

## Shifts in Functional Traits of Aquatic Insects along a Subtropical Stream in Taiwan

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**Sen-Her Shieh, Ling-Kuo Wang, and Wen-Feng Hsiao (2012)** Shifts in functional traits of aquatic insects along a subtropical stream in Taiwan. *Zoological Studies* 51(7): 1051-1065. Despite an increasing interest in the study of functional diversity, there have been no attempts to link aquatic insect traits, habitat variations, and community structure in subtropical Asian streams. We examined relationships between habitat characteristics and aquatic insect traits at 7 sites along a subtropical stream in Taiwan by an RLQ analysis and fourth-corner analysis. The habitat was described by the slope, mineral substrate size, and water chemistry, while aquatic insect traits were described using 40 categories of 9 functional traits. The 1st axis of the RLQ analysis explained 96.5%, suggesting a strong longitudinal environmental gradient structuring the characteristics of sites and aquatic insect assemblages. The most influential habitat variables were the slope, mineral substrate size, conductivity, and alkalinity, which reflected both natural variations and human impacts along the stream. The main covariations between traits and habitat were a shift from organisms with combining features, such as crawlers, a large body size, a flat body form, and low body flexibility to organisms which are swimmers, possess high body flexibility and a small body size, and feed on fine detritus and microinvertebrates. In the results of the fourth-corner analysis, 18 of 40 trait categories were significantly correlated with environmental variables. In particular, traits relating to flow, such as body form, attachment to substrate, body flexibility, and food type, showed the strongest correlations with environmental variables. Longitudinal gradients of aquatic insect assemblages corresponded to a gradual shift from aquatic insects that preferred larger and more stable mineral substrates (rubble) to those that preferred still waters in lowlands with finer substrates. The results suggested that the low substrate heterogeneity and stability at downstream sites led to a simpler but less stable functional organization, and that functional traits should be sufficiently sensitive to detect changes in environmental variables and can be used for biomonitoring streams in Taiwan.  
<http://zoolstud.sinica.edu.tw/Journals/51.7/1051.pdf>

**Key words:** Life history traits, Habitat templet hypothesis, RLQ analysis, Fourth-corner analysis.

It has long been recognized that environmental variability in time and space shapes the distribution of organisms and their interactions and adaptations (Wiens 1986). Such spatial and temporal variability is a basic characteristic of stream ecosystems. A fundamental topic in current stream ecology is to understand and identify environmental variables and how they structure organism assemblages. The traditional approach to understanding relationships

between environmental variables and organism assemblages is to describe parameters of community structure, such as taxonomic richness, abundance, diversity index, and evenness, and to relate those parameters to environmental variables using correlation, regression, and/or ordination techniques. Although this approach may detect responses of individual species and metrics to an environmental stress, it can be difficult to determine which ecological functions are driving

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those responses. An alternative approach is to assign species to different functional groups based on their functions in ecosystems and investigate how these functional groups change with environmental variables. Although this approach provides a stronger link between functional groups and ecosystem functions than does the taxonomic composition approach, the reduction of species to a small number of groups represents a loss of potentially important ecological information (Charvet et al. 1998). Furthermore, it is often difficult to determine exactly which types of functional groups to use, because species display multiple traits they might not be easily assigned to discrete categories, and some changes in habitat may induce responses in 1 type of functional group classification, but not another (Hausner et al. 2003).

Recently, to overcome these shortcomings, the functional trait approach combining a wide variety of morphological, behavioral, and life-history traits, such as food, feeding habits, body size, fecundity, voltinism, and dispersion, was developed to determine relationships between community structure and environmental variability. This approach has been a central subject in recent stream ecological studies (Díaz et al. 2008, Tomanova et al. 2008, Dolédec et al. 2011). A trait is called functional when it indirectly impacts an organism's fitness via its effects on growth, reproduction, and survival (Violle et al. 2007). Consequently, shifts in habitat will lead to changes in the representation of functional traits and thus affect the presence of species. This idea was derived from the habitat templet hypothesis on multiple trait responses of organisms to natural environmental disturbances (Southwood 1988, Townsend and Hildrew 1994). The hypothesis is based on the premise that specific functional traits will be selected under a particular set of selective forces because species have evolved within limitations of their environments. Therefore, functional traits and environmental variables will be correlated to some degree (Ricklefs 1991). This approach has led to a shift from the general biomonitoring of species toward the biomonitoring of ecosystem functions (Ghilarov 2000).

