Discriminant analysis of age to enable rapid estimation of 0-age Japanese eels *Anguilla japonica*

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Abstract — Anguillid eels are declining worldwide, so monitoring of their annual recruitment is important, but labor-intensive. Efficient ways to assess the abundance of their new recruits (0-age) each year would therefore be useful. Three discriminant analysis methods were evaluated for their ability to identify 0-age eels based on the total length (TL) of eels that included various age classes from 0-age to ≥2-age. Japanese eels were sampled every month from 2007 to 2009 in the Imagawa River, an inlet river of Hamana Lake in Japan, and the ages of 223 eels (50 to 250 mm TL) were determined using otolith annuli analyses. All three discriminant analyses could predict 0-age eels more accurately than 1-age and ≥2-age eels based on TL and catch date data. Canonical discriminant analysis appeared to be best for predicting 0-age eels, with all eels determined by otolith analysis as 0-age being correctly predicted (100%) in the leave-one-out cross validation method, although 2.4% of 1-age eels were wrongly predicted as 0-age. Studies of the recruitment patterns, habitat use, or ecology of 0-age recruits in a river system would be possible without additional aging efforts after a discriminant analysis function was created for a river system.

Key words: *Anguilla japonica*, age, discriminant analysis, elvers, growth, otolith

Introduction

Northern Hemisphere anguillid eels including the Japanese eel, *Anguilla japonica*, have shown declining recruitment in recent decades causing worldwide concern for these catadromous fishes (Dekker et al. 2003, Tsukamoto et al. 2009). After being spawned offshore, their recruitment stage glass eels enter estuaries and then freshwater and begin their juvenile stage. Monitoring of recruitment variations of glass eels that move inland with tidal currents has been difficult due to temporal fluctuations in glass eel migration patterns that require long-term sampling. An alternative method for evaluating yearly recruitment levels might be surveys to determine the relative abundance of newly recruited 0-age eels at the late glass eel, elver, or early yellow eel stage. Such surveys could be conducted using an electric shocker at specific locations after the recruitment season has ended and would take much shorter periods of time than surveys for glass eels using plankton nets, dip nets or set nets. However, to study annual recruitment, a reliable method needs to be found to identify the 0-age recruits from each year.

Age determinations of fish are frequently made using the annual rings in their sagittal otoliths (Campana and Neilson 1985) and these techniques have become an essential tool in fish ecology and fisheries science (Campana 2005). Many studies have provided evidence that the otoliths in various fishes likely form annual rings (Campana 2005), and they appear to typically do so in anguillid eels also (Berg 1985, Chisnall and Kalish 1993, Oliveria 1996, Pease et al. 2003, Lin and Tzeng 2009). Otolith analyses are time consuming and too labor intensive for evaluating the age structure of large numbers of fish, such as collections of young recruits and young-of-the-year to assess yearly recruitment patterns or as part of other types of life history studies. In addition, otolith age determination is lethal to the fish, which makes it a detrimental technique from a conservation perspective.

For anguillid eels, the ecology of 0-age eels, which are defined as eels in the first year after recruitment into their continental growth habitats, is also important because their behavior during this early period may have an important effect on their life in the following long-term growth period. During this 0-age period, most eels appear to decide whether or not to migrate upstream into freshwater or remain in saline waters (Edeline 2007, McCleave and Ediline 2009, Yokouchi et al. 2012). It has been found using otolith microchemistry analyses that Japanese eels, European eels *Anguilla anguilla* and American eels *Anguilla rostrata*, can spend their growth

periods in coastal marine, estuarine or freshwater habitats, because they have a "facultatively" catadromous life history (Tsukamoto et al. 1998, Tsukamoto and Arai 2001, Tzeng et al. 2002, Morrison and Secor 2004, Kotake et al. 2004, Daverat et al. 2006, Lamson et al. 2006, 2009). Thus the inshore and upstream migration of 0-age eels at the transition point from oceanic to estuarine and freshwater habitats is a phase when each young eel must choose the type of habitat where they will settle and begin feeding and growth.

