Integrated habitat monitoring of environmental features to site the releasing of cultivated *Oncorhynchus masou formosanus*

W Kuan¹, Y Chen², C Yeh³ and Y Huang⁴

¹Department of Safety, Health, and Environmental Engineering, Ming Chi University of Technology, New Taipei, Taiwan
²Department of Industrial Engineering and Management, Ming Chi University of Technology, New Taipei, Taiwan
³Department of Water Resources Engineering and Conservation, Feng Chia University, Taichung, Taiwan
⁴Department of Aquaculture, National Taiwan Ocean University, Keelung, Taiwan

E-mail: whkuan@mail.mcut.edu.tw

**Abstract.** The Formosan landlocked salmon (*Oncorhynchus masou formosanus*), an endangered species, had ever inhabited in the upper branches of stream Da-Chia of Taiwan in 1940s. However, the distribution of the salmon gradually declined and was only observed in stream Chi-Chia-Wan and stream Kao-Shan in last decades. Shei-Pa National Park has ever devoted to restoring the salmon and releasing the cultivated breeds into several historic rivers. Therefore, a comprehensive and systematic monitoring and assessment of the habitat candidates is indispensable to gain the successful propagation of released salmon. This study presents a methodology for determining the optimal juveniles’ releasing sites in association with the water quality and channel’s physical features. Five historic inhabiting streams with nine monitoring sites for cultivated breeds release were selected. Three reference sites were chosen as various levels, i.e. the best, the mild interfered, and the worst circumstance for the salmon, to evaluate the candidate sites. The three reference sites located in the stream where the Formosan salmon could naturally propagate. The results indicated that the overall habitat quality of stream Lo-Ye-Wei (potential releasing site Y#1) was superior to the other sites. The increase of fish population in the Lo-Ye-Wei was significant with a total of 1269 fish in 2012 over 2.5 times the amount of 519 fish in 2011. This significant boost of salmon population in Lo-Ye-Wei indicated that the siting strategy proposed in this study is feasible for the salmon release.

1. **Introduction**

The Formosan landlocked salmon (*Oncorhynchus masou formosanus*) is an endangered species enrolled in the red list of the International Union for Conservation of Natural resources. Originated during the Ice Age and endemic to Taiwan, the Formosan salmon were widely spread over the six upper streams of the Dachia Stream in 1940s. However, the extent of their habitat has been gradually reduced to three streams [1]. The population of the Formosan salmon ever declined to the lowest of about 200 individuals in 1984, while recovered to 1000 - 5000 individuals during the last decade [2]. The Shei-Pa National Park administration consecutively endeavors to conserve and restore the
Formosan salmon by hatching and releasing the sub-adult fishes to the upstream tributaries. The optimal site to release juvenile fishes was vital to successfully restore the Formosan salmon populations because suitable habitats provide the survival resources and circumstances [3]. Generally, habitats characteristics can be categorized of water quality, physical features and biotic components [4]. Hsu et al [2] indicated that fish tend to choose habitats with suitable combinations of two or more features rather than positions with preferred levels of a single attribute of habitat. Therefore, holistic monitoring and evaluation are imperative route to understand the habitat preference, thereby to determine optimal releasing sites.

An integrated project financially supported by Shei-Pa National Park administration was conducted for two years, aimed to monitor the water quality, physical features of stream channel, fish distribution, aquatic insects, and riparian vegetation. Additionally, the overall evaluating data was combined into an ecological database and compared to that of stream Chi-Chia-Wan in which the Formosan salmon naturally propagate in order to propose ecologically suggestions for the restoration of the Formosan salmon. This study revealed the perspectives of water quality and channel’s physical features associated with salmon distribution to determine the releasing site of sub-adult fishes.

