Aquaculture and Genetic Structure in the Japanese Eel *Anguilla japonica*

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Abstract

Both commercial catches of natural eel and eel seeds for aquaculture have decreased over four decades. These declines may indicate a severe biomass reduction of the Japanese eel (*Anguilla japonica*). Although the causes of this reduction are not clear, more knowledge is necessary of the basic biology of the Japanese eel, including knowledge of their migration and population dynamics and structure, which will contribute to resource management. We studied the genetic structure of *A. japonica* by the random amplified polymorphic DNA (RAPD) technique. Glass eel were collected from Taiwan, Kagoshima, Ibaraki, and Miyagi. The frequencies of most RAPD bands were similar among the four locations, but the frequencies of 1100bp band amplified by the Operon A10 primer showed nearly significant difference between Miyagi and the others. Additional genetic studies are needed to determine the population structure of the Japanese eel.

Introduction

The annual eel consumption in Japan currently amounts to 120,000 to 130,000 tons. In other words, the per capita consumption is about 5 eel a year. *Unajuu*, or *Unadon*, a dish of charcoal-grilled eel filets served with sweet soy sauce on rice, is as important in Japan as sushi. Eighteen percent of the eel consumed in Japan is produced in the country (23,211 tons, aquaculture; and 817 tons, wild in 1999), and the remainder is imported from China, Taiwan, and Malaysia. Only 0.6% of the total is wild adult eel. Therefore, eel aquaculture is very important to the success of the eel industry. The Prefectures of Aichi, Kagoshima, Shizuoka, and Miyazaki are major producers. However, the recent decline of glass eel catches in East Asia has caused serious problems in eel aquaculture in Japan and Taiwan. Although scientists at the National Research Institute of Aquaculture have reared hatched eel larvae for more than 250 days (Tanaka *et al.*, 2000), complete artificial propagation, from eggs to glass eel, has not been successful. At this time, seeds for aquaculture are totally dependent on wild glass eel from East Asia and Europe. This paper deals with the decline of natural eel resources and the genetic structure of the Japanese eel (*Anguilla japonica*).

Decline of Natural Eel Resources

The Japanese eel has been a major target species for aquaculture in East Asia, and there have been many studies of eel aquaculture and related topics, such as disease control, artificial maturation, propagation, and searches for spawning sites. However, little attention has been given to natural populations and the resource management of eel. Biomass is considered proportional to the catch per unit effort (CPUE). The CPUE in eel fisheries is not available in Japan. Available resource data come from yearly commercial catches of natural eel, which may have a correlation with the biomass. The annual catch of natural eel in the inland waters of Japan was around 3,000 tons between 1901 and 1941 (Matsui, 1952). According to statistics of the Ministry of Agriculture and Forestry and the Ministry of Agriculture, Forestry and Fisheries (name of the ministry since 1978), about 3,000 tons of natural eel were caught annually in the 1960s. However, the annual catches started to decline in 1970 to ca. 2,000 tons in 1979 (Fig. 1). The decline has continued in the 1980s and 1990s. Since 1993, the catch has been less than 1,000 tons a year. The one-third reduction in commercial catch of natural eel during the past four decades may imply a severe reduction of the eel biomass.

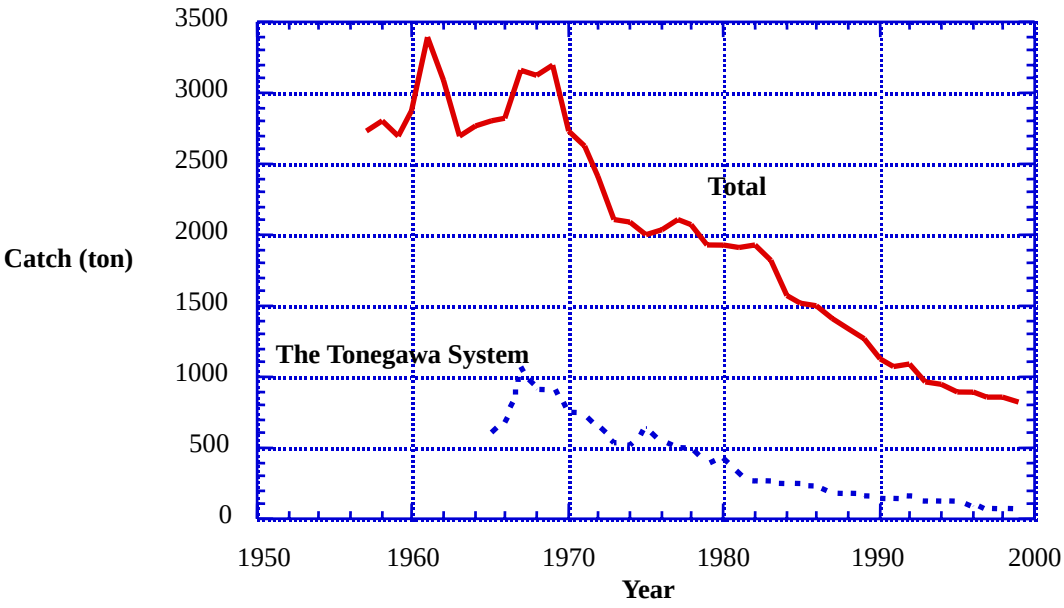

Figure 1. Annual catch of natural eel in Japan.

