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Potential Contributions by Escaped Cultured Eels to the Wild Population of Japanese Eel *Anguilla japonica* in the Kao-Ping River

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ABSTRACT

Due to Typhoon Mindulle a lots of cultured Japanese eel *Anguilla japonica* escaped from aquaculture ponds to the Kao-Ping River in July, 2004. To evaluate whether the escaped cultured eels contributed to the wild silver eel population, the compositions in age, sex ratio, and migratory histories indicated by otolith Sr:Ca ratios of the silver eels in the river were examined from during 1999 to 2006. Mean ages were younger in 2004 to 2006 than in 1999 to 2003 and the sex ratio of the eels had significantly changed from female-dominated before 2004 to male-dominated in 2004 and 2005 (Test of homogeneity, χ_1^2 = 69.5 and 103.5, all $p < 0.001$). However, after 2006 the sex ratio reverted to their previous conditions (Test of homogeneity, $\chi_5^2 = 8.22$, $p > 0.1$). The dominant migratory types of the silver eels also significantly changed from brackish-water type in 1999 ~ 2003 to freshwater type in 2004 ~ 2006 (Goodness of fit, $\chi_1^2 = 252$, $p < 0.001$). This indicated that the effects of escaped eels on the wild eel population were of not more than two years duration. Some escaped eels have silvered and left the river to potentially contribute to the wild spawning stock within two years.

***Key words**: Japanese eel,* Anguilla japonica*, Cultured eel, Silvering, Otolith Sr:Ca ratios.*

INTRODUCTION

Japanese eel, *Anguilla japonica*, is a catadromous fish, widely distributed in the coastal waters, lagoons, estuaries, and rivers in Taiwan, China, Japan, and Korea (Tesch, 2003). It is an important aquaculture fish species in East Asia (Liao, 2001). Artificial propagation of the eels is not completely successful yet and the eel fry for aquaculture all come from elvers in the estuary. The overfishing of elvers may be one of the reasons leading to the decline of the eel population in the natural environment (Tzeng and Chang. 2001; Tatsukawa, 2003). The sex ratio of the wild eel is predominately female but cultured eels are mostly male, enabling the use of sex ratio as an indicator to discriminate cultured from wild eels (Han and Tzeng, 2006).

The Kao-Ping River is the largest river in southern Taiwan and the most important fishing ground for yellow and silver eels (Tzeng and Chang, 2001). Typhoon Mindulle attacked Taiwan on July 2, 2004 and resulted in the escape of about 30,000 yellow eels of 1.5- year-old from eel farms to the Kao-Ping River. These cultured eels can be identified by their sex ratio, body color patterns, and the Sr:Ca ratios and elemental composition of the otolith (Chu *et al.*, 2006). However, little study has shown whether cultured eels escaped from eel farms can adapt to the natural environment, become silver eels, and

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contribute to the wild eel spawning stock.

The mean age of wild silver eels was 5.3 years for males and 6.4 years for females to reach mature stage, i.e. the silver eel (Tzeng *et al.*, 2003). But escaped cultured eels might mature younger than wild silver eels (Beullens *et al.*, 1997). In addition, cultured eels were reared in fresh water but wild eels in the river comprise freshwater, seawater and estuarine residents (Tzeng *et al.*, 2002). Therefore, the difference in mean ages and migratory histories indicated by otolith Sr:Ca ratios may be used to discriminate wild and cultured eels from collected samples.

This study aims to evaluate whether the escaped cultured eels can become sexually mature silver eel in the wild and thus contribute to the spawning silver eel population. The sex ratio, age, and habitat residency of silver eels collected in the Kao-Ping River were examined from 1999 to 2006. The age was determined by counts of otolith annuli and migratory history was determined by the temporal change of otolith Sr:Ca ratios by electron probe micro-analyzer. The feasibility of enhancement of the declining Japanese eel population by releasing cultured eels was also discussed.