Aquatic insects are good candidates for investigating how changes in community structure in stream ecosystems might alter ecosystem functioning, because they occur at various levels of the food chain (Rosenberg and Resh 1993). In particular, community structures of aquatic insects show substantial responses to various types of

human impacts. According to the habitat templet hypothesis (Townsend and Hildrew 1994), it is assumed that species with a small body size, a short life span, rapid population growth, and high mobility (swimmers) should be found in temporally variable (unstable) habitats, such as sandy substrate, thus favoring resilience, and that species in more-constant (stable) habitats, such as rubble, have larger body sizes and longer individual life spans. In addition, in habitats with fluctuating flow, such as lower-order streams, resistance features should be particularly favored, including firm attachment mechanisms, flexible body forms, and streamlined or flattened shapes.

However, information on functional traits of aquatic insects responding to environmental gradients has mainly come from Europe (Charvet et al. 2000, Stutzner et al. 2004 2005). To our knowledge, there have been no similar studies examining relationships between traits of aquatic insects and habitat variables in subtropical Asian streams. The objective of this study, therefore, was to link functional traits of aquatic insects to environmental gradients in a subtropical stream in Taiwan. Streams of Taiwan are characterized by high hydrological variability because of the steep topography and high level of rainfall associated with typhoons (Wu and Kuo 1999, Tew et al. 2002). Thus, organisms frequently experience abiotic disturbances and may respond over evolutionary time by developing morphological, physiological, and/or life-history traits that minimize the impacts of disturbances. We examined how aquatic insect assemblages changed with longitudinal gradients of the stream in terms of functional traits, abundance, and taxonomic composition of aquatic insects. We also determined how these functional traits varied with habitat changes to ascertain which traits were likely to be associated with habitat modification. We expected that habitat structural characteristics, such as mineral substrate size and reach slope, would be strongly related to functional traits.

## MATERIALS AND METHODS

### Study area

The Wu Stream, located in central Taiwan (Fig. 1), is the 4th largest river in Taiwan in terms of drainage area (about 2025.6 km<sup>2</sup>), with a length of approximately 119.1 km, a mean annual discharge of 112.78 m<sup>3</sup>/s (Anonymous 2006), and

annual average precipitation of 2100 mm. The source of the stream is near the Central Mountain Range (CMR; at an elevation of 2596 m) and flows westward through mountains and foothills of Nantou County, then Taichung City and Chungwai Township in the plains area. The CMR blocks the winter monsoon which brings cool and moist air from the Asian mainland and the temperate ocean. Of the total annual rainfall, 85.8% occurs in May-Sept. and 14.2% in Oct.-Apr. Typhoons often occur in summer and fall and cause flooding. Thus, hot, wet summers and cool, dry winters characterize the climate of the river basin. The annual mean air temperature is about 22-23°C on the plains. The stream is used for agricultural, industrial, and drinking water supplies, recreation, and power generation, resulting in multiple point and non-point discharges. These modifications have led to habitat degradation, resulting in alterations in flow, temperature regimes, and water quality.

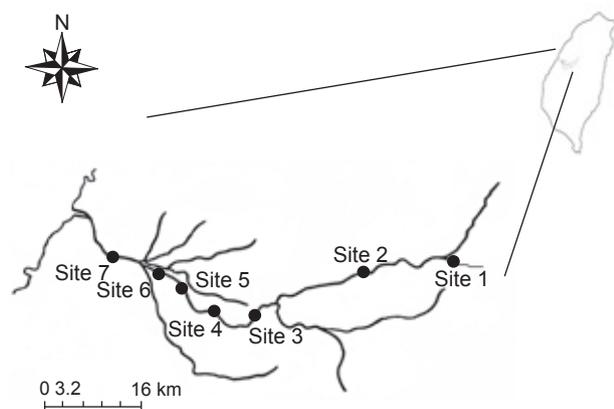
Seven sites were selected along the Wu Stream for the study (Fig. 1). Site 1 is on a 1st-order tributary stream at an elevation of 485 m. This site is located upstream of major agricultural and urban areas. The riparian zone of the site is dominated by natural broadleaf and planted coniferous forests, and therefore, is relatively pristine. Site 2 is on a 3rd-order stream at an elevation of 412 m. The site is located in a recreation area with a few agricultural activities. Site 3 is located in an agricultural area, with orchards (e.g., peach, plum, and betel palm trees) and rice paddies scattered throughout the area. Site 3 is on a 4th-order stream at an elevation of 238 m. Site 4 is on a 4th-order stream at an

