151
11. Hayashi A, Dotsu K, Ohta M (1995) Reliability of surface reading methods to count otolith opaque zones on aging in marbled rockfish *Sebastiscus marmoratus* compared with the cutaway reading method. Nippon Suisan Gakkaishi. 61:1−5 (in Japanese with English abstract)
12. Haas RE, Recksiek CW (1995) Age verification of winter flounder in Narragansett Bay. Trans Am Fish Soc. 124:103−111
13. Peltonen H, Raitaniemi J, Parmanne R, Eklund J, Nyberg K, Halling F (2002) Age determination of Baltic herring from whole otoliths and from neutral red stained otolith cross sections. ICES J Mar Sci. 59:323–332
14. Dwyer KS, Walsh SJ, Campana SE (2003) Age determination, validation and growth of Grand Bank yellowtail flounder (*Limanda ferruginea*). ICES J Mar Sci. 60:1123−1138
15. Sekigawa T, Takahashi T, Takatsu T, Nishiuchi S, Sasaki M, Shiokawa F (2003) Age and growth of *Sebastes vulpes* in the coastal waters of western Hokkaido, Japan. Fish Sci. 69:575−580
16. Atsuchi S, Masuda Y, Akamo H, Iori K (2004) Age and growth of Japanese flounder *Paralichthys olivaceus* from the coastal waters around Kagoshima Prefecture, Japan, using transverse sections of otoliths. Nippon Suisan Gakkaishi. 70:714−721 (in

Japanese with English abstract)

17. Doiuchi R, Kokubo T, Ogawa M (2007) Age and growth of threeline grunt *Parapristipoma trilineatum* along the south-western coast of Kii Peninsula, Japan. Fish Sci. 73:777−783
18. Yabuki K (1989) Age determination of yanagimushigarei *Tanakius kitaharai* (Pleuronectidae) from otoliths in the sea of Japan off Kyoto Prefecture. Nippon Suisan Gakkaishi. 55:1331–1338
19. Polat N, Bostanci D, Yilmaz S (2001) Comparable age determination in different bony structures of *Pleuronectes flesus luscus* Pallas, 1811 inhabiting the Black Sea. Turk J Zool. 25:441–446
20. Forsberg JE (2001) Aging manual for Pacific halibut: procedures and methods used at the international Pacific halibut commission (IPHC). IPHC, Seattle
21. Beamish RJ, Fournier DA (1981) A method for comparing the precision of a set of age determinations. Can J Fish Aquat Sci. 38:982−983
22. Powers JE (1983) Some statistical characteristic of ageing data and their ramifications in population analysis of ocean pelagic fish. NOAA Tech Rep NMFS. 8:19−24
23. Tokai T (1997) Maximum likelihood parameter estimates of a mesh selectivity logistic model through Solver on MS-Excel. Bull Jpn Soc Fish Oceanogr. 61:288−298 (in Japanese with English abstract)
24. Gorie S (2001) Estimation of the parameters in von Bertalanffy’s growth formula by MS-Excel. Suisanzoshoku. 49:519−527 (in Japanese with English abstract)
25. Akamine T (2004) Statistical test and model selection of fish growth formula. Bull Jpn Soc Fish Oceanogr. 68:44−51 (in Japanese with English abstract)

FIGURE CAPTIONS

Fig. 1 Locations of sampling stations used for bottom trawl surveys [2] (black circles), and three fisheries ports (black triangles) in Tokyo Bay, Japan

Fig. 2 Example of different aging results obtained for the same sagittal otolith of *Pseudopleuronectes yokohamae* in Tokyo Bay by (a) surface-reading method and (b) cross-section method. White dots indicate annuli and broken line indicates the sectioning line. Bars are 1 mm

Fig. 3 Monthly changes in percentage occurrences of opaque (■) and translucent (□) zones at otolith edges in *Pseudopleuronectes yokohamae* in Tokyo Bay, as estimated by (a) surface-reading method and (b) cross-section method. n indicates sample size

Fig. 4 Frequencies of occurrence of discrepancies in age for (a) males and (b) females as determined by the surface-reading method and cross-section method. Age discrepancy was obtained by subtracting the age estimated by the surface-reading method from that estimated by the cross-section method. n indicates sample size