MATERIALS AND METHODS

Sample collection

Japanese eels were collected by both eel tubes and shrimp nets in the lower Kao-Ping River in southwestern Taiwan (120°50'E and 22°40'N) during the period from 1999 to 2006. Total length (*TL*, to 1 mm), total weight (*TW*, to 0.1 g), and gonad weight (*GW*, to 0.1 g) of the eels were measured and sex was determined by gross inspection of the gonads (Han *et al.*, 2003). The sex ratio is defined as total number of females divided by total number of eels examined. Silver eels were identified by their eye size and black color in the body surface and pectoral fins (Han *et al.*, 2001, 2003).

Gonado-somatic index and condition factor

The gonado-somatic-index (*GSI*) was calculated as $GSI = GW \times (BW)^{-1}$. The relationship between total length and total weight of the eel was assumed to have a multiplicative error structure as $TW = a \times (TL)^{b} e^{\varepsilon}$, where *a, b* are constants, and ε is the error term.

This formula was fitted by taking logarithmic transformation:

$Log_e(TW) = log_e(a) + b^* \, log_e(TL) + \varepsilon$. The estimates for $log_e(a)$ and *b*, $\hat{A}$ and $\hat{b}$, were obtained by least squares linear regression. $\hat{b}$ could be directly used because it is an unbiased estimator for *b*. But a nearly unbiased estimator of *a* was given by: $\hat{a} = \exp(\hat{A} + 0.5 * MSE)$ where MSE is the mean sum of square errors obtained from the log-transformed linear regression above (Hayes, *et al.*, 1995).

Once the parameters in length-weight relationship were estimated, the relative condition factor (*CF*) was calculated according to Froese (2006): $CF = TW \times (\hat{a}TW^{\hat{b}})^{-1}$. *CF* was used to infer the condition or fatness of the eels and was then compared between 1999 ~ 2003 and 2004 ~ 2006 in both sexes.

Measurement of Sr:Ca ratios in otolith and age determination

The sagittal otoliths of the silver eels caught from 2004 ~ 2006 were randomly selected for Sr:Ca ratio analysis and age determination. The Sr:Ca ratios in the otoliths of the silver eels were analyzed by electron probe micro-analyzer (EPMA, JEOL JXA-8900R) with conditions similar to Lin *et al.* (2005). According to the temporal changes in otolith Sr:Ca ratios, the migratory types were classified into 3 types, i.e. freshwater, brackish water, and seawater residents (Tzeng *et al.*, 2002). The composition of silver eel migratory histories from 2004 to 2006 was compared with that from previous years referring to Tzeng *et al.*, (2003). After the EPMA analysis, the otoliths were re-polished to remove the carbon coating and were etched by 5% ethylene diamine tetra-acetate (EDTA) to enhance the annulus structure (Tzeng *et al.*, 2003). Ages were then determined by counting the number of annuli appearing on the otolith under a light microscope (Fig. 1).

Data analysis

The numeral variables being compared were tested for normality of distribution and homogeneity of variance by Kolomogrov-Smirnov and Barlett's tests, respectively. If these assumptions were not satisfied, the variables were then compared by non-parametric methods. The total lengths of silver eels between sexes, the *GSI* and *CF* between 1999 ~ 2003 and 2004 ~ 2006 were compared by the non-parametric Mann-Whitney U test. The sex ratio, namely proportion of females, in 1999 ~ 2003 remained about 71.7% and was set as a reference point for comparison with the proportion of female eels among 2004, 2005, and 2006 with the Chi-square test. The homogeneity of sex composition of silver eels caught among 2004, 2005 and 2006 was also tested with the Chi-square test of homogeneity (Daniel, 1987). Similarly, the migratory types were first tested for homogeneity between sexes. If the migratory types did not differ significantly between sexes, they were pooled and were then compared to the period from 1998 to 1999 (Tzeng *et al.*, 2003). The ages between migratory types were compared with the non-parametric Mann-Whitney U test for both sexes. The ages of the silver eels, where migratory history types were pooled, were compared to the previous level of 6.43 years in females and 5.33 years in males (Tzeng *et al.*, 2003) by the nonparametric Wilcoxon Signed Rank test. The significance level α of all tests was set at 0.05.