2. Methods

2.1. Study sites
The study was conducted in the Shei-Pa National Park situated at the central of Taiwan, as figure 1 displayed. Five streams including Lo-Ye-Wei, Yu-Shan, I-Ka-Wan, La-Shan and Si-Jie-Lan stream were chosen as the potential releasing Creek because the salmon had ever inhabited there several decades ago. Because the Formosan salmon moves up and down stream in short distance [5], several potential releasing sites for monitoring ecological variables were selected depending on the conditions of a stream. In Lo-Ye-Wei and Yu-Shan stream up- and down-stream connected with each other, four potential sites were selected and denoted as site Y#1, Y#2, Y#3 and Y4. Three potential sites S#1, S#2 and S#3 were chosen in Si-Jie-Lan stream. One potential site I#1 and L#1 was chosen in I-Ka-Wan and La-Shan stream, respectively.

![Figure 1](image_url)

**Figure 1.** Potential releasing sites (blue dot) and reference sites (red dot) of the Formosan salmon in Shei-Pa National Park. R#1 denoted as the mild interfered site, R#2 represented as the best superior site, and R#3 with the worst circumstances for the salmon.
To determine the optimal releasing location, three sites on Chi-Chia-Wan stream in which the Formosan salmon can naturally propagate were also screened out for references, as figure 1 revealed. Reference site R#1 situated on the middle of the Chi-Chia-Wan stream bordering a main road, camping zone, and flower farms was denoted as the mild interfered site for the Formosan salmon. Reference site R#2 located on Kao-Shan stream surrounded by natural forest without human activity was denoted as the best site for the Formosan salmon. Reference site R#3, denotes as the worst level site because of the substantial use of fertilizer on the agricultural farms along the riparian Yu-Shan stream. Although this site situated in the conjugated and adjacent creek with the stream R#1 located, none of the Formosan salmon was ever observed at this site (R# 3) since 1984. In contrast, there is stable salmon population monitored at R#1 and R#2 sites.

2.2. Sampling and analysis
Site monitoring was conducted at bi-monthly intervals from 2010 to 2012. Each sampling sites is located at the center of the respective stream. Field measurements of temperature, dissolved oxygen, pH, and conductivity were conducted using a portable meter. Approximately 5 liters of surface water was acquired from the stream site, stored in polyethylene bottles at 4°C, and returned to the laboratory for chemical analysis. The samples were stored at 4°C prior to analysis, which was conducted within the specified time following standard methods. Measurements of NO₃ were conducted using ion chromatography ( Dionex ICS-1500) with an AS4A-SC column. The concentrations of NO₂ and NH₄⁺ were determined using a Perkin Elmer model Lambda 16 UV-vis spectrometer using the sulfanilamide-naphthlenediamine and distillation phenate addition methods, respectively. Phosphate was measured by the standard method 4500. The total organic carbon (TOC) in the samples was measured using a total organic carbon analyzer (O-I Analytical 1010).

To attain a natural balance, the physical features of stream channel constantly vary with time evolution. The description of the channel progression was performed through the monitoring of cross and longitude sections, substrate composition, as well as physical habitat patterns. A 20-metre transecting rope perpendicular to stream flow was established over the stream and the width of stream was measured firstly. The stream depth, current velocity, substrate composition, and physical habitat pattern were individually observed at 1/4, 1/2, 3/4 positions of length along the transecting rope. The substrate composition was classified to 6 types depending on the grain size, i.e. smooth surface (<0.2 cm), gravel (0.2 – 1.6 cm), pebble (1.6 – 6.4 cm), rubble (6.4 – 25.6 cm), small boulder (25.6 – 51.2 cm), and large boulder (>51.2 cm). Based on the Froude number (Fr =V/(gH)¹/²), the physical habitat pattern was defined as pool (Fr < 0.095), run (0.095 < Fr < 0.255), riffle (0.255 < Fr < 1), and rapid (Fr>1).

Since the endangered population of the Formosan salmon, underwater observation is the best and only way to count its occurrence in the streams. That is a non-destructive measurement and has minimal impact on the fish population and environment. This method has been used to evaluate fish abundances [6] and to investigate their habitat use, particularly concerning riverine salmonid populations [7].