Eels recruit into the continental habitat as glass eels and then grow through the elver stage into yellow eels during their first year, so it is possible to distinguish glass eels and small elvers as 0-age using pigmentation stages. However, during the first year the elvers likely grow into yellow eels, but the criterion between elvers and yellow eels has not been defined by the past studies. A way to distinguish 0-age eels without using their pigmentation stages would be helpful to be able to distinguish all sizes of 0-age individuals from each recruitment year class.

This study aimed to construct an easy method to discriminate 0-age eels that uses only their size and sampling time. We collected eels in the Imagawa River that flows into Hamana Lake along the central Pacific coast of Japan and determined their ages using otolith analysis. Using these data, several discriminant analyses for estimating 0-age Japanese eels from their total length and sampling month were constructed and compared to determine which type of analysis may be the best for using with these types of data sets.

Materials and Methods

Samples

Samples of 1,376 Japanese eels were collected every month from 2007 to 2009 in the Imagawa River, an inlet river of Hamana Lake in Shizuoka Prefecture, Japan (Fig. 1), by set nets and an electric shocker. The sampling locations covered brackish and non-tidal zones. 1,119 live samples were immediately brought to the Fisheries Laboratory, Graduate School of Agricultural and Life Sciences, The University of Tokyo, near the channel of the lake, and used for morphological measurements. Their total lengths (TL) were measured to the nearest 0.1 mm after they were anaesthetized by 30 ppm eugenol. Then they were sacrificed and preserved as frozen samples. No 0-age eels were found among eels larger than 200 mm TL in the Imagawa River using otolith analyses (Yokouchi et al. 2008), thus this study used eels <250 mm TL to avoid missing any 0-age eels. 3–22 individuals were subsampled randomly from every 10 mm size interval group of eels 50 to 250 mm TL in size and used for age determination by otolith analysis.
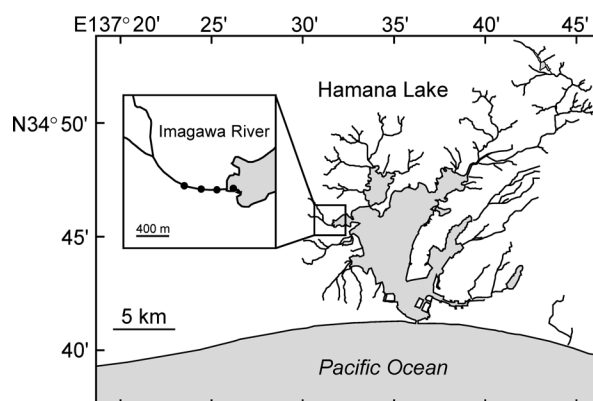
Fig. 1. Map of the location of the Imagawa River on the west side of Hamana Lake along the Pacific coast of Japan where the study was conducted.

Otolith analysis

The sagittal otoliths were embedded in epoxy resin (Epofix, Struers) and ground to expose the core. The otolith surface was polished with OP-S (Struers) on a polishing wheel and then was etched with 0.3 M HCl. Age determination was conducted by counting annual rings on the otolith using an optical microscope (Nikon, Optiphoto) because the deposition of annual rings in Japanese eels has been validated (Lin and Tzeng 2009). The distinct transition check at a radius of about 150 μm from the core (elver mark) was assigned as age=0 because it was thought to be associated with recruitment to growth habitat (Arai et al. 2003). In this study, the age of eels is represented as years spent in the continental habitat and the recruitment into the habitat is defined as the beginning of 0-age.