Fig. 5 The von Bertalanffy growth curves for (a) male and (b) female *Pseudopleuronectes yokohamae* in Tokyo Bay. Symbols and vertical bars denote mean standard length and standard deviation, respectively, at each age. Solid and dashed lines indicate growth curves based on ages as determined by the surface-reading method and the cross-section method, respectively

Table 1 Parameters of the von Bertalanffy growth curve for males and females of *Pseudopleuronectes yokohamae* in Tokyo Bay, based on ages as estimated by two age determination methods

Method	Sex	L_∞ (mm)	K (per year)	t_0 (years)
Surface-reading				
	Males	301.1	0.590	− 0.245
	Females	449.0	0.257	− 0.826
Cross-section				
	Males	305.6	0.533	− 0.355
	Females	409.9	0.321	− 0.634

Fig. 1

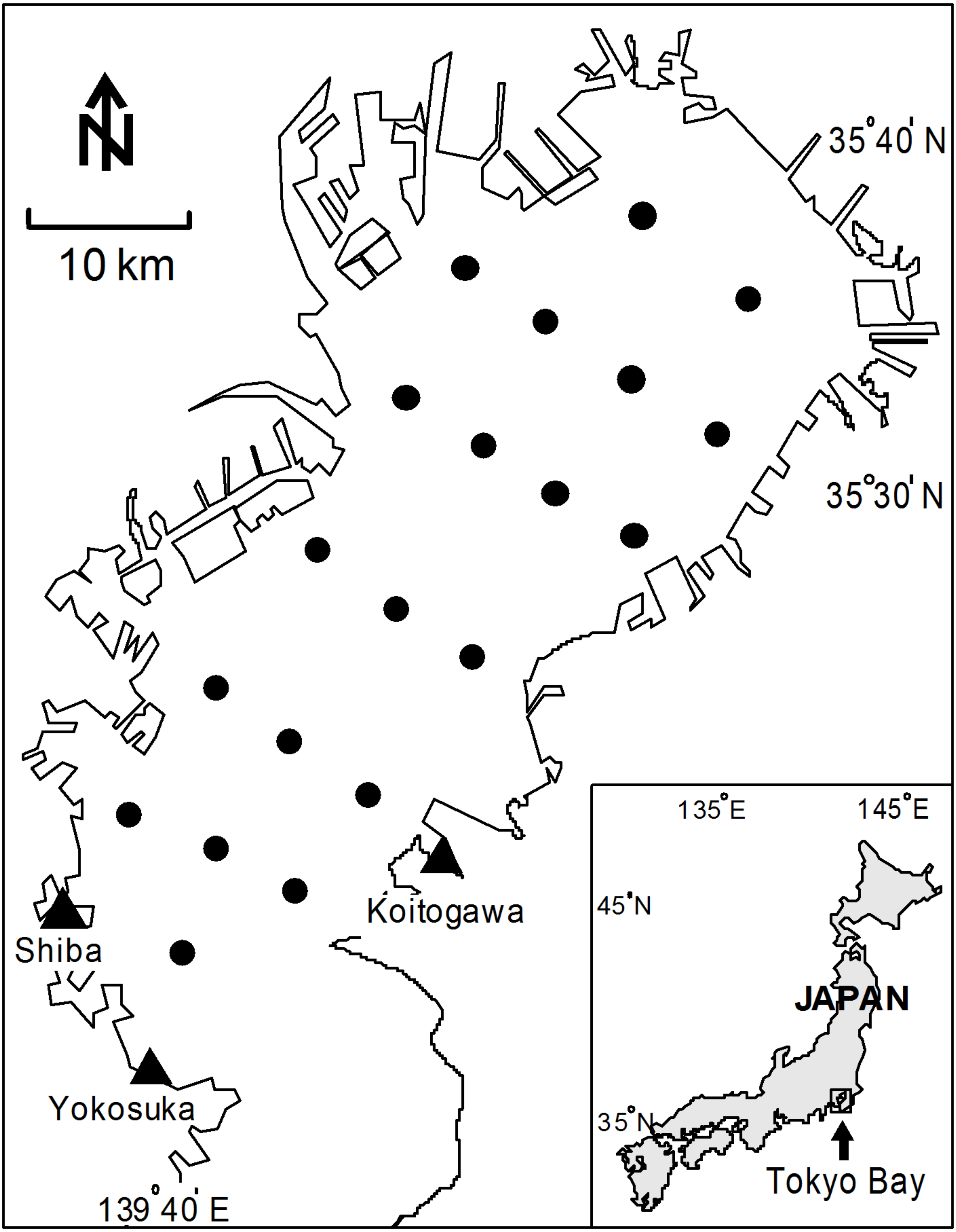

N
10 km
35°40' N
35°30' N
Koitogawa
Shiba
Yokosuka
139°40' E
135°E
145°E
45°N
35°N
JAPAN
Tokyo Bay