The Formosan salmon populations were counted by snorkeling during the daytime. Counting started at the downstream end at each site (~ 300 m long at a maximum) and was completed in the upstream. The counting sites distance was determined by the geographical barriers, i.e. artificial dams and largely areal pool that significantly restrict the Formosan salmon movement to avoid double counting by different counting group. Each counting group comprised of three researchers, two snorkelers and one recording on bank. Two trained snorkelers slowly swam upstream in parallel with the main channel and counted fish outwards and towards the bank nearest to them. At the abundant sites underwater filming was simultaneously performed during the snorkelers’ counting.

2.3. Determine criteria and statistical analyses
To determine the suitable releasing site is a rather difficult task because the complicated causality among variety ecological factors. The European Council Directive 2006/44/EC of 6 September 2006
was aimed to protect and improve the quality of fresh waters that support or could support, certain species fish, including salmonid. Chen [9] and Chang [10] proposed the local guidelines for water quality of the stream the Formosan salmon inhabit. Through underwater observation, Tsao et al [11] indicated that the suitable microhabitat depended on the life stages of the salmon. The preliminary screening of the suitable releasing site was carried out by conducting the box-whisker plot for all monitoring variables, subsequently subject to the rule out based on the relative criteria proposed in precedent literatures.

To elucidate the relationship between ecological variables and salmon populations, the monitoring data was firstly adjusted to normal distribution using Johnson transformation method. The transformed data was then used to find the significant candidate predictors for predicting the salmon population using multiple stepwise regression method (α= 0.15) by Minitab software V.16.

3. Results and discussions

The hypothesis of siting is that the most suitable releasing site could have similar habit features with that of the stream section the salmon naturally propagate. And by analogy, the unsuitable releasing site could possess the characteristics resulting in the depressed salmon population. In this study, three levels of reference site were referred to the best, the mild-interfered, and the worst site for salmon. Besides, several ecological variables have been widely investigated regarding the suitable extent for salmon living in literatures. Both the reference sites’ conditions and suitable extent of ecological variables were incorporated into the determination of Formosan releasing site. Based on the literature suggestions [12-15], ten environmental features, 6 items of water quality and 4 items of physical features, were selected as indices from 32 ecological variables monitored in the field.

Although the food availability of the Formosan salmon was not discussed in the study, it’s indeed an important factor for the salmon population. Aquatic invertebrates were the major prey in the diet of the Formosan salmon [16]. When water quality degrades, the taxa richness of insect, ratio of scrapers to filtering collectors, and ratio of shredders to total insects would decrease, which led to the decrease of salmon food availability.

The box-whisker plot of 6 water quality factors including temperature, pH, phosphate, total ammonium, nitrite, and nitrate were displayed in figure 2. The indicated suitable extent of these six water variables was marked as blue shadow in figure 2. Each specific site was marked as red circle if its water quality condition fell out of the suitable extent.

The pH value of stream water beyond the range of 6.5 and 8.5 could deteriorate the surface cell of fish gill, subsequently hinder the respiration of the salmon [10]. Water temperature was indicated as one of the most important factors for the Formosan life. The optimum temperature for the salmon living ranges from 5-17°C [8]. The temperature of hatchery is narrow down to the extent of 7-12.5°C. The high temperature leads to the low dissolved oxygen and the lethal effect on the salmon eggs. The dissolved phosphate relates to eutrophication in a stream. The unpolluted aquatic system the Formosan salmon living is 0.01 mg/L [9].

In contrast, nitrogen was believed as the more crucial element than phosphorus to the salmon, even though both were regarded as the nutrient to the eutrophication in aquatic ecosystem [17]. The European Council issued a Directive stating the concentration of nitrite, non-ionized ammonia, and total ammonium must not exceed 3 μg as N/L, 4.1 μg as N/L, and 0.03 mg as N/L, respectively, for the streams supporting salmonid fisheries. Exposing to the continuous high levels of ammonia, the growth rate and disease resistance of salmon reduce, while the gill ventilation, metabolic rate, and erratic movements increase [18]. Nitrite converts oxyhemoglobin to methemoglobin, which is incapable of oxygen transport, leading to the toxicity to salmon [17]. Therefore, the potential releasing site with nitrogen species concentration similar to the superior reference site R#2 were marked as green circle (figure 2) and referred to the highly potential sites for releasing.