elevation of 188 m and is in agricultural and urban areas. The major impacts on this site are municipal and agricultural. Sites 5-7 are on 4th- and 5th-order streams and are located in the Taichung metropolitan area. They are at elevations of 18-94 m. These sites are a combination of municipal, agricultural, and industrial settings. Thus, site 1 represents almost natural conditions, whereas sites 2-4 have some human impacts, and sites 5-7 are the most impacted by humans.

### Sampling methods

Aquatic insects were sampled monthly from the 7 sites using Surber samplers (with an area of 30.5 × 30.5 cm and a mesh size of 0.28 mm) from Mar. 2005 to Feb. 2006. Five Surber samples were randomly taken from a riffle section in a 100-m stream section and were pooled together as a sample unit. Aquatic insects were picked from the samples and preserved with 95% ethanol in a zip-lock bag. In the laboratory, all aquatic insects were identified to the lowest level according to the available literature, including Chang (1992), Kang (1993), Morse et al. (1994), Dudgeon (1999), and Kawai and Tanida (2005).

On each sampling occasion, water temperature, pH (EcoSan pH6 Meter, Eutech Instruments Pte Ltd, Singapore), conductivity (EcoSan CON5 Conductivity Meter), alkalinity (Alkalinity HI3811, HANNA, Woonsocket, Rhode Island, USA), and sulfate (Sulfate HI38000, HANNA) were also measured. The stream substrate composition was estimated by a method described by Mangum (1985). First, 3 plots (1 × 1 m) were randomly selected within riffles at each sampling site. The plot was further divided into 100 grids (10 × 10 cm for each grid), and the substrate percent composition was recorded. Substrates were classified as boulder (> 30 cm), rubble (7.5-30 cm), gravel (0.3-7.5 cm), and sand-silt (< 0.3 cm). Slopes of each study reach were measured with surveying gear (FUJI-KOH S302W™, Tokyo, Japan). These environmental variables are regarded as major components of the habitat template in stream systems because the mineral substrate type is a primary determinant of the aquatic insect community structure (Minshall 1984, Beisel et al. 1998). In addition, discharges were obtained from gauge stations nearby at sites 2, 3, and 5. Other water chemical variables, such as dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia nitrogen, total nitrogen, total phosphorus,



**Fig. 1.** Locations of the 7 study sites in the Wu Stream (inset showing its position within Taiwan).

suspended solids, and coliform groups were obtained from the Environmental Protection Agency of Taiwan. These variables, however, were only available at sites 2, 3, 5, and 7, so they were not used in the following statistical analyses.

### Functional traits of aquatic insects

Very little is known about functional traits of most aquatic insect species in tropical Asia. In the present study, the limited available information was used to assess some key traits for aquatic insect taxa. To describe the functional composition of aquatic insect assemblages, 40 categories of 9 functional traits were used (Table 1). These traits included trophic (food and feeding habits), physiological (respiration), morphological (maximum body size, body flexibility, and body form), behavioral (mobility and attachment to substrate), and life-cycle features (aquatic stages). These traits were selected because they reflected a site's food resources and oxygen availability and were linked to flow constraints and substrate modifications. Within the database, each taxon was coded according to its affinity for each category of a trait using a fuzzy coding approach (Chevenet et al. 1994). Each taxon was assigned an affinity to each category for a given trait. An

affinity score of 0 indicates no affinity, while an affinity score of 3 indicates a high affinity for a given trait category. Trait information generally includes expert knowledge and various literature sources (Chang 1992, Kang 1993, Morse et al. 1994, Dudgeon 1999, Kawai and Tanida 2005).