Data analysis

To construct discriminant functions for the age estimation of young Japanese eels, we used datasets of age, TL and sampling month of 223 individuals that were aged using otolith analysis. The age of individuals were categorized as three classes that were 0-age, 1-age or ≥2-age. Functions for canonical discriminant analysis, quadratic discriminant analysis and discriminant analysis using Maharanobis distance were constructed using the data, which were processed in the MASS package in the statistical program R (R Development Core Team 2005). For distinguishing among age groups, canonical discriminant analysis uses two canonical variates, which are linear combinations of features (TL and collection month in this case). In contrast, the quadratic discriminant and discriminant analyses using Maharanobis distance are based on non-linearity in distinguishing among age groups. To discriminate age groups, the former uses a quadratic equation and the later uses the Maharanobis distance from each of the age group centroids in the TL-collection month plane.
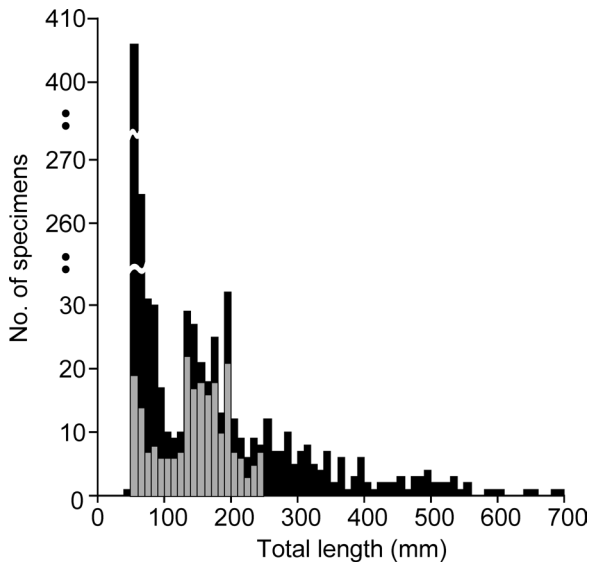
For comparing the precision of each analysis, we used

the leave-one-out cross validation method that uses a single observation from the original sample as the validation data, and the remaining observations as the training data.

Results

Age and total length

The TL used for body measurement ranged from 49.9–

Fig. 2. Length frequency distribution of total length of the Japanese eels, *Anguilla japonica*, caught in the Imagawa River from 2007 to 2009. Individuals used for both otolith analysis and body measurement are shown with gray bars and individuals used only for body measurements with black bars.