The Formosan salmon at various life stages showed the difference preference to the physical features of habitat [19]. Figure 3 showed the monitoring results of physical features at each site. The adult salmon favored slow and shallow sites (pool type of physical habitat pattern) for resting, and fast
velocity and deep sites (riffle type) for feeding. The juvenile salmon preferred to inhabit at site with deep water and moderate current (run type) [11]. The large boulder substrate could provide the refuge for the adult salmon in the flooding event, demonstrated being the crucial factor for salmon population [2]. The channel slope indicated the overall condition of a stream rather a microhabitat feature. A certain slope provided the food source for salmon and water flow for maintaining water physic-chemical quality, however, the steep slope of stream leaded to the severe erosion of channel and even caused the drought or shallow stream in the dry season. Therefore, 3% - 7% of channel slope was suggested a suitable extent for salmon inhabiting [11]. In figure 3 the criterion of channel slope was used both for selecting the highly potential releasing site (green circle) and ruled-out site (red circle).

Figure 2. Box-whisker plot for water quality variables in reference and candidate sites. The blue shadow area represents the suitable extent for the Formosan salmon survival. The red circles indicate the conditions were ruled out based on the salmon survival conditions published in literatures. The green circles express the superior conditions for the salmon’s life.

The releasing of the juvenile and/or sub-adult salmon have ever launched since 2006. There were 250 cultivated salmon released at site S#1 in 2006, 160 at site S#3 in 2007, 300 at site I#4 in 2008, 100 at site S#3 and 150 at site Y#1 in 2009. In 2010, an enhanced releasing task was conducted to rapid enlarge the population of salmon in stream, therefore, 120, 180, and 350 cultivated salmon were released at site S#3, S#1, and Y#1, respectively. According to the monitoring of released and wild-born salmon, the salmon population significantly increased in Lo-Ye-Wei stream from a total of 1269 salmon in 2012 over 2.5 times the amount of 519 salmon in 2011, which was originated from the 150 (released in 2009) and 350 (released in 2010) parental salmon released at site Y#1. Besides, the natural habitat was also found to expand to approximate 2 Km downstream of releasing site Y#1. However, none of the other released streams Si-Jie-Lan (S#1, S#2, and S#3), I-Ka-Wan (I#1), and La-Shan (L#1) showed a significant wild-born salmon population. The monitoring of fish population revealed that the releasing task at site Y#1 of Lo-Ye-Wei stream is quite successful and the wild-born salmon could stably propagate and expand their living habitat to the downstream site Y#2, even Y#3.
Figure 3. Box-whisker plot for channel physical features in reference and candidate sites. The blue shadow area represents the suitable extent for the Formosan salmon survival. The red circles indicate the conditions were ruled out based on the salmon survival conditions published in literatures. The green circles express the superior conditions for the salmon’s life.

Table 1. Integrated evaluation of releasing sites.

| Stream site | Se-Jei-Lan stream | Lo-Ye-Wei & Yu-Shan stream | I-Ka-Wan | La-Shan |
|-------------|-------------------|----------------------------|----------|---------|
| S#1         | S#2              | S#3                        | Y#1      | Y#2     | Y#3     | Y#4 | I#1 | L#1 |
| Temp.       | -                | -                          | -        | -       | -       |     |     |     |
| pH          | -                | -                          | -        | -       | -       |     |     |     |
| PO₄³⁻       | -                | -                          | -        | -       | -       |     |     |     |
| NH₄⁺        | -                | +                          | +        | -       | -       | -   |     |     |
| NO₂⁻        | +                | +                          | +        | -       | +       | +   |     |     |
| NO₃⁻        | +                | +                          | +        | -       | +       | +   |     |     |
| Pool        | -                | -                          | +        | -       | +       | +   |     |     |
| Run         | -                | -                          | -        | -       | -       | -   |     |     |
| Slope       | +                | -                          | -        | +       | -       | -   |     |     |
| Large boulder | +              | +                          | +        | +       | +       | +   |     |     |
| Overall evaluation | -2 | -2 | +3 | +6 | +1 | -4 | -3 | -1 | +1 |
| Salmon observed | 2 | n.o.{superscript:5} | 5 | 853 | 289 | n.o.{superscript:3} | n.o.{superscript:3} | n.o.{superscript:3} | n.o.{superscript:3} |

* Census of the Formosan salmon monitored in Oct. 2012.  
{superscript:3} The salmon population was observed downstream site Y#2 but away from Y#3.  
{n.o.} No salmon observed.

