Factors Controlling Changes in Epilithic Algal Biomass in the Mountain Streams of Subtropical Taiwan

Yi-Ming Kuo¹, Hwa-Lung Yu², Wen-Hui Kuan³, Mei-Hwa Kuo⁴, Hsing-Juh Lin⁵,⁶*

¹ Laboratory of Basin Hydrology and Wetland Eco-restoration, School of Environmental Studies, China University of Geosciences, 430074 Wuhan, China, ² Department of Bioenvironmental Systems Engineering, National Taiwan University, Taipei 10617, Taiwan, ³ Department of Safety, Health and Environmental Engineering, Ming Chi University of Technology, New Taipei City 24301, Taiwan, ⁴ Department of Entomology, National Chung Hsing University, Taichung 402, Taiwan, ⁵ Department of Life Sciences and Research Center for Global Change Biology, National Chung Hsing University, Taichung 402, Taiwan, ⁶ Biodiversity Research Center, Academia Sinica, Taipei 115, Taiwan

hjlin@dragon.nchu.edu.tw

Abstract

In upstream reaches, epilithic algae are one of the major primary producers and their biomass may alter the energy flow of food webs in stream ecosystems. However, the overgrowth of epilithic algae may deteriorate water quality. In this study, the effects of environmental variables on epilithic algal biomass were examined at 5 monitoring sites in mountain streams of the Wuling basin of subtropical Taiwan over a 5-year period (2006–2011) by using a generalized additive model (GAM). Epilithic algal biomass and some variables observed at pristine sites obviously differed from those at the channelized stream with intensive agricultural activity. The results of the optimal GAM showed that water temperature, turbidity, current velocity, dissolved oxygen (DO), pH, and ammonium–N (NH₄–N) were the main factors explaining seasonal variations of epilithic algal biomass in the streams. The change points of smoothing curves for velocity, DO, NH₄–N, pH, turbidity, and water temperature were approximately 0.40 m s⁻¹, 8.0 mg L⁻¹, 0.01 mg L⁻¹, 8.5, 0.60 NTU, and 15°C, respectively. When aforementioned variables were greater than relevant change points, epilithic algal biomass was increased with pH and water temperature, and decreased with water velocity, DO, turbidity, and NH₄–N. These change points may serve as a framework for managing the growth of epilithic algae. Understanding the relationship between environmental variables and epilithic algal biomass can provide a useful approach for maintaining the functioning in stream ecosystems.

Introduction

The upstream reaches of the Dajia River, located in the Wuling basin of the Shei-Pa National Park in central Taiwan at approximately 1800 m above sea level, are the only habitats of the Taiwanese masu salmon (Formosan landlocked salmon; Oncorhynchus masou formosanus).
Because of its limited population and narrow distribution, the The International Union for Conservation of Nature (IUCN) listed the Taiwanese masu salmon as a critically endangered species in 1996 [1]. Epilithic algae are one of the major primary producers in streams and play major roles in controlling energy flow of food webs in stream ecosystems [2–4]. However, the overgrowth of epilithic algae may deteriorate water quality [5]. The variation of epilithic algal biomass may indirectly affect the distribution and population of the Taiwanese masu salmon.

The production and dynamics of an epilithic algal composition in stream ecosystems are also largely influenced by physical variables such as geochemical conditions, flow rate, current velocity, light, and water temperature [6–8]. Water quality variables (electrical conductivity, pH, total dissolved solids, chemical oxygen demand, biochemical oxygen demand, and nutrients such as phosphorus and nitrogen from the surrounding lands) also play major roles in regulating the production rate and species composition of epilithic algae in streams [9–13]. However, in complex stream ecosystems, the dynamics of epilithic algal biomass may nonlinearly interact with the combination of abiotic and biotic factors. A technique should be employed to describe the nonlinear relationships between epilithic algal biomass and aforementioned factors.

A generalized additive model [14], which is an extension of a generalized linear model [15], enables analyzing nonlinear effects such as additive functions and smooth components in explanatory variables. GAM has been extensively applied in ecological studies such as modeling habitat suitability and ecological relationship [16–19], algal bloom analysis in Lake Taihu [20], landslide susceptibility analysis [21], geomorphological distribution modeling in a complex terrain [22–23], and air pollution research [24–25]. Ecological data tend to extremely noisy and heterogeneous. Therefore, the GAM may facilitate improving our understanding of dynamics and controlling factors of epilithic algal biomass.

Despite the availability of strong evidence of effects of nutrients and environmental factors on epilithic algal growth in numerous rivers, most studies on epilithic algae have been conducted in low-altitude streams of temperate climate regions [26–29]. In the current study, chlorophyll a (Chl-a) was used to estimate epilithic algal biomass [30]. Water quality, environmental variables, and Chl-a were examined in high-altitude streams of subtropical climates. The specific study objectives were to apply the GAM to determine which key abiotic and biotic factors (as explanatory variables) considerably influence epilithic algal biomass and provide insight into how algal biomass nonlinearly responds to the data range of aforementioned key factors. Evaluating and maintaining the growth of epilithic algae, which consequently provides a habitat with sufficient food for Taiwanese masu salmon, necessitates the long-term monitoring of the temporal variations in epilithic algal biomass and key controlling factors.