694 mm and the TL of samples for the otolith and discriminant analyses ranged from 54.3–248 mm (Fig. 2). The otolith analyses of these eels indicated that the number of 0-age, 1-age, 2-age, 3-age and 4-age eels were 57, 82, 68, 15 and 1, respectively. Within this 50–250 mm size range, the larger eels included a higher proportion of older eels (Fig. 3). The TL were significantly different among age groups (Kruskal Wallis, $p<0.05$, Fig. 4a) and the TL at 0-age, 1-age and ≥2-age was significantly correlated to sampling month (0-age eels, $r=0.78$, $p<0.001$; 1-age eels, $r=0.41$, $p<0.001$; ≥2-age, $r=0.36$ $p<0.001$, Fig. 4b).
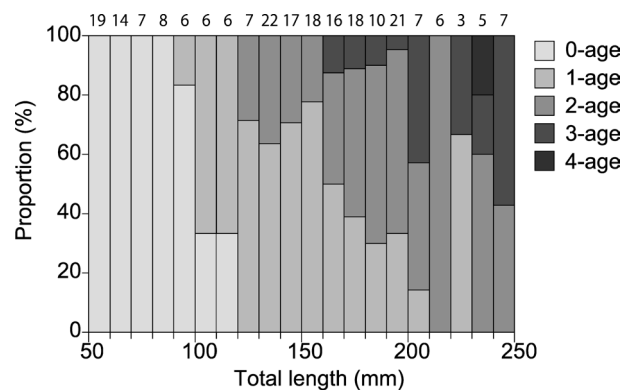
Fig. 3. Proportion of each age-group of eels determined from otolith analyses that were in each 10 mm interval of total length for the eels from the Imagawa River that were used in the discriminant analyses calculations. The numbers above bars show the number of specimens used for age determination by otolith annual rings.
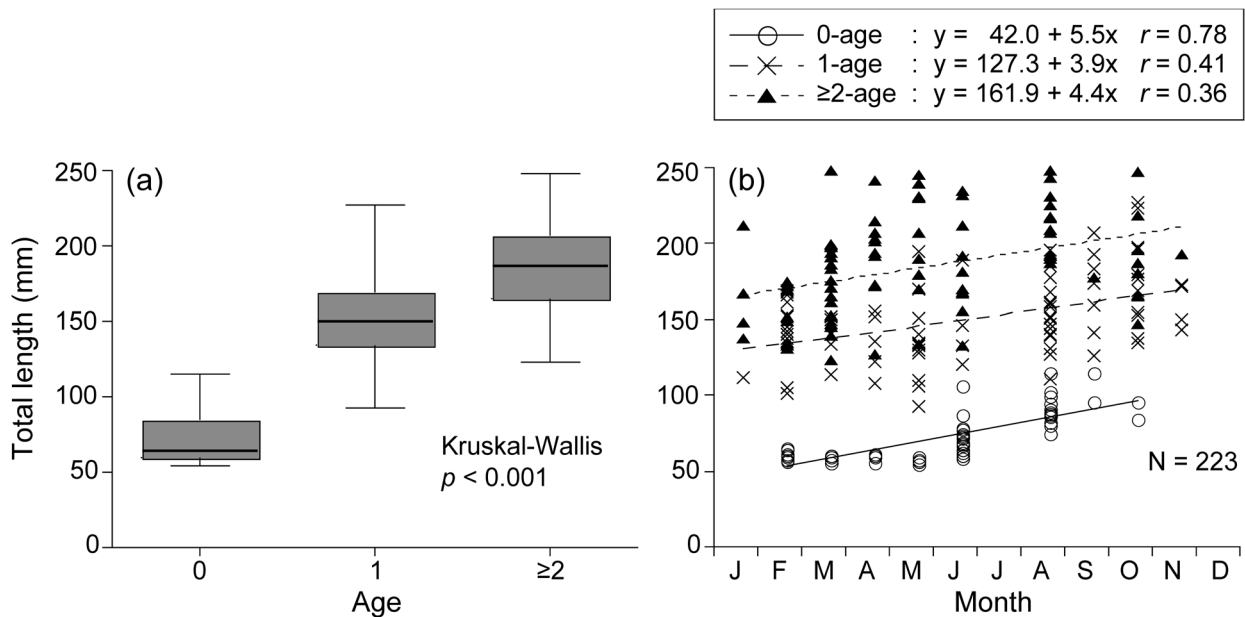
Fig. 4. Total length in each age (a) and a plot of the total lengths of 0-age, 1-age and ≥2-age eels during each month of the years (b) in the Imagawa River. The boxes and thick lines in (a) indicate the middle 50% and median, respectively, and whiskers show the total range. Regression lines in (b) are shown for each age category.

Table 1. Number of fish predicted as 0-age, 1-age and ≥2-age by three kinds of discriminant analysises. Predicted group membership indicates the proportion of fish predicted by leave-one-out cross validation within each age group. Proportions predicted correctly are written in bold.

	Actual age*	Predicted group membership		
		0-age	1-age	≥2-age
Canonical discriminant analysis	0-age	**100.0**	0.0	0.0
	1-age	2.4	**70.7**	26.8
	≥2-age	0.0	26.2	**73.8**
Quadratic discriminant analysis	0-age	**94.7**	5.3	0.0
	1-age	1.2	**73.2**	25.6
	≥2-age	0.0	28.6	**71.4**
Discriminant analysis using Mahalanobis distance	0-age	**94.7**	5.3	0.0
	1-age	1.2	**75.6**	23.2
	≥2-age	0.0	26.2	**73.8**

*Actual age was determined using otoliths.