The annual commercial catch of natural seeds, which includes the glass eel, has decreased even more obviously. In statistics from the Ministry of Agriculture, Forestry and Fisheries of Japan, glass eel are listed in two categories: 1) the coastal catch for aquaculture, and 2) aquaculture seeds from the inland waterways, which include glass eel and juveniles up to one-year-old. Figure 2 shows the sum of the two categories, which is the annual catch of natural eel seeds for aquaculture in Japan. During the 1960s, up to 150 tons of natural eel seeds were caught annually. The catch ranged from 50 to 100 tons in the 1970s and declined to fewer than 50 in the 1980s. Currently, the catch has been around 20 tons (Fig. 2). The seed catch is one-fifteenth what it was 30 years ago. The reduction of the seed and natural eel catch indicates that the eel biomass has decreased.

The Tonegawa drainage system near the Tokyo Metropolitan area used to be known as a major production area for eel seeds. In 1958, a total of 179 tons of eel seeds were caught in the drainage system (Kasebayashi, 1960). The seed catch in the Tonegawa drainage was 76% of the total catch in 1969 (Fig. 2). These seeds were transported to Shizuoka and Aichi Prefectures for aquaculture. Fewer than 10 tons were caught in the Tonegawa drainage in 1999. Huge amounts of eel seeds used to be caught in the Tonegawa drainage. If the catch of eel seeds was the same today as it was forty years ago in the Tonegawa drainage alone, there would be no shortage of glass eel for aquaculture in Japan. Moreover, the natural eel catch in the Tonegawa drainage was one-third of the total in Japan several decades ago. Therefore, the Tonegawa drainage system used to be a major habitat for the Japanese eel, and it could possibly be used today to sustain eel resources. Kasebayashi (1960) suggested that the ongoing river development in the Tonegawa system might negatively affect the eel population. The reduction in the eel population coincided with the construction of dams in the lower reaches of the river. The construction might cause eutrophication of Lake Kasumigaura (220 km^2) because of the lower exchange between freshwater and seawater.

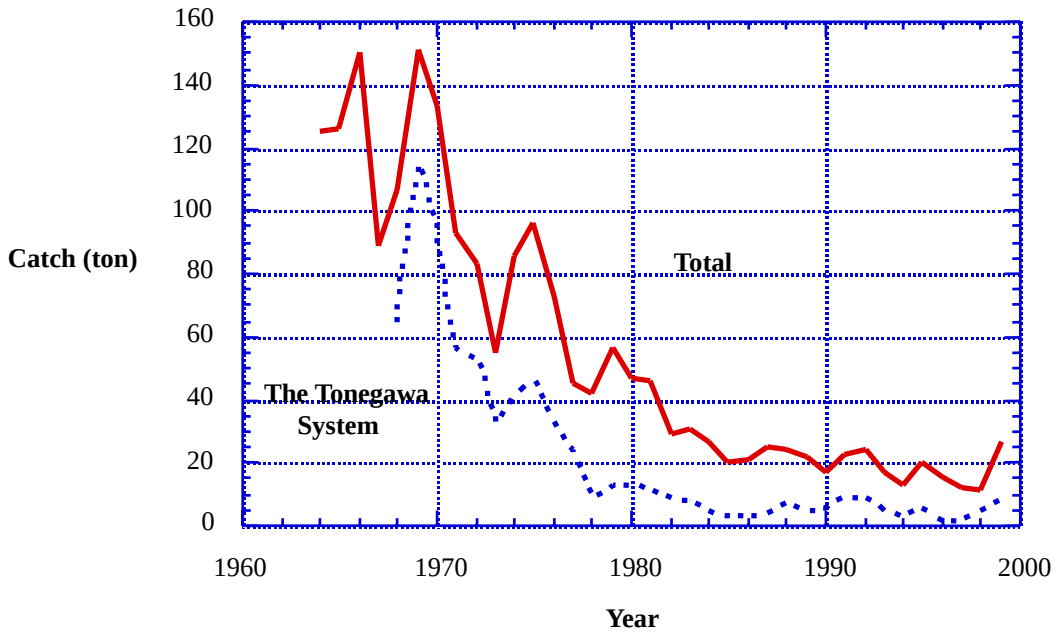

Figure 2. Annual catch of natural seeds for aquaculture in Japan. The annual catch is the sum of glass eel catch around coasts and seed catch (glass eel and juveniles up to one-year old) from the inland waterways.