Materials and Methods

Study area

The upstream reaches of the Dajia River located in the Wuling basin of the Shei-Pa National Park comprises 3 third-order streams [Chichiawan (CCW), Yousheng (YS), and Kaoshan (KS) Streams] and 2 second-order streams [Taoshan West (TW) and Taoshan North (TN) Streams] (Fig 1). The CCW and KS Streams are the only habitats of the Taiwanese masu salmon. The CCW, YS, and KS Streams are characterized as short, straight, and steep channels, respectively, and are often influenced by heavy storms. The mean discharge of the upstream Dajia River in the dry season was 1.84–2.30 m$^3$ s$^{-1}$ and that in the wet season was 2.58–2.96 m$^3$ s$^{-1}$ [31]. The mean annual water temperature was 12˚C, ranging from 10˚C in winter to 18˚C in summer [32]. The mean annual precipitation is 1640 mm and the mean monthly rainfall typically did
not exceed 40 mm in the dry season of October–April. However, the mean rainfall frequently exceeded 300 mm month$^{-1}$ in the wet season of May–September [5].

Epilithic algae were monitored at 5 sites (Fig 1) that reflected the different cover levels of riparian vegetation and agricultural activities (Table 1). The permission for setting up these 5 monitoring sites was issued by the Shei-Pa National Park Authority. Diatoms were the most dominant taxa and contributed 85% of the total cell numbers of the epilithic algal communities in the streams of the Wuling basin [32]. Site 1 was located in the TW Stream. The watershed of

Table 1. Comparison of site characteristics in the upstream watershed of the Dajia River. Locations of study sites are shown in Fig 1. Sites 2 and 3 are located in CCW Stream.

| Site   | Elevation (m) | Channel slope (m km$^{-1}$) | Channel width (m) | Channel condition | Surrounding land use (refer to Tsai et al., 2013) | Stream attribute (length/area) |
|--------|---------------|-----------------------------|-------------------|-------------------|-----------------------------------------------|-----------------------------|
| Site 1 | 1900          | 41.6                        | 3–4               | Natural           | Pristine forest                               | TW Stream (13.8 km/41.6 km²) |
| Site 2 | 1790          | 128                         | 30–35             | Natural           | Moderate agricultural activity, some natural riparian forest | CCW Stream (15.3 km/76 km²) |
| Site 3 | 1742          | 132                         | 23–30             | Natural           | Pristine forest                               | KS Stream (10.6 km/40 km²)   |
| Site 4 | 1776          | 140                         | <10               | Natural           | Intensive agricultural activity, no natural riparian vegetation | YS Stream (11.4 km/31 km²)   |
| Site 5 | 1770          | 68                          | 10–15             | Channelized       |                                               |                             |

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the TW Stream vegetated by a pristine riparian forest. Sites 2 and 3 were located in the central and down reaches of the CCW Stream, respectively. Yeh [33] reported that the streambed comprised a high proportion of pebble (42%) in winter, but was dominated by rubble (26%) and boulders (21%) in summer. The upper reach of the CCW Stream is bordered by a riparian forest, but the central reach, where Site 2 was located, is developed for agriculture including an area of 104 ha of vegetables, apples, peaches, and pears [32]. Site 4 was located in the KS Stream in the pristine down reach of the CCW Stream. According to Yeh [33], the streambed was dominated by pebble (39%) and rubble (27%) in winter, but had a high proportion of boulders (44%) in summer. The surroundings of the KS Stream are vegetated by natural forests (no agriculture); therefore, this stream can be considered to be in a pristine state [34]. Site 5 was located in the downstream reach of the YS Stream. The streambed is dominated by gravel (39%) and pebble (39%) [33]. The YS Stream demonstrates relatively high nutrient concentrations and has been channelized and developed for agriculture since 1970s. Since Site 2 bordered by a riparian forest, Sites 1–4 were considered pristine forests, whereas Site 5 can be attributed to the agricultural activity.

Sample collection

At each site, samples of epilithic algae were collected bimonthly from randomly selected cobbles (n = 6) in the riffle zone during June 2006–June 2011 (5 y). On each cobble, a 12.5-cm² transparent steel frame was placed to define a sampling area of an algal patch. Four algal patches (total surface area of 50 cm² on each cobble) were scraped with a toothbrush, and the toothbrush and cobbles were subsequently washed with 50–100 mL of filtered stream water [32]. In the laboratory, the algal samples were homogenized and then centrifuged at 3500 rpm for 10 min to concentrate them to 5 mL. A 3-mL subsample was extracted for Chl-a concentration in 90% acetone for 24 h at 4˚C in the dark and analyzed spectrophotometrically [35].

At each sampling site, the water temperature (TEMP), pH, conductivity (EC), turbidity, and dissolved oxygen (DO) of the stream were measured in situ by using YSI 600XLM multi-parameter monitoring systems and portable meters (YSI Inc., Yellow Springs, OH, USA). The current velocity was measured at the upstream 1 cm of the selected cobble using a velocimeter (FlowTracker handheld-ADV, SonTek/YSI Inc., San Diego, CA, USA). Water samples were immediately placed on ice in a cooler and returned to the laboratory for analyzing nitrate–nitrogen (NO₃⁻–N), ammonium–nitrogen (NH₄⁺–N), sulfate (SO₄²⁻), orthophosphate (PO₄³⁻), total organic carbon (TOC), and dissolved silicate (SiO₂) according to the standard methods of the American Public Health Association [36]. Aquatic insects were collected from 6 random samples using a Surber net sampler (30.5 × 30.5 cm net with a mesh size of 250 μm) at each site. Insects were preserved in 75% ethyl alcohol and then determined the biomass and identified organisms to the lowest possible taxonomic level using available keys [37] in laboratory. No endangered or protected species were involved in this study.