Fig. 2

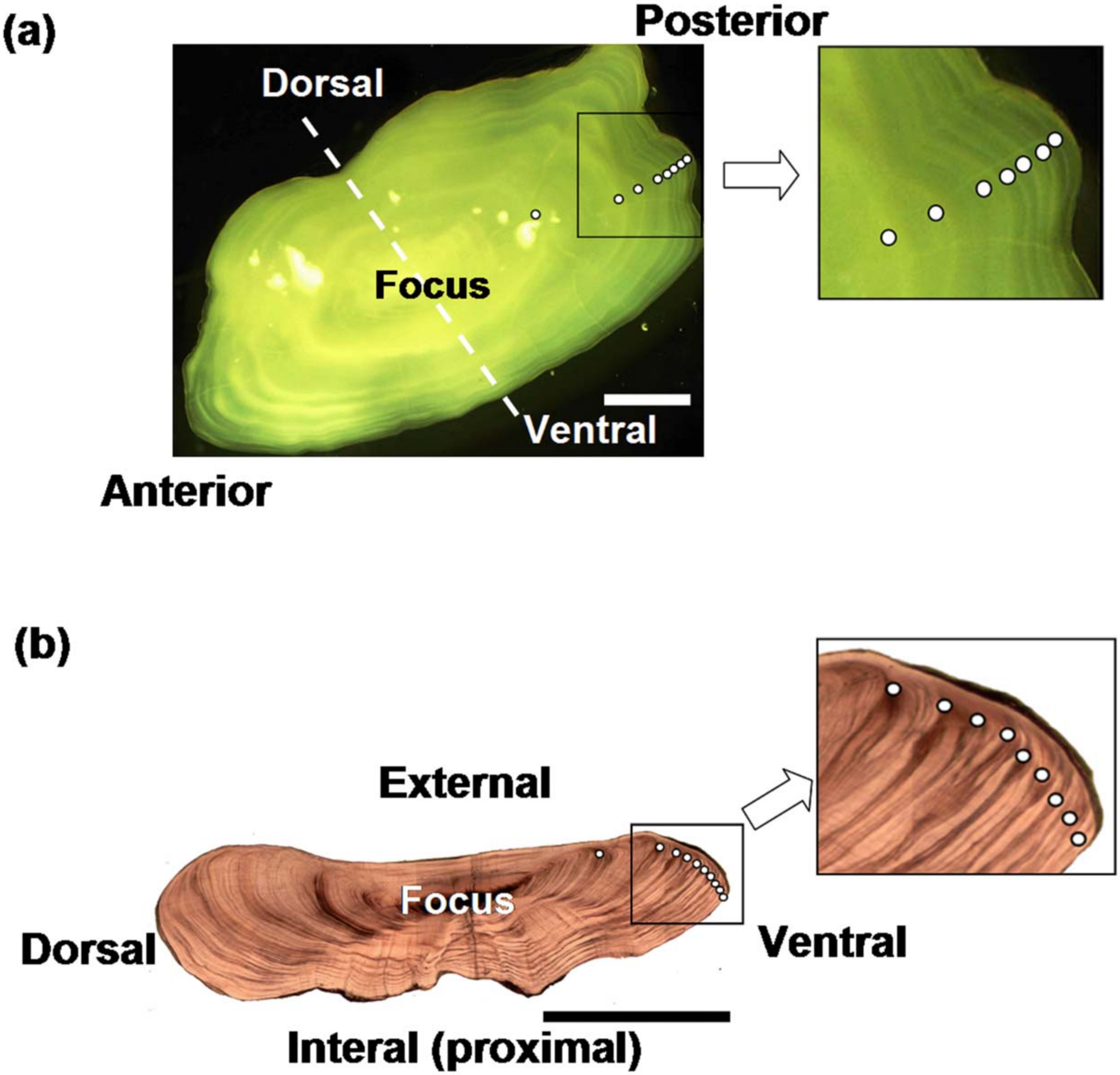

(a)
Posterior
Dorsal
Focus
Ventral
Anterior
(b)
External
Focus
Dorsal
Ventral
Interal (proximal)