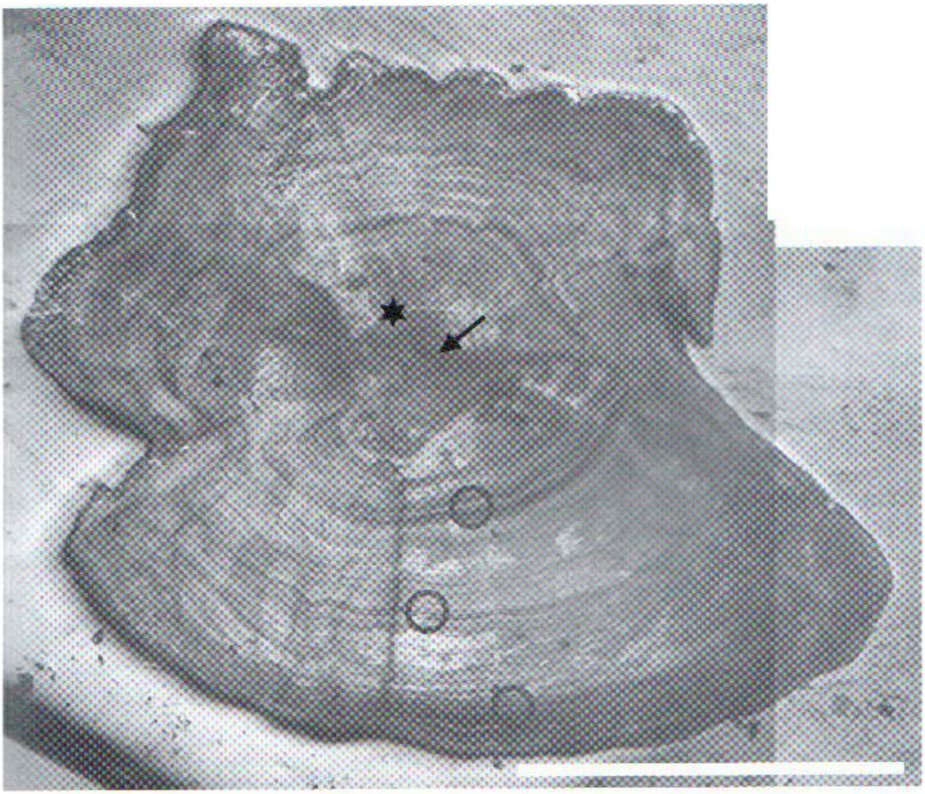

Fig. 1. Annuli in an otolith of *Anguilla japonica* (L_T = 504 μm) under reflected light. Arrow = primordium, circles = annuli and the star = the metamorphosis check deposited at the transition from leptocephalus to glass eel. Scale bar = 1 mm.

RESULTS

Total lengths and sex ratios

A total of 320 silver eels, including 152 females and 168 males, were caught in the lower reaches of Kao-Ping River from 1999 to 2006 (Table 1). Mean total length (TL ± SD) of silver females was 610 ± 90 mm in the period from 1999 to 2006, which was significantly larger than males (533 ± 56 mm) (Mann-Whitney U test, $p < 0.001$). The sex ratio (proportion of silver female eels) did not differ significantly among 2004 (24.5%) and 2005 (22.4%) (Test of homogeneity, $\chi_5^2 = 8.22$, $p > 0.1$). However, the sex ratios in 2004 and 2005 were respectively significantly lower than the mean from 1999 to 2003 (71.7%, $\chi_1^2 = 69.5$ and 103.5, all $p < 0.001$). The sex ratio in 2006 returned to the previous level, being not significantly different from 71.7% ($\chi_1^2 = 4.3$, $p > 0.05$) (Table 1).