Generalized additive model

The GAM is a regression model that assumes that response variables are dependent on the smoothing splines of independent variables instead of linear coefficients [14]. The GAM used in this study is as follows:

\[
g(\mu_i) = \alpha + \sum_{j=1}^{n} S_j(X_{ij}) + \text{factor}(ENV_i) + \sum_{j=1}^{n} S_j(X_{ij}) : \text{factor}(ENV_i)
\]

where \(g\) is the specified link function. In addition, \(\mu_i = E(Y_i)\) is the expected values of the response variable \(Y_i\), where \(Y_i\) is the ith Chl-a concentration; \(\alpha\) is the intercept; and \(X_{ij}\) is the
ith value of explanatory variable $X_i$. The $i$ ranges from 1 to 135 because each site had data of 27 samples. $S_j(X_{ij})$ is the smooth function (smoothing spline) of ith value of explanatory variable ($X_j$), and $n$ is the total number of explanatory variable. Moreover, $ENV_i$ is a nominal explanatory variable representing types of surrounding environments. The term $factor(ENV)$ adds or subtracts only a constant value from the smoother.

**GAM variables**

The Chl-a concentration at the 5 sites served as the response variable in the GAM. Water quality variables ($SO_4^{2-}$, $PO_4^{3-}$, $NH_4-N$, $NO_3-N$, $TOC$, $SiO_2$, pH, EC, turbidity, and DO) and environmental variables (canopy cover, water temperature, current velocity, and the number of aquatic insects) served as explanatory variables in the GAM. Although the Site 2 has moderate agricultural activity, the natural riparian vegetation along the river band can retard non-point source pollution to degrade the water quality. Sites 1–4 can be attributed to the Environment 1 (natural riparian vegetation) and Site 5 can be attributed the Environment 2 (anthropic disturbance without riparian vegetation). The Environments 1 and 2 served as nominal explanatory variables are used in GAM models for investigating the effects of surrounding environment conditions on stream Chl-a concentration.

**Analytical procedure**

A crucial step in applying GAMs is selecting an appropriate level of the “smoother” for a predictor. This can be achieved by specifying the level of smoothing by using the concept of effective degrees of freedom. A reasonable balance must be maintained between the total number of observations and the total number of degrees of freedom used when fitting the model. During model selection in the GAM, the Akaike information criterion [38] was used to determine the optimal GAM model. The types of the specified link function and distributions of response variable were considered. In each backward selection step, cross-validation was applied to estimate the optimal degrees of freedom for each smoother. Finally, variance inflation factor (VIF) analysis [39] was performed to detect multicollinearity in each set of explanatory variables. In the optimal GAM model, the VIF values of explanatory variables were controlled within 5 [40]. Using multicollinear explanatory variables would generate an error or warning message in GAM outputs. GAM was employed using the Brodgar Version 2.7.4 statistical package (Highland Statistics Ltd., Newburgh, UK), which is based on the statistical software language “R” Version 3.0.2. The R library “mgcv” allows for the automatic application of the cross-validation method in the GAM.

**Results**

**Descriptive statistical analysis**

Table 2 showed the mean and coefficient of variation (CV) of the response and explanatory variables for each site. High CVs (CV > 50%) were observed for the concentrations of Chl-a, $SiO_2$, $PO_4^{3-}$, $NH_4-N$, and $NO_3-N$, velocity, turbidity, and number of aquatic insects. The highest Chl-a concentration determined in the YS Stream (Site 5) surrounded by intensive agricultural activities was 6–9 times higher than that in other streams (Fig 2). Fig 3 illustrates seasonal fluctuations in explanatory variables during the study period and the magnitudes of the water temperature, EC, pH, $NO_3-N$, and $SO_4^{2-}$ at Site 5 were also higher than other four sites. Canopy cover was highest at Site 4, surrounded by a pristine forest. Fluctuations in TOC, $SO_4^{2-}$, and $SiO_2$ followed the same patterns at all sites before April 2009, after which they considerably varied until the end of the study period.
GAM results

Several GAM models combining with various explanatory variables were executed; however, only the numerical outputs of significant explanatory variables in the optimal model were listed in Table 3. The optimal GAM involved a Poisson distribution and log link function. The optimal model explained 91.0% of deviance, indicating that 91.0% of the total sum of squares was explained by the optimal GAM ($R^2 = 0.942$ and RMSE = 5.12). The residual variance was 309 and AIC was 607.

The optimal GAM model showed that the water temperature, turbidity, current velocity, DO, pH, and NH$_4^+$–N were the main factors explaining the long-term dynamics of Chl-a concentration in the study area (Table 2). Among six significant explanatory variables, DO is

Table 2. Mean and coefficient of variation (CV) of variables for each site. Units of the last 7 variables are mg L$^{-1}$.