Precision of age prediction by discriminant analyses

In the canonical discriminant analysis, the correct prediction rates of 0-age, 1-age and 2≥age eels were 100%, 70.7% and 73.8%, respectively (Table 1). In the quadratic discriminant analysis, the discrimination rates of 0-age, 1-age and ≥2-age eels were 94.7%, 73.2% and 71.4% (Table 1). In the discriminant analysis using Mahalanobis distance, the discrimination rate of 0-age, 1-age and ≥2-age eels were 94.7%, 75.6% and 73.8% (Table 1).

Evaluating the data qualitatively in Figure 4b suggests that the ability to discriminate 0-age eels may be greater during some months of the primary recruitment and upstream migration season. It is evident that there is a clear separation in the TL of 0-age eels and those of other ages from February to May, as well as in October. From June to September however, some 0-age and 1-age eels have very similar or overlapping TL values. The 1-age and ≥2-age eels overlapped in TL throughout most of the year except in January and November when there were low sample sizes.

Discussion

All of the discriminant analysis methods in the present study had high accuracy in discriminating 0-age eels, although they were less accurate in discriminating 1-age and ≥2-age eels. The TL of 1-age or ≥2-age eels were heavily overlapped in samples from some months, but the TL of the 0-age eels only overlapped with that of the 1-age eels within the 90–120 mm size range (Fig. 3). This suggested that the eels had a large amount of variation in their growth rates

after recruitment to their growth habitats and the variation increased as they grew older. The growth of freshwater eels is related to their sex (Tzeng et al. 2000), and histological observations have found that sexual differentiation in *A. japonica* occurs around 200 mm (Satoh et al. 1962, Chiba et al. 1993). Even though that size of eels was included in the range of samples examined in this study, the sex of the larger eels was not included in the discriminant analyses because it is difficult to distinguish testes and ovaries until eels reach about 300 mm without using histological methods (Yokouchi 2010). If the sex of the larger eels is also considered in the discriminant analysis, it might account for some of the variability and help increase the accuracy of discriminating 1-age and ≥2-age eels, although this would require labor-intensive histological methods. Regardless of this, the discriminant analyses in this study that estimated age from their TL and collection month can clearly detect 0-age eels.

The canonical discriminant analysis predicted the actual 0-age eels that were determined by otolith aging as being 0-age with 100% precision (0% error), but some actual 1-age eels were predicted as 0-age at a 2.4% error rate. Although quadratic discriminant analysis and discriminant analysis using Mahalanobis distance predicted actual 0-age eels as 0-age with 94.7% precision (5.3% error), actual 1-age eels were predicted as 0-age with a 1.2% error rate. Considering the sum of the two error prediction rates with 0-age eels being as 1-age and 1-age eels as 0-age, the sum of the canonical discriminant analysis was lowest (2.4%) among the three analyses, indicating that it was best for 0-age estimation in the present study.