Migratory histories

The migratory histories of 17 female and 27 male silver eels caught after the attack of Typhoon Mindulle, i.e. from 2004 to 2006, were determined using otolith Sr:Ca ratios. Silver eels in the sample were classified as two types: (1) freshwater type (FW): the otolith Sr:Ca ratios after elvers stage were all below 4.0×10^{-3}, indicating a consistent residency in freshwater (Fig. 2a) and (2) brackish-water type (BW): the otolith Sr:Ca ratios in the yellow and silver eel stage fluctuated around 4.0×10^{-3}, indicating a movement between fresh-and brackish waters (Fig. 2b). The compositions in migratory histories of silver eels during this period did not differed between sexes (Test of homogeneity, $\chi_5^2 = 5.45$, $p > 0.1$) and therefore the sex was pooled. More than 80% of the eels examined were of the FW eels while the remaining 20% were the BW eels. Moreover, the proportion of BW silver eels during 2004 to 2006 was significantly lower than 90% ($\chi_1^2 = 252$, $p < 0.001$), which is the proportion of BW eels during 1998 to 1999 before Typhoon Mindulle (Tzeng *et al.*, 2003). FW silver eels increased and the BW ones decreased significantly, implying possible contribution of the escaped cultured eels rearing in freshwater ponds.

Ages of the silver eels

The mean age of FW female silver eels was 2.9 ± 0.5 year and 2.4 ± 0.5 years for males, while female BW silver eels averaged 5.3 ± 0.8 years and 4.0 ± 1.4 years for males collected during 2004 to 2006. FW silver eels of both sexes during the period 2004 to 2006 were significantly younger than BW type silver eels (Mann-Whitney U test, $p < 0.05$) (Fig. 3). When the migratory histories of the silver eels from 2004 to 2006 were pooled, the ages of both sexes were significantly younger than for the years from 1998 to 1999 (Tzeng *et al.*, 2003, Wilcoxon Signed Rank test, $p < 0.001$), which was used as a standard age before escapement

Table 1. Sample size (N) and mean total length (*TL* ± SD, mm) and weight (*TW* ± SD, g), by sex, of silver Japanese eels caught in the lower Kao-Ping River from 1999 to 2003 (pooled), 2004, 2005 and 2006. Sex ratio (SR) = proportion of silver females; different superscripts indicate statistically significant differences (Chi-square test, $\alpha = 0.05$). The months and numbers in brackets indicate the months and sample size for microchemistry analysis and age determination

Year	SR	Female			Male		
		N	TL	TW	N	TL	TW
99' ~ 03'	71.7[a]	104	621 ± 72	421 ± 158	41	546 ± 67	245 ± 93
04' (Jul ~ Dec)	24.5[b]	23 (4)	599 ± 112	381 ± 275	71 (15)	527 ± 43	196 ± 61
05' (Nov)	22.4[b]	13 (4)	537 ± 60	241 ± 87	45 (3)	540 ± 54	230 ± 80
06' (Jan ~ Sep)	52.2[a]	12 (9)	609± 154	417 ±310	11 (9)	497 ± 54	176 ± 87
Overall	47.5	152 (17)	610 ± 90	399 ± 196	168 (27)	533 ± 56	216 ± 83