| Variables            | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 |
|----------------------|--------|--------|--------|--------|--------|
|                      | Mean   | CV     | Mean   | CV     | Mean   | CV     | Mean   | CV     | Mean   | CV     |
| Chl-a (mg m$^{-2}$)  | 9.70   | 99.0   | 8.43   | 132.2  | 13.14  | 108.1  | 9.30   | 127.7  | 78.80  | 107.3  |
| Velocity (m s$^{-1}$)| 0.70   | 83.8   | 0.606  | 69.6   | 0.77   | 79.6   | 0.60   | 68.7   | 0.57   | 112.4  |
| Canopy cover(%)      | 42.2   | 15.4   | 75.39  | 23.5   | 38.5   | 11.7   | 79.0   | 9.38   | 47.0   | 12.0   |
| Turbidity (NTU)      | 0.72   | 144.8  | 1.693  | 121.6  | 1.24   | 140.1  | 1.63   | 125.0  | 5.63   | 189.1  |
| Temperature (°C)     | 11.42  | 23.7   | 12.36  | 24.5   | 13.33  | 18.9   | 12.45  | 24.2   | 16.16  | 23.8   |
| EC (μS cm$^{-1}$)    | 176.1  | 46.3   | 194.5  | 22.5   | 218.2  | 18.7   | 194.6  | 22.1   | 289.1  | 14.7   |
| pH                   | 8.03   | 9.4    | 7.96   | 7.40   | 8.12   | 6.5    | 7.97   | 7.3    | 8.70   | 5.3    |
| Aquatic insect (No. m$^{-2}$) | 214.5 | 110.4  | 199.7  | 120.8  | 221.6  | 107.5  | 199.7  | 120.8  | 81.67  | 130.8  |
| DO                   | 8.95   | 11.5   | 8.72   | 12.97  | 8.60   | 10.1   | 8.70   | 12.8   | 8.26   | 16.6   |
| SiO$_2$              | 3.116  | 50.1   | 4.419  | 63.4   | 3.734  | 58.2   | 4.345  | 63.9   | 3.766  | 78.3   |
| PO$_4^{3-}$          | 0.004  | 150.0  | 0.075  | 140.3  | 0.042  | 158.8  | 0.072  | 176.6  | 0.004  | 148.0  |
| NH$_4$–N             | 0.011  | 170.3  | 0.011  | 166.3  | 0.015  | 176.5  | 0.009  | 181.1  | 0.019  | 180.5  |
| NO$_3$–N             | 0.225  | 92.0   | 0.200  | 86.5   | 0.317  | 73.2   | 0.206  | 83.8   | 1.475  | 46.7   |
| SO$_4^{2-}$          | 22.70  | 23.2   | 28.33  | 26.1   | 28.93  | 22.8   | 28.40  | 25.6   | 37.26  | 24.1   |
| TOC                  | 0.710  | 43.3   | 0.812  | 46.7   | 0.778  | 39.3   | 0.815  | 45.7   | 1.082  | 37.0   |

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Fig 2. Seasonal variations in the Chl-a concentration for bimonthly observations at the 5 sites during 2006–2011. Y-axis in the right is only for Site 5.

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Fig 3. Seasonal variations in abiotic and biotic variables for bimonthly observations at the 5 sites during 2006–2011.

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related to the photosynthetic production and respiratory consumption of the epilithic algal biomass. DO is the result from epilithic algal growth rather than the factor affects their variations. However, other five variables can be attributed to water quality and environmental variables. The VIFs for these six explanatory variables (1.05 ≤ VIF ≤ 1.64) did not exceed the VIF threshold. Table 3 shows the smoothers for each significant variable and all smoothers are highly significant at the 0.001 level. Since the turbidity has a high CV, an individual smoother was used to describe the relationships between the turbidity and epilithic algal biomass for each Environment. The estimated degrees of freedom (edf shown on the Y-axis) for all smoothers are in the ranges of 2.00 and 4.00, indicating that these variables are moderately nonlinear relationships with Chl-a concentration (Table 3).

The intercept had a value of 2.234 (Table 2) and was significantly different from 0 at the 0.1% level. The notation “factor (Environment 2)” denotes the Chl-a concentration at Environment 2. Its estimated regression parametric coefficient was 1.705, indicating that the Chl-a concentration at Environment 2 was 1.705 mg m⁻², higher than that at Environment 1. The p-values for the environment levels indicated which monitoring environments were significantly different from the baseline, namely Environment 1. The Chl-a concentration observed at Site 5 (Environment 2) with intensive agricultural activity was significantly different from those observed at the other four sites (Environment 1 with natural riparian vegetation/forest) at the 0.1% level.

**Discussion**

**Relationships between epilithic Chl-a concentration and explanatory variables**

The mean velocities at the 5 sites ranged from 0.55 to 0.70 m s⁻¹, which can be considered moderate to high velocity [41–42]. The Chl-a concentration was not affected by velocity when velocity is smaller than 0.55 m s⁻¹. However, an increasing velocity reduces epilithic algal biomass once the velocity is greater than the threshold of 0.55 m s⁻¹. Water velocity was the important variable regulating epilithic algal composition [43]. Tsai et al. [44] developed a process-based model to investigate how storm-induced velocity influenced the variations of

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Table 3. The numerical outputs (parametric coefficients and approximate significance of smooth terms) of the optimal GAM model.

| Nominal variables | Parametric coefficients | Smoothers | Approximate significance of smooth terms |
|-------------------|-------------------------|-----------|-----------------------------------------|
|                   | Estimate | Standard errors | t-value | Pr(>|t|) | edf | Ref.df | Chi Square | p-value |
| Intercept         | 2.234    | 0.054             | 41.28   | <2e-16 *** |
| factor(ENV₂)      | 1.705    | 0.155             | 10.97   | <2e-16 *** |
| s(Velocity)       | 2.656    | 3.181             | 39.54   | 2.37e-08 *** | 2.656 | 3.181 | 39.54 | 2.37e-08 *** |
| s(pH)             | 4.000    | 4.000             | 94.35   | <2e-16 *** |
| s(DO)             | 2.192    | 2.815             | 55.73   | 8.56e-12 *** |
| s(Temperature)    | 1.998    | 2.000             | 59.93   | 9.70e-14 *** |
| s(NH₄)            | 2.806    | 2.961             | 20.05   | 1.02e-05 *** |
| s(Turbidity):factor(ENV₁) | 2.992 | 3.000 | 56.78 | 6.51e-11 *** |
| s(Turbidity):factor(ENV₂) | 3.000 | 3.000 | 84.99 | <2e-16 *** |