The canonical discriminant analysis did not tend to mis-
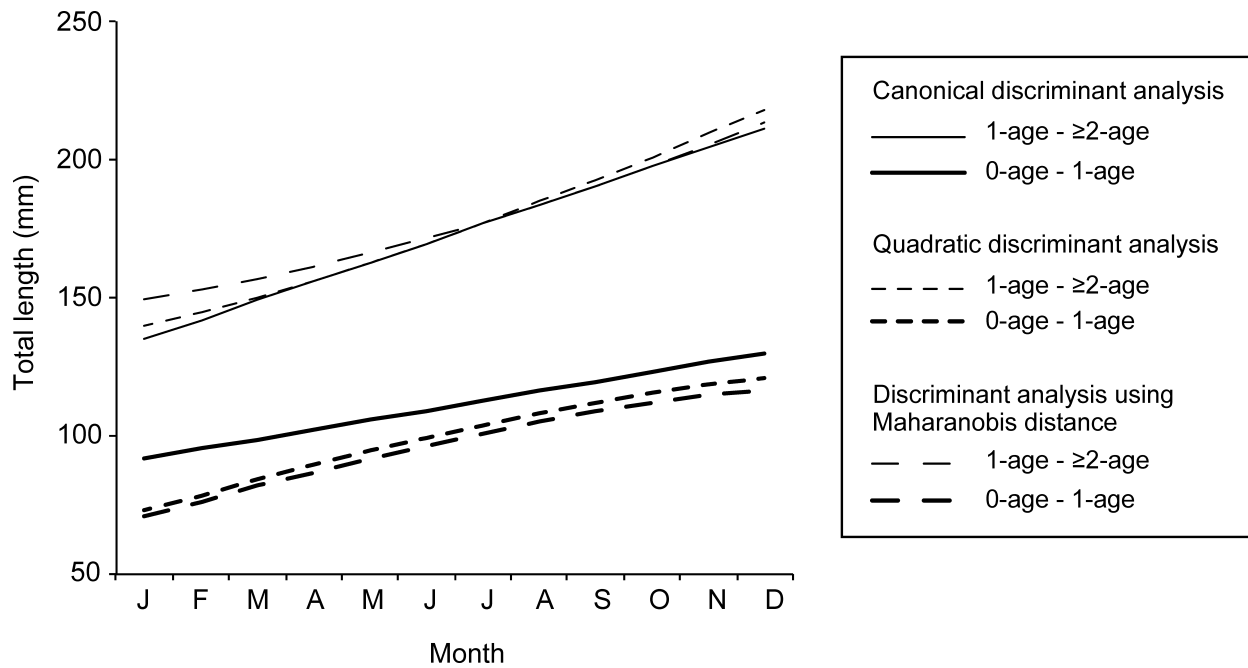
Fig. 5. The boundaries of total length between 0-age and 1-age eels, and 1-age and ≥2-age eels in each month estimated from canonical discriminant analysis, quadratic discriminant analysis and discriminant analysis using Maharanobis distance.

Appendix A. The range of total length of 0-age, 1-age and ≥2-age eels in each month estimated from canonical discriminant analysis, quadratic discriminant analysis and discriminant analysis using Maharanobis distance.

	Canonical discriminant analysis			Quadratic discriminant analysis			Discriminant analysis using Maharanobis distance		
	0-age	1-age	≥2-age	0-age	1-age	≥2-age	0-age	1-age	≥2-age
Jan	≤ 91.9	92.0 - 135.1	135.2 ≤	≤ 73.2	73.3 - 139.8	139.9 ≤	≤ 70.7	70.8 - 149.4	149.5 ≤
Feb	≤ 95.4	95.5 - 142.0	142.1 ≤	≤ 78.8	78.9 - 144.7	144.8 ≤	≤ 76.3	76.4 - 152.9	153.0 ≤
Mar	≤ 98.8	98.9 - 149.0	149.1 ≤	≤ 84.3	84.4 - 150.2	150.3 ≤	≤ 81.8	81.9 - 156.9	157.0 ≤
Apr	≤ 102.3	102.4 - 155.9	156.0 ≤	≤ 89.5	89.6 - 156.2	156.3 ≤	≤ 86.9	86.8 - 161.2	161.3 ≤
May	≤ 105.8	105.9 - 162.8	162.9 ≤	≤ 94.5	94.6 - 162.8	162.9 ≤	≤ 91.9	92.0 - 166.1	166.2 ≤
Jun	≤ 109.2	109.3 - 169.7	169.8 ≤	≤ 99.3	99.4 - 169.7	169.8 ≤	≤ 96.6	96.7 - 171.3	171.4 ≤
Jul	≤ 112.7	112.8 - 176.7	176.8 ≤	≤ 103.8	103.9 - 177.1	177.2 ≤	≤ 101.1	101.2 - 177.1	177.2 ≤
Aug	≤ 116.2	116.3 - 183.2	183.3 ≤	≤ 108.0	108.1 - 184.8	184.9 ≤	≤ 105.2	105.3 - 183.4	183.5 ≤
Sep	≤ 119.6	119.7 - 190.5	190.6 ≤	≤ 111.9	111.1 - 192.7	192.8 ≤	≤ 108.8	108.9 - 190.1	190.2 ≤
Oct	≤ 123.1	123.2 - 197.5	197.6 ≤	≤ 115.4	115.5 - 201.0	201.1 ≤	≤ 112.1	112.2 - 197.4	197.5 ≤
Nov	≤ 126.6	126.7 - 204.4	204.5 ≤	≤ 118.5	118.6 - 209.4	209.5 ≤	≤ 114.8	114.9 - 205.1	205.2 ≤
Dec	≤ 130.0	130.1 - 211.3	211.4 ≤	≤ 120.9	120.1 - 218.0	218.1 ≤	≤ 116.7	116.8 - 213.4	213.5 ≤