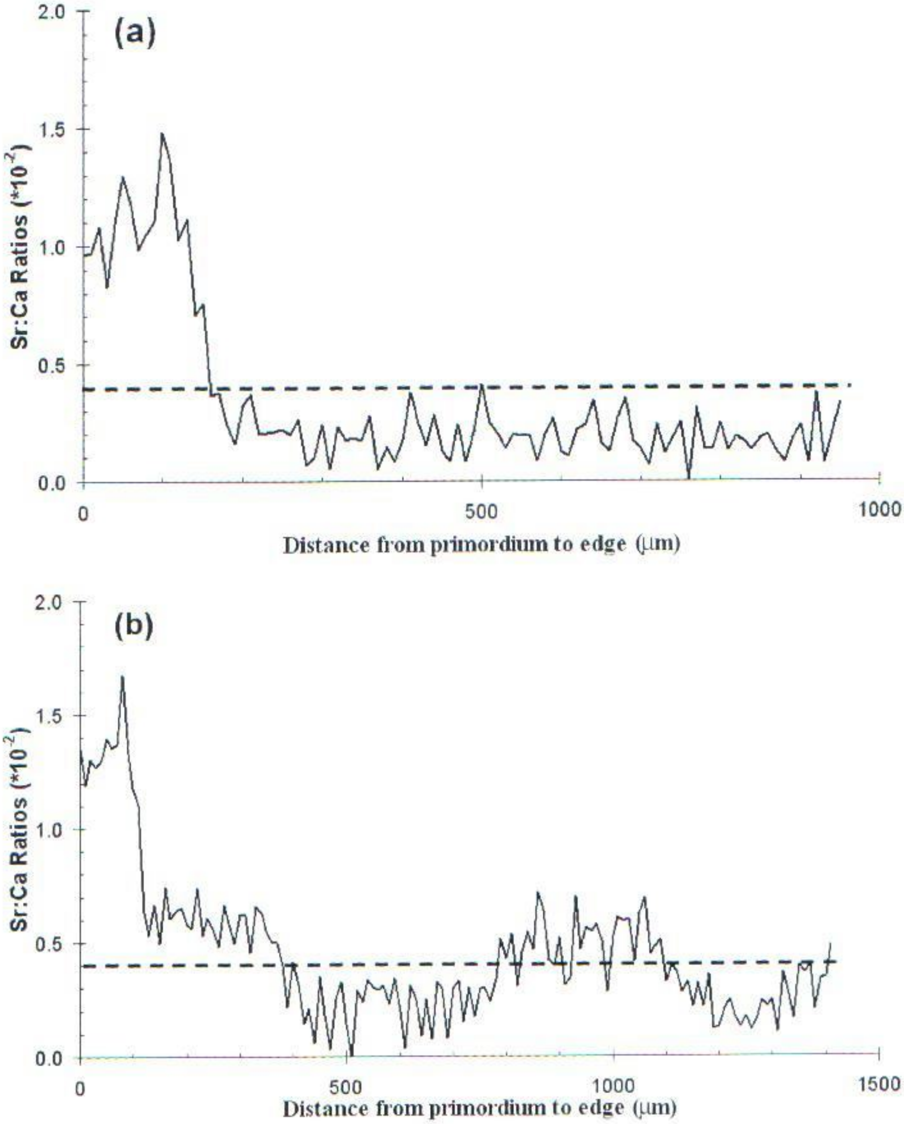

Fig. 2. Sr:Ca ratios in silver Japanese eel otoliths. (a) Freshwater type (Male, L_T = 478 μm). (b) Brackish water type (Female, L_T = 560 μm). The arrow indicates the metamorphosis check.

of cultured eels. This suggests that the escaped cultured eels possibly able to become silver eels at a younger age in the wild than do wild eels.

Gonado-somtic index (*GSI*) and condition factor (*CF*)

The mean gonado-somatic-index (GSI ± SD) and relative condition factor (CF ± SD) of female eels was 9.48 ± 5.87 × 10^{-3} and 1.02 ± 0.17 during 1999 to 2003 and 8.64 ± 6.02 × 10^{-3} and 0.96 ± 0.16 during 2004 to 2006, respectively. Mean *GSI* and *CF* of males was 1.51 ± 0.71 × 10^{-3} and 1.05 ± 0.14 during 1999 to 2003, respectively and 1.52 ± 0.58 × 10^{-3} and 0.98 ± 0.12 during 2004 to 2006 (Table 2). The mean *GSI* was