Significant code: ***: 0.001.
epilithic algal biomass in the CCW Stream. They determined that the algal biomass was considerably reduced by a high storm-induced velocity. A high velocity may cause rocks to tumble and sediment substrata on the streambed to be scoured [45–46] and change the community structure of epilithic algae [47]. However, a low or moderate velocity may reduce the thickness of the diffusion boundary layer and increase nutrient uptake [41–42], and consequently increase algal biomass or biodiversity [48]. Heath et al. [49] showed that decreased velocity reduced nutrient dilution capacity and then indirectly increased benthic cyanobacterial blooms. Epilithic algal biomass was increased with velocity (velocity \(0.60 \text{ m} \cdot \text{s}^{-1}\)), but further increase in velocity reduced the biomass [50]. Therefore, variations in velocity result in different relationships with epilithic algal biomass because once the flow velocity reaches a critical level, the shear stresses induce the algae to detach from the epilithic algal mat [51].

CV values of turbidity at these 5 sites are greater than 120 showing high fluctuations. Therefore, turbidity may have a complicated relationship with epilithic algal biomass (Table 2). For both Environments 1 and 2, epilithic algal biomass was increased with turbidity (when turbidity <0.60 NTU) and then decreased with turbidity after that value. However, at Environment 1 only four observations (shown as ticks on the X axis) of epilithic algal biomass were increased with turbidity (when turbidity >2.3 NTU). Increases in turbidity can reduce the available light reaching the streambed and thus diminish the stream temperature, possibly affecting epilithic algal growth rates [52–53]. Therefore, turbidity considerably influenced variations in net community production and respiration rates of epilithic algae [54]. However, Figueroa-Nieves et al. [55] reported that the interaction between hydrology and turbidity might control algal biomass in nutrient-rich agricultural streams. The effects of nutrients and light availability on epilithic algal biomass are evident only after accounting for the large-scale constraints of land use, such as the levels of riparian vegetation and agricultural activity [41].

Water temperature sharply and negatively (when water temperature <14˚C) correlated with epilithic algal biomass (Fig 4). However, when water temperature was greater than 15˚C, the curve became a slightly increasing trend with an uncertainty expanded (i.e. the confidence interval is becoming wider). Two previous studies conducted in the streams of the Wuling basin support our results. Yu and Li [32] indicated that abundances of some epilithic algal species (diatoms such as \(A. \text{atomus}\) and \(\text{Planothidium}\)) in the streams were negatively correlated with water temperature. Tsai et al. [41] found that increasing temperature stimulated algal growth at sites with a moderate or low canopy cover, but it restrained algal growth at sites with a dense canopy cover. The epilithic diatoms are dominated at lower temperatures and consequently are the most abundant group during the winter and spring [56]. Variations in water temperature and canopy cover probably cause different correlations between temperature and algal growth rates.

In this study, the DO concentrations during the study period were greater than 6.5 mg/L, which can be attributed to "good" water quality [57]. Fig 4 shows that the epilithic Chl-a concentration is not related to DO when the DO level ranges between 7.0 and 8.0 mg/L and is decreased with DO when the DO level is higher than 8.0 mg/L. DO levels in surface waters are affected by a number of interacting processes including: photosynthesis and respiration of aquatic organisms [58], bacterial respiration, carbonaceous and nitrogenous deoxygenations, nitrification, reaeration, and sediment oxygen demand. Epilithic algae play an important role in photosynthetic production and respiratory consumption of DO [59], and consequently regulate the diurnal variation in DO concentration [60]. In addition, higher TOC and lower DO levels were obviously found at Site 5 indicating that bacteria consumed oxygen while breaking down organic matter, and then reduced DO levels in the Wuling streams.

Fig 4 shows that the epilithic Chl-a concentration is negatively and linearly correlated with \(\text{NH}_4–\text{N}\) when \(\text{NH}_4–\text{N}\) is greater than 0.010 mg/L (edf is 2.987). However, in most samples
(82%) the NH₄⁻N concentrations were less than 0.010 mg/L and positively related to epilithic Chl-α concentration. The NH₄⁻N concentrations in the YS Stream (Site 5) derived mainly from agricultural runoff were mostly higher than other four sites. Ammonium is readily bioavailable for algae uptake. Therefore, ammonium significantly affects epilithic Chl-α concentration in this study. Tien et al. [61] determined that epilithic algae in biofilms were negatively correlated with ammonium concentrations and positively correlated with pH in the Erh-Jen River, which showed the similar correlations in this study.