### Statistical analysis

An RLQ analysis was used to link functional traits of aquatic insects to environmental variables through the taxonomic composition. It is a 3-table co-inertia analysis (Dolédec et al. 1996). Three separate ordinations of the R (environmental variable), L (taxonomic composition), and Q (functional trait) tables were performed prior to the RLQ analysis. First, the aquatic insect abundance table (L table) containing the number of individuals in each taxon occurring at each sample unit was analyzed by a correspondence analysis (CA), an eigen analysis approach that provides joint scaling of sample units and taxon scores. The sample unit and taxon scores were used to link the R and Q tables, as sample units were shared by R and L and taxa were shared by Q and L. In this analysis, null samples (no taxa) and taxa with only 1 individual were excluded from the analysis to reduce the effect of rare taxa. In

**Table 1.** Functional traits and categories for taxa used in this study. 'Code' is the label used in the figures

| Trait             | Category (code)         | Trait                           | Category (code)           |
|-------------------|-------------------------|---------------------------------|---------------------------|
| Food type         | Fine detritus (FP)      | Body flexibility                | < 10° (F1)                |
|                   | Coarse detritus (CP)    |                                 | 10-45° (F2)               |
|                   | Microphyte (Mip)        |                                 | > 45° (F3)                |
|                   | Macrophyte (Map)        | Body form                       | Streamlined (SL)          |
|                   | Microinvertebrate (Mii) |                                 | Flattened (FL)            |
| Feeding habit     | Macroinvertebrate (Mai) | Attachment to soil or substrate | Cylindrical (CY)          |
|                   | Collector-gatherer (CG) |                                 | Spherical (SP)            |
|                   | Shredder (SH)           |                                 | Swimmers (SW)             |
|                   | Scraper (SC)            |                                 | Crawlers (CR)             |
|                   | Collector-filterer (CF) |                                 | Burrowers (BU)            |
| Respiration       | Piercer (PI)            | Aquatic stage                   | Temporary attachment (TA) |
|                   | Predator (PR)           |                                 | Permanent attachment (PA) |
|                   | Tegument (TG)           |                                 | Eggs (EG)                 |
|                   | Gills (GI)              |                                 | Larvae (LA)               |
| Maximum body size | Plastron (PL)           | Body armor                      | Pupae (PU)                |
|                   | Stigmata (ST)           |                                 | Adults (AD)               |
|                   | < 5 mm (S1)             |                                 | Soft-bodied (SF)          |
|                   | 5-10 mm (S2)            |                                 | Sclerotized (SZ)          |
|                   | 10-20 mm (S3)           |                                 | Case/shell (CA)           |
|                   | 20-40 mm (S4)           |                                 |                           |
|                   | > 40 mm (S5)            |                                 |                           |

total, 48 sample units from 7 sites and 30 taxa were included in this analysis. The 2nd procedure was to investigate relationships between sample units and environmental variables (R table). For the quantitative dataset of variables, a principal component analysis (PCA) was conducted, using weights obtained from the result of the CA thereby linking the R to the L table. A dummy variable (season) was used as an environmental variable to describe temporal variations in assemblages and functional traits of aquatic insects. In this analysis, therefore, totally 12 environmental variables were used. The 3rd step was to analyze the Q table of functional traits with row weights obtained from results of the CA using a fuzzy correspondence analysis (FCA). The RLQ analysis then combined these 3 separate analyses and maximized the covariation between environmental variables and taxonomic traits. The sample unit scores in the R table constrained the sample unit scores in the L table and the taxon scores in the Q table constrained the taxon scores in the L table. Within these constraints, a co-inertia analysis selected axes that maximized the covariance between the R and Q tables. As a result, the environmental variables were related to functional traits. The significance of relationships between environmental attributes (R) and functional traits (Q) was investigated with a Monte Carlo test (with 999 random permutations). The null hypothesis was independent between the R and Q tables, and the alternative hypothesis was that they were related (Dolédec et al. 1996). All environmental variables (except pH values) and aquatic insect abundances were  $\log_{10}(x+1)$  transformed to approach a normal distribution (Legendre and Legendre 1998).