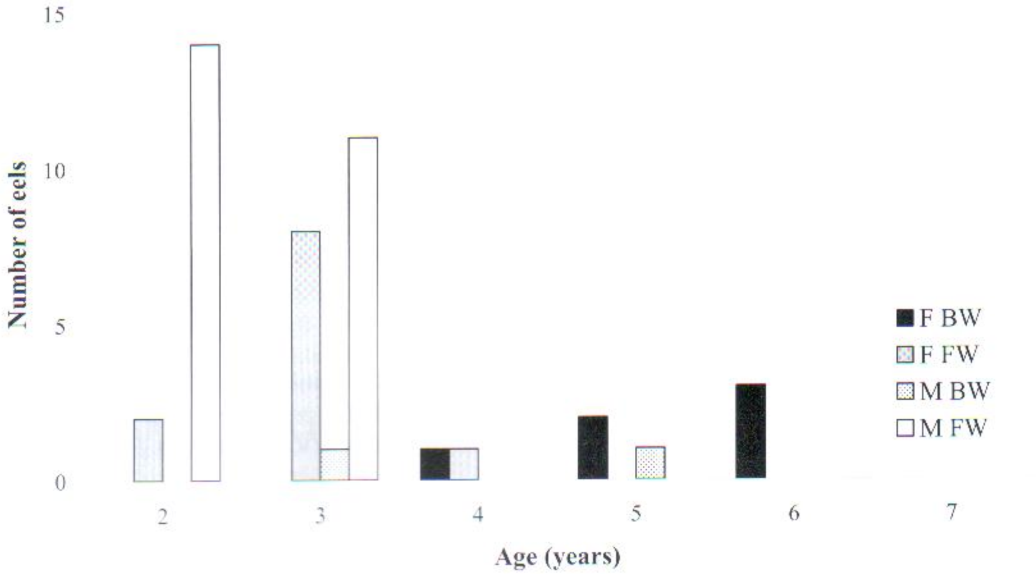

Fig. 3. Age distribution, by sex and migratory type, of silver Japanese eels caught in the Kao-Ping River from 2004 to 2006. F = females, M = Males, BW = brackish-water type, FW = freshwater type.

Table 2. Comparisons of gonado-somatic index (*GSI*) and relative condition factor (*CF*) of silver eels caught between 1999 ~ 2003 and 2004 ~ 2006 in both sexes

	Female		Male	
Period	99' ~ 03'	04' ~ 06'	99' ~ 03'	04' ~ 06'
N^a	95	48	30	127
GSI ($\times 10^{-3}$)	$9.48^{ab} \pm 5.87$	$8.64^{a} \pm 6.02$	$1.51^{c} \pm 0.71$	$1.52^{c} \pm 0.58$
CF	$1.02^{a} \pm 0.17$	$0.96^{a} \pm 0.15$	$1.05^{c} \pm 0.14$	$0.98^{d} \pm 0.12$

[a] N = sample size.
[b] Different superscripts in *GSI* and *CF* indicated significant difference between periods (Mann-Whitney U test, $\alpha = 0.05$).

not significantly different between the two periods for both sexes (Mann-Whitney U test, all $p > 0.3$). The mean *CF* did not differ significantly between different periods for females (Mann-Whitney U test, $p = 0.09$), but was significantly smaller during 2004 to 2006 than in 1999 ~ 2003 for the males (Mann-Whitney U test, $p = 0.0043$). The *GSI* of the silver eels was evidently not influenced by the escapement of cultured eels, which might imply the *GSI* of the cultured and wild silver eels were similar in both sexes in the wild environment. But the *CF* was lower after the escapement, at least for males.

DISCUSSION

Sex ratio skewed to female

The sex determination mechanism of the anguillid eels is not fully understood, but the population density is one possible factor influencing the sex of eels (Tesch, 2003; Davey and Jellyman, 2005; Han and Tzeng, 2006). Eels in the high-density culturing ponds were male-dominated, ranged from 70% to 90% male (Beullens *et al.*, 1997; Tesch, 2003; Davey and Jellyman, 2005). Wild eels in the Kao-Ping River were female-dominated in previous years (Han *et al.*, 2001; 2003; Tzeng *et al.*, 2003) and the population density was estimated to be low (Chu, *et al.*, 2006; Han and Tzeng, 2006), which might favor the development of females. Although other environmental factors such as temperature and salinity might affect the sex determination of the eels (Davey and Jellyman, 2005), it seems unlikely that these factor had changed drastically in the Kao-Ping River in 2004, so as to favor the sex determination for the males. Thus, the dramatic change in silver eel sex ratios in the Kao-Ping River might indicate the influx of cultured eels that escaped from eel farms as proposed in the previous study (Chu *et al.*, 2006).

Changes in compositions of migratory history and age

The migratory history composition of silver eels during 2004 to 2006 was dominated by FW eels, which was different from the dominance of brackish- and seawater-types eels with less than 10% FW silver eels in the years before 2004 (Tzeng *et al.*, 2003). Cultured eels in the Kao-Ping River were reared in freshwater ponds until being flushed into the river before Typhoon Mindulle, and thus the migratory histories of the cultured eels are freshwater-type. Consequently, the change in dominance of FW silver eel indicates that these escaped cultured eels possibly became silver eels.