The recharge of agricultural nonpoint source pollution associated with lime-rich fertilizers can result in increased pH in aquatic systems. The pH affects most chemical and biological processes in water, and is one of the most important environmental factors limiting the distribution of species in aquatic habitats. Average pH values at the study sites were slightly alkaline, ranging from 7.96 to 8.70. Fig 4 showed that epilithic algal biomass sharply increases with the pH when the pH value is greater than 8.8; otherwise, the algal biomass slightly increases with the pH when pH value is less than 8.8. The change in pH affects the aqueous equilibria (such
as ammonia, hydrogen sulfide, and dissolved metals) and is directly related to the availability and absorption of nutrients [62]. As algal bloom, more carbon dioxide is removed by photosynthesis of epilithic algae than is added by respiration, thus elevating pH levels in water and leading to the enhancement of the algal growth [63]. Therefore, Kivrak and Uygun [13] also found that some of diatom taxa were positively correlated with pH. Epilithic algal species composition was significantly correlated with pH in a pristine subalpine stream [64]. pH is a major environmental variable determining diatom distributions in water bodies in temperate areas [65] and exerts a strongest effect on epilithic algal species composition [66].

Suitable habitat for epilithic algae

Site 5 located in an area with intensive agricultural activity has the highest Chl-a concentration. The contribution of nutrient enrichment on epilithic algal growth under certain conditions is limited by environmental variables such as temperature and light availability. Complex habitats with diverse combinations and ranges of water quality and environmental variables result in different correlations between these variables and epilithic algal biomass.

The change points of smoothing curves for velocity, DO, NH$_4$–N, pH, turbidity, and water temperature were approximately 0.40 m s$^{-1}$, 8.0 mg L$^{-1}$, 0.01 mg L$^{-1}$, 8.5, 0.60 NTU, and 15$^\circ$C, respectively. When aforementioned variables were greater than relevant change points, the epilithic algal biomass was sharply increased with pH and water temperature, and sharply decreased with water velocity, DO, turbidity, and NH$_4$–N. However, epilithic algal biomass at the Environment 1 (Sites 1–4) was increased with turbidity when turbidity was greater than 2.30 NTU. These change points may serve as a framework for managing the growth of epilithic algae, especially diatoms, in the Wuling streams. Many streams suffer from overgrowth of epilithic algae due to anthropogenic nutrient loading [67]. If administrators of the Shei-Pa National Park understand the relationship between environmental variables and epilithic algal biomass, the optimal levels of epilithic algal biomass in the streams can be effectively maintained.

Supporting Information

S1 File. This file contains the raw data used in modeling.
(XLSX)

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Author Contributions

Conceptualization: YMK HLY.
Data curation: WHK MHK HJL.
Formal analysis: YMK HLY.
Funding acquisition: YMK HJL.
Investigation: WHK MHK HJL.
Methodology: YMK HLY.
Project administration: YMK HJL.
Resources: WHK MHK HJL.
Software: YMK HLY.
Supervision: HJL.
Visualization: YMK HLY.
Writing – original draft: YMK.
Writing – review & editing: HJL.

References
1. Kottelat M. 1996. Oncorhynchus formosanus. In: the IUCN red list of threatened species. Version Feb 2015. Available at http://www.iucnredlist.org. Accessed 20 July 2015.
2. Pan Y, Stevenson RJ, Hill BH, Kaufmann PR, Herlihy AT. Spatial patterns and ecological determinants of benthic algal assemblages, stream slope, TP, TN and riparian canopy coverage. J. Phycol. 1999; 35: 460–468.
3. Hill W, Dimick SM. Effects of riparian leaf dynamics on periphyton photosynthesis and light utilization efficiency. Freshw Biol. 2002; 47: 1245–1256.
4. Holomuzki JR, Feminella JW, Power ME. Biotic interactions in freshwater benthic habitats. J N Am Benthol Soc. 2010; 29: 220–244. doi: 10.1899/08-044.1
5. Lin HJ, Peng TR, Cheng IC, Chen LW, Kuo MH, Tzeng CS, et al. A trophic model of the subtropical headwater stream habitat of the Formosan landlocked salmon. Oncorhynchus formosanus. Aquat Biol. 2012; 17: 269–283.
6. Hill WR, Knight AW. Nutrient and light limitation of algae in two northern California streams. J Phycol. 1988; 24: 125–132.
7. Quinn JM, Cooper AB, Stroud MJ, Burrell GP. Shade effects on stream periphyton and invertebrates: an experiment in streamside channels. New Zealand J Mar Fresh. 1997; 31: 665–683.
8. Soininen J, Könönen K. Comparative study of monitoring South-Finnish rivers and streams using macroinvertebrate and benthic diatom community structure. Aquat Ecol. 2004; 38: 63–75.
9. Van Nieuwenhuyse EE, Jones JR. Phosphorus–chlorophyll relationship in temperate streams and its variation with stream catchment area. Can J Fish Aquat Sci. 1996; 53: 99–105.
10. Mosisch TD, Bunn SE, Davies PM, Marshall CJ. Effects of shade and nutrient manipulation on periphyton growth in a subtropical stream. Aquat Bot. 1999; 64: 167–177.
11. Scrimgeour GJ, Chambers PA. Cumulative effects of pulp mill and municipal effluents on epilithic biomass and nutrient limitation in a large northern river ecosystem. Can J Fish Aquat Sci. 2000; 57: 1342–1354.
12. Bowman MF, Chambers PA, Schindler DW. Epilithic algal abundance in relation to anthropogenic changes in phosphorus bioavailability and limitation in mountain rivers. Can J Fish Aquat Sci. 2005; 62: 174–184.
13. Kivrak E, Uygun A. The structure and diversity of the epipelagic diatom community in a heavily polluted stream (the Akarçay, Turkey) and their relationship with environmental variables. J Freshw Ecol. 2012; 27(3): 443–457.
14. Hastle TJ, Tibshirani RJ. Generalized Additive Models. London: Chapman and Hall; 1990.
15. McCullagh P, Nelder JA. Generalized linear models. 2nd ed. London: Chapman & Hall; 1989.
16. Austin MP. Spatial prediction of species distribution: an interface between ecological theory and statistical modelling. Ecol Model. 2002; 157: 101–118.
17. Brotons L, Thuiller W, Araújo MB, Hérzeg AH. Presence-absence versus presence-only modelling methods for predicting bird habitat suitability. Ecography. 2004; 27: 437–448.
18. Wintle BA, Elith J, Potts JM. Fauna habitat modelling and mapping: a review and case study in the lower hunter central coast region of NSW. Austral Ecol. 2005; 30(7): 719–738.
19. Heinänen S, Rönkä M, Von Numers M. Modelling the occurrence and abundance of a colonial species, the arctic tern Sterna paradisaea in the archipelago of SW Finland. Ecography. 2008; 31(5): 601–611.
20. Tao M, Xie P, Chen J, Qin B, Zhang D, Niu Y, et al. Use of a Generalized Additive Model to Investigate Key Abiotic Factors Affecting Microcystin Cellular Quotas in Heavy Bloom Areas of Lake Taihu. PLoS ONE. 2012; 7(2): e32020. doi: 10.1371/journal.pone.0032020 PMID: 22384128
21. Park NW, Chi KH. Quantitative assessment of landslide susceptibility using high-resolution remote sensing data and a generalized additive model. Int J Remote Sens. 2008; 29: 247–264.