To test relationships between environmental variables and functional traits, we carried out a fourth-corner analysis through the link provided by the abundance of taxa (Dray and Legendre 2008). The analysis allows statistical tests of the significance of links between all combinations of functional traits and environmental variables in a correlation-type analysis. Thus, relationships between functional traits and environmental variables can be obtained. The analysis relates the R table of environmental variables to the Q table of functional traits by way of the L table of taxon occurrences. Five different ecological hypotheses tested by permutation were described by Dray and Legendre (2008). They recommended a combination of permutation tests to reduce type I errors. Herein, we used their model 2 to test

the null hypothesis that taxon abundances and environmental variables were unrelated. After that, we tested model 4 which states the null hypothesis that taxon abundances and traits are unrelated. If both permutation tests were significant, then we could reject the null hypothesis that functional traits were unrelated to environmental characteristics. All analyses were carried out using the ade4 software package (Chessel et al. 2011) available in R 2.14.1 (R Development Core Team 2011).

## RESULTS

Measured environmental variables exhibited a longitudinal trend along the stream (Table 2). Values of water temperature, conductivity, alkalinity, BOD, COD, ammonia nitrogen, coliform groups, total nitrogen, and total phosphorus all increased downstream. Concentrations of sulfate and DO decreased downstream. All these indirectly reflected that human impacts, such as agricultural activities and domestic sewage effluents, increased downstream. Furthermore, there was a trend in the mineral substrate composition. The percent composition of boulders and rubble decreased downstream, except for boulders at site 5 and rubble at sites 3 and 6. Inversely, percent compositions of sandy substrate increased downstream. Total discharge increased downstream, but the variation in discharge decreased downstream. The results suggested that the longitudinal gradient of the stream showed a shift from a more-heterogeneous and stabler substrate composition and fluctuating flow to more homogeneous and unstable substrate composition and constant flow.

In total, 43 taxa were collected during the sampling period. The mean taxon richness per sample unit was highest at site 1 with 13.2 taxa (standard deviation, 5.4) and lowest at site 7 with 1.7 taxa (1.2). Taxon richness per sample unit at sites 2-6 ranged 5.0-8.8 taxa. Dominant taxa changed along the longitudinal gradient, with site 1 dominated by *Glossosoma* sp. (which accounted for 35% of individuals at the site), *Neoperla* sp. (10%), and Chironomidae (9%); sites 2-6 by *Baetis* spp., *Hydropsyche* spp., *Cheumatopsyche* sp., and Chironomidae; and site 7 by *Baetis* spp. and Chironomidae (Table 3). Pollution-sensitive taxa, such as all stonefly nymphs and some caddisfly larvae, including *Agapetus* sp., *Apsilochorema* sp., *Glossosoma* sp., *Goera* sp., *Goerodes* sp., *Psychomyia* sp., *Rhyacophila* spp., and

*Stenopsyche* sp., were only found at sites 1 and/or 2.

The Monte-Carlo permutation test indicated that there was a highly significant ( $p < 0.001$ ) association between environmental variables and functional traits. The 1st 2 axes of the RLQ analysis explained 96.5% and 2.3%, respectively, of the total variance from the data matrix that crossed environmental characteristics and functional traits. Because the RLQ analysis represented the partial ordination of environmental characteristics, taxon abundances, and functional traits, proportions of the variance attributed to each matrix were compared to those resulting from their separate analyses. The 1st RLQ axis (with an eigenvalue of 0.404, covariance of 0.636, and correlation of 0.533) accounted for 95.9% (6.311/6.584) of the variation in the separate analysis of the R table, 75.3% ( $0.533/(0.501)^{1/2}$ ) in the separate analysis of the L table, and 65.3% (0.226/0.346) in the separate analysis of the Q table (Table 4). Similarly, the 2nd RLQ axis accounted for 84.7%, 47.1%, and 79.2% of the variation, respectively.