Fig. 3

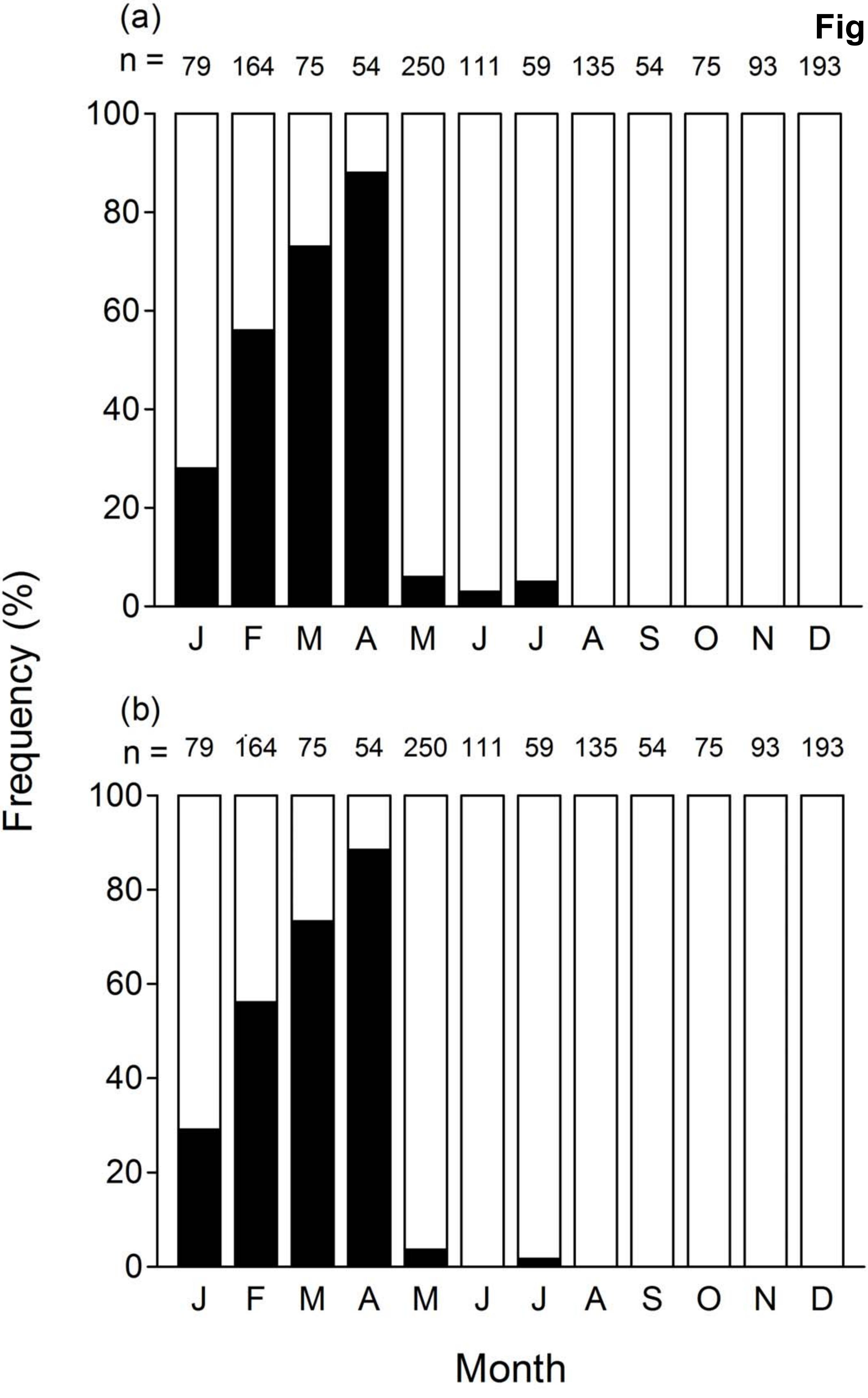

(a)
n = 79 164 75 54 250 111 59 135 54 75 93 193
100
80
60
40
20
0
J F M A M J J A S O N D
(b)
n = 79 164 75 54 250 111 59 135 54 75 93 193
100
80
60
40
20
0
J F M A M J J A S O N D
Frequency (%)
Month