22. Brenning A, Grasser M, Friend D. Statistical estimation and generalized additive modeling of rock glacier distribution in the San Juan Mountains, Colorado, USA. J Geophys Res. 2007; 112: F02S15.

23. Brenning A. Benchmarking classifiers to optimally integrate terrain analysis and multispectral remote sensing in automatic rock glacier detection. Remote Sens Environ. 2009; 113: 239–247.

24. Schlink U, Herbarth O, Richter M, Dorling S, Nunnari G, Cawley G, et al. Statistical models to assess the health effects and to forecast ground-level ozone. Environ Modell Softw. 2006; 21(4): 547–558.

25. Carslaw DC, Beever SD, Tate JE. Modelling and assessing trends in traffic-related emissions using a generalised additive modelling approach. Atmos Environ. 2007; 41(26): 5289–5299.

26. Dodds WK. Jones JE, Welch EB. Suggested classification of stream trophic state: distributions of temperate stream types by chlorophyll, total nitrogen, and phosphorus. Wat. Res. 1998; 5: 1455–1462.

27. Dodds WK. Smith VH. Lohman K. Nitrogen and phosphorus relationships to benthic algal biomass in temperate streams. Can. J. Fish Aquat. Sci. 2002; 59: 865–874.

28. Allan JD. Autotrophs. In: Stream Ecology, Structure and Function of Running Waters. Springer, Netherlands, 2006.

29. Soininen J. Niemelä P. Inferring the phosphorus levels of rivers from benthic diatoms using weighted averaging. Arch. Hydrobiol. 2002; 154: 1–18.

30. Baulch HM, Turner MA, Findlay DL, Vinebrooke RD, Donahue WF. Benthic algal biomass—measurement and errors. Can J Fish Aquat Sci. 2009; 66(11): 1989–2001.

31. Chung LC, Lin HJ, Yo SP, Tseng CS, Yeh CH, Yang CH. Relationship between the Formosan landlocked salmon Oncorhynchus masou formosanus population and the physical substrate of its habitat after partial dam removal from the Kaoshan Stream, Taiwan. Zool Stud. 2008; 47: 25–36.

32. Yu SF, Lin HJ. Effects of agriculture on the abundance and community structure of epilithic algae in mountain streams of subtropical Taiwan. Botanical Studies. 2009; 50: 73–78.

33. Yeh CH. Long-term ecological monitoring and ecosystem modeling in the Wuling area—the study of stream morphology and physical habitat change on the environmental factors. Technical report, Shie-Pa National Park Administration, Miaoli, Taiwan. (in Chinese). 2006.

34. Hsu CB, Tzeng CS, Yeh CH, Kuan WH, Kuo MH, Lin HJ. Habitat use by the Formosan landlocked salmon Oncorhynchus masou formosanus. Aquat Biol. 2010; 10: 227–239.

35. Lobban CS, Chapman DJ, Kemer BP. Experimental phycology laboratory manual. USA: Cambridge University Press; 1988.

36. Clesceri LS, Greenberg AE, Eaton AD. Standard methods for the examination of water and wastewater. 20th edn. American Public Health Association: Washington, DC, 1998.

37. Merritt RW. Cummings K.W. (Eds.). An Introduction to the Aquatic Insects of North America. Kendall/Hunt Publishing Company, Dubuque, 1996.

38. Akaike H. A new look at the statistical model identification. IEEE Trans Autom Control. 1974; 19: 716–723.

39. Zuur AF, Ieno EN, Smith GM. Analysing ecological data. Springer; 2007.

40. Ritter A, Regaldo CM, Muñoz-Carpena R. Temporal common trends of topsoil water dynamics in a humid subtropical forest watershed. Vadose Zone J. 2009; 8(2): 437–449.

41. Biggs BJF, Goring DG, Nikora VI. Subsidy and stress responses of stream periphyton to gradients in water velocity as a function of community growth. J Phycology. 1998; 34: 598–607.