The ages of FW silver eels were significantly younger than those of BW ones, which contradicted the expectation that silver eel age would be older in freshwater than in brackish and seawater because the eels need more time to reach maturation size in a less-productive freshwater habitat than brackish and seawater habitats (Morrison and Secor, 2003; Kotake *et al.*, 2005). Moreover, when the migratory histories were pooled, both sexes of silver eels collected during 2004 to 2006 were also significantly younger than those in the previous study, 5.3 years for males and 6.4 years for females (Han *et al.*, 2001; Tzeng *et al.*, 2003). The escaped culture eels were reared approximately 1.5 years before the coming of typhoon Mindulle (Chu *et al.*, 2006). The younger ages of silver eels after 2004 indicated the escaped eels contributed to the silver eels in the wild.

However, the duration of influence by the escaped cultured eels was probably not long. A male-dominated sex ratio was only apparent in 2004 and 2005 and recovered to female-dominated in 2006, suggesting that the escaped cultured eels disappeared in the period from 2004 to 2006 due to either death from failure to adapt and find appropriate habitats or becoming silver eels and following downstream migration. The possibility that cultured eels escaped during 2005 and 2006 might be small because the proportion of males declined gradually in this period. Although the coming of typhoons is common during summer in Taiwan, the escapement of cultured eels is not a common event.

Conditions needed to become silver eels

A critical body condition, such as a certain level of body fat content, was considered to be required for silvering of the eels and was used for long-term migration to the spawning ground and for further development of the gonads (van Ginneken and van den Thillart, 2000; van Ginneken and Maes, 2005). Moreover, field observations indicated that the length, rather than age, was probably more important in the silvering and spawning migration of eels (Vøllestad, 1992; Oliveira, 1999; Jellyman, 2001).

Since the silvering is highly related to the length and body fat content, the eels probably undergo silvering as long as they reach a critical size with a sufficient fat storage, even with a relatively younger age, which has been observed in the plaice *Hippoglossoides platessoides* (Roff, 1982). Cultured Japanese eels generally grow faster and are fatter than wild eels (Han *et al.*, 2000). Cultured European eels *A. anguilla* could develop to silver eels at a younger age and larger size (Beullens *et al.*, 1997). Exotic *A. anguilla* raised in eel farms in Japan sometimes escaped into the wild and were found moving downstream as silver eels, which had a higher growth rate than in their native habitats (Okamura *et al.*, 2002; Miyai *et al.*, 2004). Thus better growth potential and faster energy accumulation provided in eel farms might accelerate the silvering of the Japanese eels (Durif *et al.*, 2005).

The silvering process is also controlled by exogenous environmental factors such as water temperature, light, salinity, pressure, atmosphere depressions, increasing water level and lunar cycle (Okamura *et al.*, 2002; Miyai *et al.*, 2004). Once the large, fat cultured eels escaped into the river, they probably experienced the environmental cues necessary for silvering, which might be lacking in the eel farm. Consequently, escaped cultured Japanese eels with sufficient body condition could become silver eels similar to *A. anguilla* in capacity (Beullens *et al.*, 1997) and in Japan (Okamura *et al.*, 2002; Miyai *et al.*, 2004).

Different *GSI* and *CF* between cultured and wild eels

Even if the escaped cultured eels did become silver eels in the wild, were there any differences in body condition between cultured and wild silver eels? The insignificant differences in mean *GSI* for silver eels of both sexes between 1999 and 2003 and 2004 and 2006 suggests that the escaped culture eels might be able to attain a crude reproductive output (Gunderson, 1997) similar to that of wild eels of both sexes. However, the slightly lower mean *CF* in the males after 2004 suggests that the fatness of the male cultured eels was lower than for wild males. Mark-recapture experiments conducted in the Kao-Ping River found that the total mass of cultured eels generally decreased after release. The guts of these eels were usually empty, and the food remains were only found in two out of the 23 recaptured eels after a half year of releasing (Han, *unpublished data*; Lin, *unpublished data*). Cultured eels may have difficulty in finding sufficient food or suitable habitats which might result in reduced *CF*.