Results of the RLQ analysis showed the main environmental gradients of the stream from upstream to downstream (Figs. 2, 3). The right part of RLQ axis 1 outlined traits associated

with upstream sites possessing high slope, high elevation, and large mineral substrate. Diagnostic traits included taxa with combining features, such as an aquatic adult stage, large body size, spherical body form, predatory feeding habit, flattened body form, and stigmatic respiration (Fig. 4). Corresponding taxa were pollution-sensitive organisms listed above (Fig. 5). The left part of RLQ axis 1 was indicative of higher values of conductivity, alkalinity, water temperature, pH, and sandy substrate (Fig. 2). This trend was exemplified by taxa which were piercers, that fed on macrophytes and possessed small body size ( $< 5$  mm), burrowed in the stream bottom, and were collector-filterers or swimmers (Fig. 4). Corresponding taxa were *Hydroptila* sp., *Baetis* spp., Chironomidae, *Simulium* spp., *Chimarra* sp., *Cheumatopsyche* sp., and *Hydropsyche* spp. (Fig. 5).

We compared the taxonomic and functional approaches using statistics derived from the CA and FCA. Functional traits had a relatively low data structure (i.e., low relative variance explained by the 1st FCA axes at 22.5%), while the taxon abundance had a relatively strong data structure (i.e., high relative variance explained by the 1st CA axes at 31.8%) (Table 4). This strong structure reflected clear gradients in taxon

**Table 2.** Mean values ( $\pm$  standard error) of environmental variables at the 7 study sites on the Wu Stream, Taiwan from Mar. 2005 to Feb. 2006. 'Code' is the label used in the figures