Fig. 4

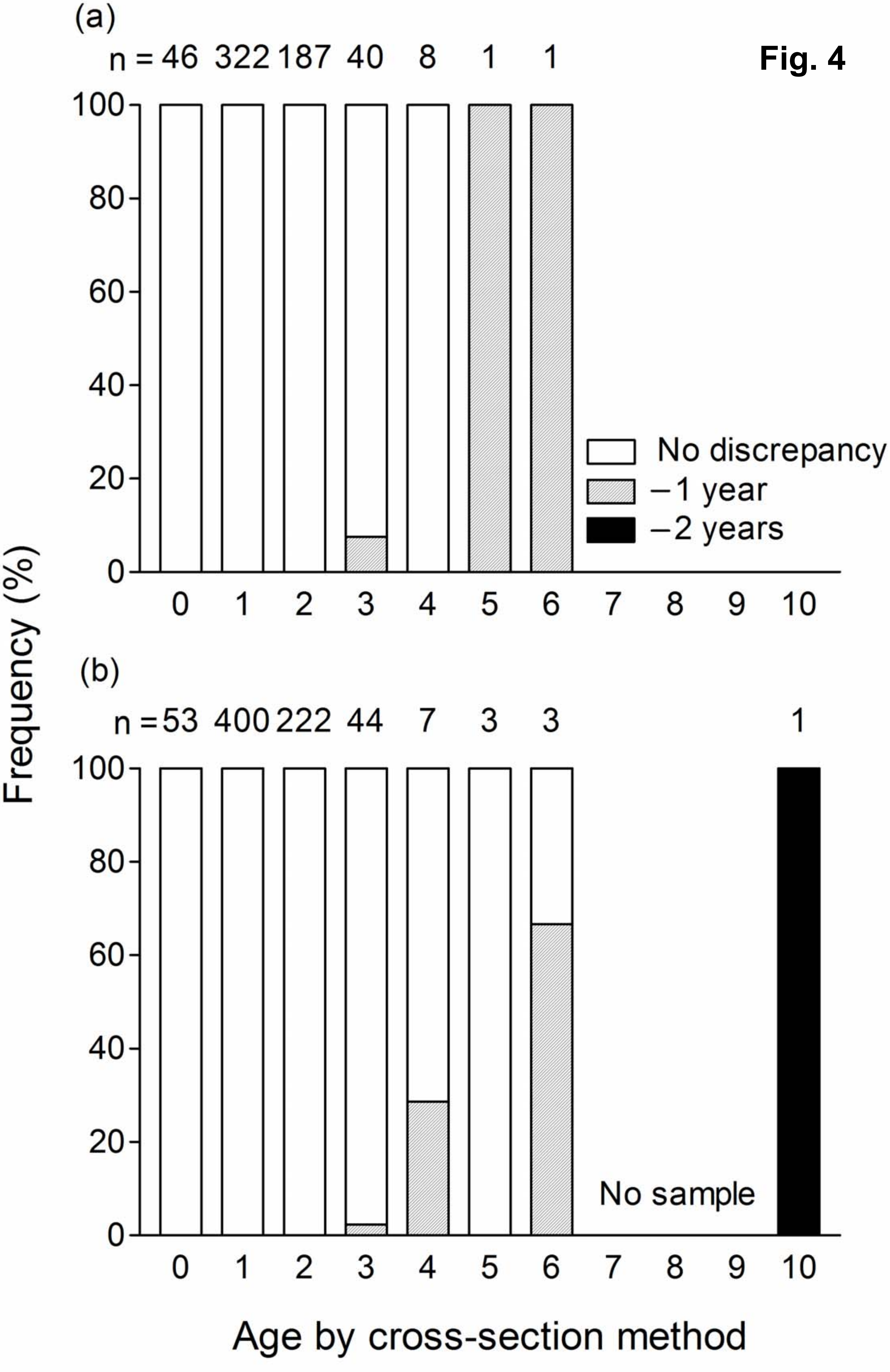
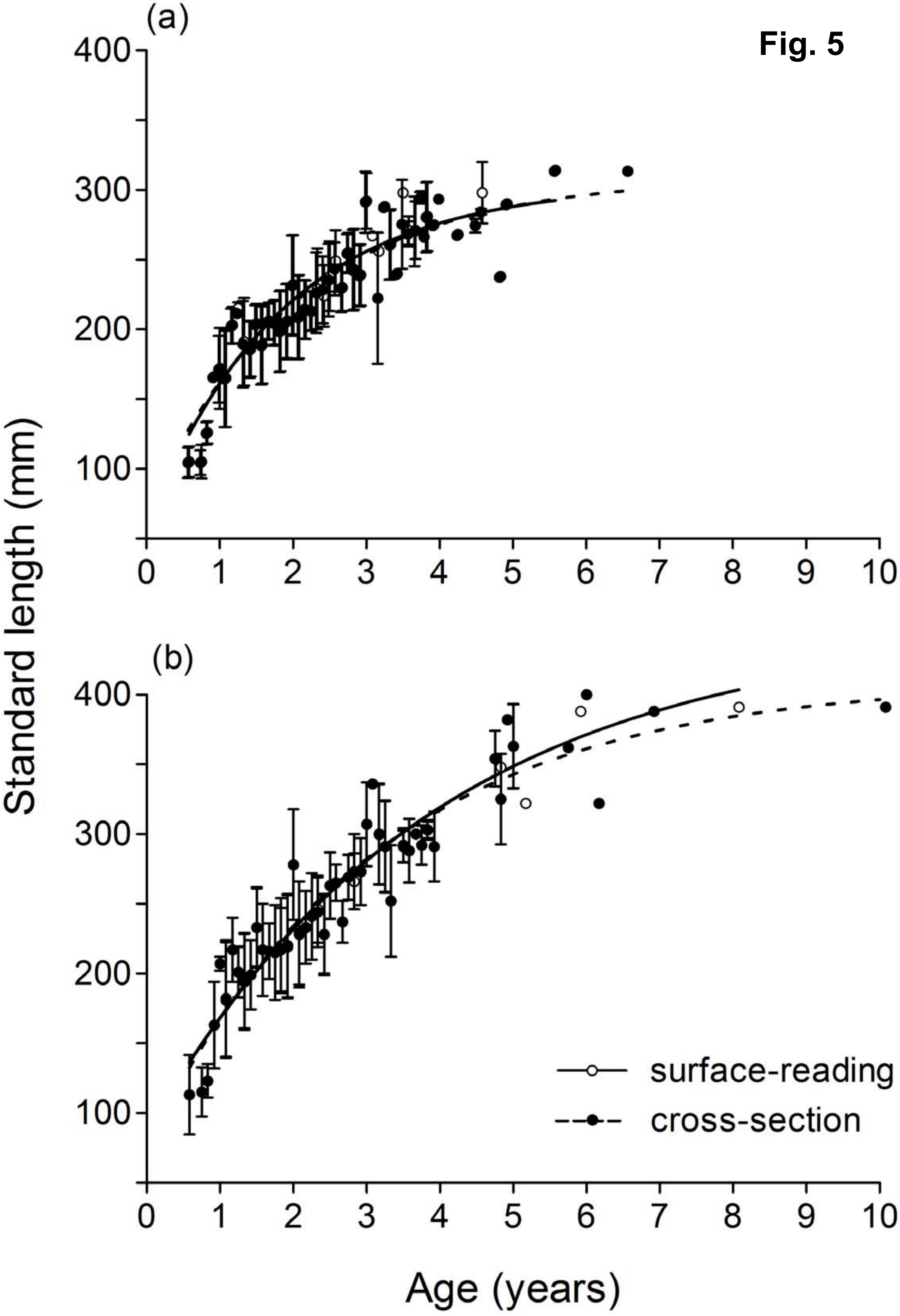

(a)
n = 46
322
187
40
8
1
1
100
80
60
40
20
0
0
1
2
3
4
5
6
7
8
9
10
No discrepancy
−1 year
−2 years
Frequency (%)
(b)
n = 53
400
222
44
7
3
3
1
100
80
60
40
20
0
No sample
0
1
2
3
4
5
6
7
8
9
10
Age by cross-section method

Fig. 5


(a)
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
(b)
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
surface-reading
cross-section
Standard length (mm)
Age (years)