Ecological impacts and potential contributions to the wild eel population

The ecological impacts of escaped culture eels on the wild population seemed apparent. About 30,000 cultured eels escaped into the local population in the lower reaches of Kao-Ping River with estimated population size of 5,000 to 20,000 eels (Han and Tzeng, 2006), seeming over the capacity the river could support. Besides, these escaped cultured eels inevitably competed with wild eels for food and habitat. The ability of escaped cultured eels to find food and habitat was likely lower indicating a lower survival rate in the wild.

Although the survival rate of cultured eels was likely lower in the wild, some individuals could become silver eels in the wild, indicating their potential contribution to the wild silver spawning population. However, can these silver escaped cultured eels find their way to the spawning ground? European eels had been transplanted from France and England estuaries to freshwaters of Baltic countries for restocking, but it is still controversial whether the restocked silver eels could find the correct path out of the Baltic Sea. Westin (1998, 2003) indicated that they face difficuty to find paths out because of lack of orientation imprinting, but Limburg *et al.* (2003) indicated that some can. Cultured Japanese eels were all caught from estuaries as elvers from the same spawning ground. Thus, the imprinting for orientation was likely the same for cultured and wild eels, as long as these escaped cultured eels came as elvers from the Kao-Ping River. Moreover, if the escaped cultured eels did join the wild silver eel population, the genetic diversity of the wild population was probably less influenced because the cultured and wild eels are all from the same panmictic population (Tseng *et al.*, 2006).

If some escaped cultured eels were able to contribute the silver eel population in the Kao-Ping River, the artificially releasing cultured eels seemed to be one possible action to replenish the declining eel population (Tatsukawa, 2003). However, before it become feasible, some points must be verified before artificially releasing cultured eels to for enhancement of the silver eel population: (1) Could escaped cultured eels that silvered eventually migrate to the spawning ground? These cultured eels may have experienced enough environmental cues to join in the spawning migration, but whether they migrate and spawn is still unknown, (2) Are the reproductive behaviors similar between cultured and wild eels? The reproductive behaviour of cultured salmons was found less active with reduced reproductive outputs (Jonsson, 1997), (3) Were the qualities of wild and cultured eel progeny similar, i.e., egg survival rate and mobility of leptocephali, which is related to the ability to find food and avoid predators? (4) Could the cultured eels provide sufficient spawning biomass, e.g., number of females? The release of cultured eels dominated by males limits the female contribution, but the appropriate female-male is unknown.

CONCLUSION

The sex ratios as proportions of females, types of migratory history, age and condition factor of Japanese eels in the Kao-Ping River differed significantly before and after the escapement of cultured eels, but the gonado-somatic index did not differ significantly. Some cultured eels have silvered in the wild and joined the spawning migration with wild eels, doing so in less than two years. Releasing appropriate numbers of cultured eels to the wild environment to enhance the potential silver eel spawning biomass might be feasible, but more comprehensive studies should be completed in advance of such a release.

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逃逸養殖鰻對高屏溪野生日本鰻族群的潛在貢獻

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2004年7月敏都利颱風肆虐，導致高屏溪河畔的鰻魚養殖池潰堤，許多養殖鰻流入溪中。為了瞭解這些逃逸養殖鰻對野生銀鰻族群的貢獻，我們檢查1999~2006年高屏溪銀鰻之年齡及性比，同時利用耳石鍶鈣比重建其洄游環境史。結果發現2004~2006年銀鰻之平均年齡比1999~2003期間顯著為小。2004年之前以雌性為優勢，然而2004~2005年改變成以雄性為優勢(均質性檢定，χ_1^2 = 69.5和103.5，機率皆小於 0.001)。可是2006年之後，性比就恢復到颱風之前的雌性優勢狀態(χ_5^2 = 8.22，機率大於 0.1)。銀鰻的洄游型也有明顯地變化，從1999~2003年期間以鹹淡水型為主，變成2004~2006年以淡水型為主(適合度檢定，χ_1^2 = 252，機率小於 0.001)。這些現象指明，逃逸養殖鰻對野生日本鰻族群的影響期間不超過兩年。有一部份逃逸養殖鰻在這兩年期間已達到銀化階段，並且有可能貢獻野生的產卵族群。

關鍵詞：日本鰻(*Anguilla japonica*)，養殖鰻，銀化，耳石鍶鈣比。

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