Genetic Structure

The first obstacle to eel resource management is the lack of information on basic eel biology, such as spawning migration and behavior, larval ecology, and natural population dynamics and structure. This basic information must first be collected. We compared the frequency difference of each RAPD band to investigate the genetic structure of the Japanese eel from Taiwan (n=10) and Japan (south to north, n=25 each: Kagoshima, Ibaraki, and Miyagi). Glass eel were collected in Tung-Kang, Taiwan, on February 26, 1998, in Tanegashima,

Kagoshima, on January 28, 1998, and in Ibaraki and Miyagi, on February 28, 1998. The total DNA was extracted from each specimen by the regular phenol-chloroform method. RAPD primers used were Operon A1 to A20 and B1 to B7 (Operon Technologies, Alameda, CA, USA). Fifty nanograms of template DNA was in the 50µl PCR solution. A ready-to-use reaction mixture (PerfectShot™ Ex Taq, TaKaRa, Shiga, Japan) was used for PCR amplification. Reactions started at 94°C for four minutes and were amplified through 35 cycles at the following parameters: one minute at 94°C, one minute at 36°C, and two minutes at 72°C, followed by a final extension step at 72°C for five minutes. PCR products were separated on 0.9 and 1.4% agarose gels and visualized by ethidium bromide staining.

Thirteen 10-base random primers (A1, A4, A5, A8, A10, A11, A12, A16, A17, A18, A20, B1, and B5) produced scorable clear bands through PCR. Frequencies of most RAPD bands were similar among the four locations. The 1100bp band amplified by the primer A10 was absent in the Miyagi samples and present in the remaining populations at different frequencies (Table 1; 0.11-0.24). The difference between Miyagi and the other three sites was nearly significant (Mann-Whitney *U* test: p=0.061). Moreover, the 1000bp band amplified by the primer B1 was present in Miyagi at the low frequency (0.08), and the other three sites did not express the band. Although the frequency of the 1500bp band amplified by the primer A11 was 0.39 in Kagoshima, the remaining sites had higher frequencies (0.70-0.82).

Table 1. Frequency of A10 RAPD markers for Japanese eel samples from Japan and Taiwan.

Location	RAPD marker (bp)						
	1000	**1100**	**1200**	**2020**	**3200**	**3300**	**3500**
Miyagi (n=24)	0.92	0.00	0.67	0.96	0.33	0.25	0.08
Ibaraki (n=25)	1.00	0.16	0.68	0.92	0.28	0.20	0.08
Kagoshima (n=25)	0.96	0.24	0.76	1.00	0.40	0.52	0.20
Taiwan (n=9)	1.00	0.11	0.56	1.00	0.22	0.22	0.00

Discussion

Adult eel and glass eel catches in Japan have declined with some fluctuations for the last 40 years. However, Japanese consumers are not aware of the problem because they can buy European eel cultured in China at reasonable prices year around. Although the causes for the reduction in the number of glass eel are not clear, there are four possibilities: 1) habitat loss due to construction of dams, 2) pollution in estuaries and rivers, 3) overfishing of glass eel, and 4) global environmental changes. Unfortunately, none of these hypotheses has been tested.

It is difficult to speculate which hypothesis is correct because the population dynamics of eel in the entire life cycle is unknown. The life history of the Japanese eel is only partially understood. Spawning sites of the Japanese eel appear to be west of the Mariana Islands in the North Pacific (Tsukamoto, 1992), and eel travel several thousand kilometers to spawn. The leptocephalous larvae travel back to freshwater habitats in Taiwan, mainland China, Korea, and Japan. A recent study showed that some larvae seemed to stay in the ocean to grow (Tsukamoto *et al.*, 1998). The spawning migration and population dynamics and structure of the Japanese eel

in nature are not well understood.

A previous allozyme study indicated that there were allelic clines at two loci in *Anguilla japonica* (Chan *et al.*, 1997). However, mtDNA studies of *A. japonica* did not reveal genetic structuring (Sang *et al.*, 1994; Ishikawa *et al.*, 2001). Although our results did not show significant genetic differentiation, additional genetic studies with a large sample size (ca. 100 individuals per location) are necessary to determine the genetic structure of the Japanese eel. Prolonged spawning periods predicted from the presence of leptocephali (Cheng and Tzeng, 1996; Tsukamoto *et al.*, 1998) may cause temporal segregation among populations.

Cumulative knowledge of the life history and ecology of the target species is the key to success for the resource management and stock enhancement efforts. The research will help not only artificial propagation but also resource conservation. Efforts to protect wild eel resources need to continue and we should not be totally dependent on the future success of artificial propagation.

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