identify 0-age eels as 1-age, but to misidentify 1-age as 0-age. The quadratic discriminant analysis and discriminant analysis using Mahalanobis distance were more likely to misidentify 0-age eels as 1-age, but not to misidentify 1-age as 0-age. These indicated that the tendency in prediction of 0-age was different between the former analysis based on linearity and the later analyses based on non-linearity. Fig. 5 and Appendix A shows the boundaries of total length between 0-age and 1-age eels, and 1-age and ≥2-age eels in each month estimated from each analyses. The canonical discriminant analysis used 91.9–130.0 mm TL as the border in distinguishing between 0-age and 1-age eels, but the quadratic discriminant analysis and discriminant analysis using Mahalanobis distance used 70.7–120.9 mm TL as the border.

These indicated that the former analyses tended to predict eels with relatively high TL values as 0-age compared to the later analyses.

The discriminant analysis function created in the present study could be applied successfully in locations with similar growth rates of eels as in the Imagawa River. However, because the growth of eels is affected by environmental water temperature (Tzeng 1994, Dou et al. 2003, Fukuda et al. 2009), this factor would have to be considered. The average annual temperature in the Imagawa River was 16.9°C (range: 7.5–25.0°C) during 2008 (Fukuda unpub. data). If temperature conditions in other rivers are similar to the Imagawa River, the present discriminant analysis could be applied to those locations.

To construct a new discriminant analysis model for eel age estimation in a different locality, any local differences in growth should be considered when choosing the best type of discriminant function for that location. Several studies reported the growth rate of Japanese eels in different locations in Japan and Taiwan and some variation was found among areas (Tzeng et al. 2000, Kotake et al. 2007, Yokouchi et al. 2008, Yokouchi 2010). Yokouchi (2010) showed that the growth rates of this species were significantly different among 11 river systems in Japan. In locations with different temperatures or growth patterns from the present study, an original discriminant analysis should be formed using the same type of data of age, TL and month in accordance with similar steps in this study. However, it would be laborious to establish an original discriminant analysis using the same methodology as the present study. Bimonthly or quarterly sampling instead of monthly sampling would be a way to reduce the effort, even if the precision of the discriminant analysis may be decreased some.

Although future studies are needed to evaluate how well this method works with less sampling effort or in areas with different eel growth patterns, the present study provides a first test of whether or not using a discriminant analysis function method would be useful in establishing a method for distinguishing 0-age or other early ages of eels in a particular area. In the case of the Imagawa River, this method showed strong potential for use with 0-age eels, but the sample sizes in some months were small for those eels late in the year. Overlap of a few older eels in some months or many eels in other months showed that distinguishing the older year classes would include a certain percentage of error. Use of the discriminant analysis function approach in several nearby rivers simultaneously to obtain larger sample sizes of the older 0-age eels may also improve the prediction accuracy of the discriminant analysis functions.

Once a discriminant analysis function and sampling method is constructed to estimate 0-age eels from eels with various ages from a particular river system, it would enable the 0-age eels to be accurately and easily distinguished from large samples of eels whose sizes were measured in the field, without needing to sacrifice the eels for aging analysis. Having this ability to survey for 0-age recruits in a river system to determine yearly differences in recruitment or to study their critical period to reside in marine, estuary and river habitats could be a useful and cost effective approach that would be much less labor intensive than sampling for glass eels continuously throughout the recruitment season.

Acknowledgements

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