Because the Formosan salmon favored the habitats with suitable combinations of two or more
features rather positions with preferred levels of a single attribute of habitat [2], a holistic assessment for each monitoring site was revealed in Table 1. Plus and minus symbols were given based on the aforementioned criteria for the Formosan salmon living, i.e., green and red circle marks in figure 2 and 3. The weighting of individual monitoring variable was not considered here. The highest grade of site Y#1 was obtained with none of the monitoring variables evaluated as minus level. After a serial releasing task conducted at S#1, S#3, Y#1, I#1, and L#1, the census of salmon in Oct. 2012 revealed that there are the highest salmon population occurred at Y#1, the releasing site of Lo-Ye-Wei. The highest salmon population at Y#1 was consistent with the result of integrated habitat assessment. The overall habitat quality of site Y#1 was similar with the superior reference site. Both cultivated parental and wild-born offspring salmon expand to the Yu-Shan stream downstream connected to Lo-Ye-Wei stream. Therefore, a significant salmon population also appeared at site Y#2 and approximate 2 Km downstream, even though the releasing overall environmental evaluation (+1 for Y#2) not as good as that at Y#1 (+6). It was also noted that the salmon population at S#3 did not show significant propagation even a relatively good grade of “+3” obtained. This phenomenon could be attributed to the channel slope far beyond the suitable extent for the salmon (figure 3).

To gain the insight of the significant ecological variables on the salmon population, a multiple stepwise regression was conducted on the population of 2-yr-old adult salmon at site Y#1 with each item of water quality and physical feature, results were in Table 2. The significant water quality variables relating to 2009 wild-born salmon population were ammonium, nitrate, and chloride, while that for incubator-born salmon were total inorganic nitrogen (TIN), pH and phosphate. The results suggested that the nitrogen species concentration in stream could dominate the salmon population and were responsible for 41% - 64% variance of all water quality variables. Regarding physical features, the independent variable (or predictor) rubble and pebble accounted for 88% and 89% of the total variance in the wild-born and incubator-born salmon population, respectively.

| 2-yr-old fish population | Predictor | R²   | Increased R² | p    |
|-------------------------|-----------|------|--------------|------|
| 2009 wild-born          | Water quality |      |              |      |
|                         | NH₄⁺      | 0.4131 | ---          | 0.005|
|                         | NO₃⁻      | 0.6447 | 0.2316       | 0.029|
|                         | Cl⁻       | 0.7895 | 0.1448       | 0.064|
|                         | Physical features |  |              |      |
|                         | rubble    | 0.8755 | ---          | 0.019|
| 2010 released (2009 incubator-born) | Water quality |  |              |      |
|                         | TIN       | 0.4611 | ---          | 0.002|
|                         | pH        | 0.7615 | 0.3004       | 0.019|
|                         | PO₄³⁻     | 0.8486 | 0.0871       | 0.085|
|                         | Physical features |  |              |      |
|                         | pebble    | 0.8875 | ---          | 0.008|
|                         | smooth surface | 0.9914 | 0.1039       | 0.039|

4. Conclusions
Integrated habitat monitoring to site the releasing of cultivated the Formosan salmon was successful to restore the salmon population in Lo-Ye-Wei stream. The overall habitat quality of site Y#1 was similar with the superior reference site. Both cultivated parental and wild-born offspring salmon expand to the Yu-Shan stream downstream connected to Lo-Ye-Wei stream. The results of multiple stepwise regression indicated that the nitrogen species concentration in stream accounts for most variance over all water quality variables; moreover, the rubble and pebble predictors even dominate
the total variance of physical features in the salmon population.