42. Townsend SA, Garcia EA, Douglas MM. The response of benthic algal biomass to nutrient addition over a range of current speeds in an oligotrophic river. Freshw Sci. 2012; 31: 1233–1243.

43. Rout J, Gaur JP. Composition and dynamics of epilithic algae in a forest stream at Shillong (India). Hydrobiologia. 1994; 291: 61–74.

44. Tsai JW, Chuang YL, Wu ZY, Kuo MH, Lin HJ. The effects of storm-induced events on the seasonal dynamics of epilithic algal biomass in subtropical mountain streams. Mar Freshw Res. 2013; 65(1): 25–38.

45. Lohman K, Jones JR, Perkins BD. Effects of nutrient enrichment and flood frequency on periphyton biomass in northern Ozark streams. Can J Fish Aquat Sci. 1992; 49: 1198–1205.

46. Biggs BJF. Eutrophication of streams and rivers: dissolved nutrient–chlorophyll relationships for benthic algae. J N Am Benthol Soc. 2000; 19: 17–31.

47. Robinson CT, Uehlinger U. Experimental floods cause ecosystem regime shift in a regulated river. Ecological Applications 2008; 18: 511–526. PMID: 18488612
48. Jørgensen SE, Patten BC, Straskraba M. Ecosystems emerging: 4. Growth Ecol Modell. 2000; 126: 249–284.
49. Heath MW, Wood SA, Brasell KA, Young RG, Ryan KG. Development of habitat suitability criteria and in-stream habitat assessment for the benthic cyanobacteria *Phormidium*. River Res. Applic. 2015; 31 (1): 98–108.
50. Horner R, Welch E, Seeley M. Responses of periphyton to changes in current velocity, suspended sediment and phosphorus concentration. Freshw Biol. 1990; 24: 215–232.
51. Uehlinger U, Büchner H, Reichert P. Periphyton dynamics in a floodprone prealpine river: evaluation of significant processes by modeling. Freshw Biol. 1996; 36: 249–263.
52. Munn MD, Osborne LL, Wiley MJ. Factors influencing periphyton growth in agricultural streams of Central Illinois. Hydrobiologia. 1989; 174: 89–97.
53. Duncan SW, Blinn DW. Importance of physical variables on the seasonal dynamics of epilithic algae in a highly shaded canyon stream. J Phycol. 1989; 25: 455–461.
54. DeNicola DM, de Eyto E, Wemaere A, Irvine K. Production and respiration of epilithic algal communities in Irish lakes of different trophic status. Arch Hydrobiol. 2003; 157: 267–298.
55. Fellowes-Davies D, Royer TV, David KM. Controls on chlorophyll-a in nutrient-rich agricultural streams in Illinois, USA. Hydrobiologia. 2006; 568: 287–296.
56. Vermaat JE. Hootsmans MJM. Periphyton dynamics in temperature–light gradient. In: Van Vierssen W, Hootsmans MJM, Vermaat JE. (Eds.), Lake Veluwe, a Macrophyte-Dominated System Under Eutrophication Stress, Geobotany 21. Kluwer Academic Publisher, Dordrecht, Boston, London, 1994, PP. 193–212.
57. Sánchez E. Colmenarejo MF, Vicente J. Rubio A, García MG, Travesio L, Borja R. Use of the water quality index and dissolved oxygen deficit as simple indicators of watersheds pollution. Ecological Indicators 2007; 7: 315–328.
58. Chapra SC. Surface Water-Quality Modeling. New York: WCB/McGraw-Hill; 1997.
59. Robinson KL, Valeo C, Ryan MC, Chu A, Iwanyshyn M. Modelling aquatic vegetation and dissolved oxygen after a flood event in the Bow River, Alberta, Canada. Can J Civ Eng. 2009; 36: 492–503.
60. Miltner RJ. A method and rationale for deriving nutrient criteria for small rivers and streams in Ohio. Environmental Management. Environ Manage. 2010; 45(4): 842–855. doi: 10.1007/s00267-010-9439-9 PMID: 20108135
61. Tien CJ, Wu WH, Chuang TL, Chen CS. Development of river biofilms on artificial substrates and their potential for biomonitoring water quality. Chemosphere. 2009; 76(9): 1288–1295. doi: 10.1016/j.chemosphere.2009.06.013 PMID: 19576617
62. Devlin RM, Witham FM. Plant physiology. Ed. 4. CBS Publishers & Distribution, 1986. New Delhi.
63. George DG, Heaney SI. Factors influencing the spatial distribution of phytoplankton in small productive lake. Journal of Ecology. 1978; 66: 133–155.
64. Lindström EA, Johansen S, Saloranta T. Periphyton in running waters—long-term studies of natural variation, Hydrobiologia. 2004; 521(1–3): 63–86.
65. van Dam H, Mertens A. Long-term changes of diatoms and chemistry in headwater streams polluted by atmospheric headwater streams polluted by atmospheric deposition of sulphur and nitrogen compounds. Freshw Biol. 1995; 34: 579–600.
66. Hill BH, Herlihy AT, Kaufmann PR, Stevenson JR, McCormick FH, Johnson, CB. Use of periphyton assemblage data as an index of biotic integrity. Journal of the North American Bentholological Society 2000; 19: 50–67.
67. Chang HY, Wu SH, Shao KT, Kao WY, Maa CJW, Jan RQ, et al. Longitudinal variation in food sources and their use by aquatic fauna along a subtropical river in Taiwan. Freshwater Biol 2012; 57: 1839–1853.