Acknowledgment
The authors would like to thank the financial support of Shei-Pa National Park in Taiwan.

References
[1] Kuan W H and Chen Y L 2014 Land-use type of catchment varying nitrogen cycle in an endangered salmon inhabited stream Environ Eng Manag J 13 971-8
[2] Hsu C B, Tzeng C S, Yeh C H, Kuan W H, Kuo M H and Lin H J 2010 Habitat use by the critically endangered Formosan landlocked salmon Oncorhynchus masou formosanus in mountain streams of subtropical Taiwan Aquat Biol 10 227-39
[3] Morrison M L, Marcot B G and Mannan R W 2006 Wildlife-Habitat Relationships: Concepts and Applications (Washington, D.C.: Island Press)
[4] Marcus D M, Young M K, Noel L E and Mullan B A 1990 Salmonid habitat relationships in the Western United States: A review and indexed bibliography GenTech Rep RM-188, USDA Forest Service
[5] Gwo H O, Huang Y S and Ueda H 2009 Site fidelity of and habitat use by the Formosan landlocked salmon (Oncorhynchus masou formosanus) during typhoon season in Chichiawan Stream, Taiwan as assessed by nano-tag radio telemetry Zool Stud 48 460-7
[6] Slaney P A and Martin A D 1987 Accuracy of underwater census of trout populations in a large stream in British Columbia N Am J of Fish Manag 7 117-22
[7] O’Neal J S 2007 Snorkel surveys Salmonid Field Protocols Handbook – Techniques for Assessing Status and Trends in Salmon and Trout Populations D H Johnson, B M Shrier, J S O’Neal, J A Knutzen, X Augerot, T A O’Neil and T N Pearsons eds. (Bethesda, MD: American Fisheries Society) pp 325-40
[8] The European Parliament and the Council Directive 2006 On the quality of fresh waters needing protection or improvement in order to support fish life Official Journal of the European Union L264 20-31.
[9] Chen H C 1998 Planning and monitoring the water quality of streams in Wuling area (Taiwan, ROC: Report of Bureau of Shei-Pa National Park)
[10] Chang S J 1989 The planning of the Formosan salmon protective area (Council of Agriculture, Executive Yuan of Taiwan, ROC) (in Chinese)
[11] Tsao E H 1995 An ecological study of the habitat requirements of the Formosan landlocked salmon (Oncorhynchus masou formosanus) Ph.D. thesis, Colorado State University, USA
[12] Lin J Y, Tsao E H, Lee T C and Yu S L 2004 Stream physical parameters and habitat requirement: The case of the Formosan salmon Eco Eng 22 305-9
[13] Ileva N Y, Shibata H, Satoh F, Sasa K and Ueda H 2009 Relationship between the riverine nitrate, Áininoren concentration and the land use in the Teshio River watershed, North Japan Sustain Sci 4 189-98
[14] Tung C P, Lee T Y, Huang J C, Perng P W, Kao S J and Liao L Y 2014 The development of stream temperature model in a mountainous river of Taiwan Environ Monit Assess 186 7489-503
[15] Nelson M C, Hocking M D, Harding J N, Harding J M, Reynolds J D and Magnan P 2015 Quantifying the effects of stream habitat on populations of breeding Pacific salmon Can J Fish Aquat Sci 72 1469-76
[16] Liao L Y, Chiu M C, Huang Y S and Kuo M H 2012 Size-dependent foraging on aquatic and terrestrial prey by the endangered Taiwan Salmon Oncorhynchus masou formosanus Zool Stud 51 671-8
[17] Lewis W M and Morris D P 1986 Toxicity of nitrite to fish: A review Trans Am Fish Soc 115 183-95
[18] Knoph M B 1992 Acute toxicity of ammonia to Atlantic salmon (Salmo salar) parr Comp
[19] Chung L C, Lin H J, To S P, Tzeng C S, Yeh C H and Yang C H 2008 Relationship between the formosan landlocked salmon Oncorhynchus masou formosanus population and the physical substrate of its habitat after partial dam removal from Kaoshan stream, Taiwan *Zool Stud* 47 25-36