|   | Code  | 1               | 2                 | 3                 | 4                | 5                 | 6                | 7                 |
|---|-------|-----------------|-------------------|-------------------|------------------|-------------------|------------------|-------------------|
| Water temperature ( $^{\circ}$ C)             | WT    | 19.2 $\pm$ 0.7  | 19.7 $\pm$ 0.6    | 22.7 $\pm$ 0.7    | 23.4 $\pm$ 0.7   | 24.6 $\pm$ 0.7    | 25.9 $\pm$ 0.6   | 26.0 $\pm$ 0.9    |
| pH  | pH    | 7.3 $\pm$ 0.1   | 7.4 $\pm$ 0.2     | 7.7 $\pm$ 0.2     | 7.8 $\pm$ 0.2    | 7.9 $\pm$ 0.1     | 7.6 $\pm$ 0.1    | 7.54 $\pm$ 0.1    |
| Conductivity ( $\mu$ S/cm)                    | Cond  | 132.1 $\pm$ 6.3 | 373.2 $\pm$ 10.9  | 368.0 $\pm$ 10.6  | 361.8 $\pm$ 13.6 | 377.9 $\pm$ 12.9  | 435.0 $\pm$ 23.8 | 502.8 $\pm$ 17.6  |
| Alkalinity (ppm)                              | Alk   | 40.9 $\pm$ 2.1  | 93.3 $\pm$ 4.0    | 88.0 $\pm$ 4.9    | 87.2 $\pm$ 3.3   | 90.7 $\pm$ 4.1    | 105.5 $\pm$ 8.0  | 111.3 $\pm$ 4.2   |
| Sulfate (ppm)                                 | Sul   | 45.0 $\pm$ 3.4  | 46.7 $\pm$ 4.1    | 45.0 $\pm$ 3.8    | 44.2 $\pm$ 3.8   | 43.3 $\pm$ 3.8    | 42.5 $\pm$ 4.1   | 41.7 $\pm$ 3.0    |
| Dissolved oxygen (mg/L) <sup>a</sup>          |       | NA              | 8.88 $\pm$ 0.19   | 8.71 $\pm$ 0.25   | NA               | 8.63 $\pm$ 0.22   | NA               | 7.08 $\pm$ 0.23   |
| Biochemical oxygen demand (mg/L) <sup>a</sup> |       | NA              | 1.74 $\pm$ 0.12   | 2.51 $\pm$ 0.63   | NA               | 2.19 $\pm$ 0.18   | NA               | 3.37 $\pm$ 0.34   |
| Chemical oxygen demand (mg/L) <sup>a</sup>    |       | NA              | 5.01 $\pm$ 0.35   | 7.65 $\pm$ 1.92   | NA               | 6.14 $\pm$ 0.57   | NA               | 9.69 $\pm$ 1.15   |
| Ammonia nitrogen (mg/L) <sup>a</sup>          |       | NA              | 0.09 $\pm$ 0.01   | 0.10 $\pm$ 0.01   | NA               | 0.11 $\pm$ 0.02   | NA               | 1.41 $\pm$ 0.20   |
| Coliform group (CFU/100 ml) <sup>a</sup>      |       | NA              | 1132 $\pm$ 542    | 10,258 $\pm$ 2391 | NA               | 12,925 $\pm$ 4944 | NA               | 38,750 $\pm$ 4784 |
| Total nitrogen (mg/L) <sup>a</sup>            |       | NA              | 1.57 $\pm$ 0.51   | 2.02 $\pm$ 0.43   | NA               | 2.77 $\pm$ 0.75   | NA               | 4.23 $\pm$ 0.40   |
| Total phosphorus (mg/L) <sup>a</sup>          |       | NA              | 0.422 $\pm$ 0.180 | 0.180 $\pm$ 0.078 | NA               | 0.288 $\pm$ 0.116 | NA               | 0.379 $\pm$ 0.019 |
| Suspended solids (mg/L) <sup>a</sup>          |       | NA              | 1488 $\pm$ 376    | 1221 $\pm$ 300    | NA               | 1248 $\pm$ 336    | NA               | 516 $\pm$ 130     |
| Elevation (m)                                 | Alt   | 485             | 412               | 238               | 188              | 94                | 39               | 18                |
| Slope (%)                                     | Slope | 5.44            | 1.12              | 0.97              | 0.13             | 0.58              | 0.26             | 0.03              |
| Discharge (m <sup>3</sup> /s) <sup>a</sup>    |       | NA              | 62.5 $\pm$ 4.5    | 169.4 $\pm$ 9.6   | NA               | 534.0 $\pm$ 21.6  | NA               | NA                |
| Substrate composition (%)                     |       |                 |                   |                   |                  |                   |                  |                   |
| Boulder                                       | Bou   | 21.0            | 6                 | 1                 | 0                | 20.3              | 1                | 0                 |
| Rubble  | Rub   | 76.3            | 40                | 49.7              | 14               | 14.3              | 80               | 0                 |
| Gravel  | Gra   | 2.7             | 33                | 42.3              | 4                | 0                 | 16.3             | 0                 |
| Sand/silt                                     | Sand  | 0.0             | 21                | 7                 | 82               | 65.4              | 2.7              | 100               |

<sup>a</sup>Variables were not used in the statistical analyses. NA, not available.

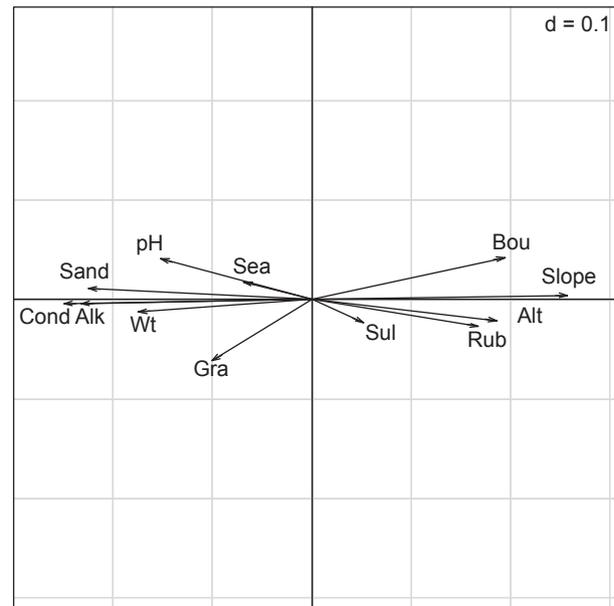
**Table 3.** Relative composition (%) of total aquatic insects collected at the 7 study sites on the Wu Stream, Taiwan from Mar. 2005 to Feb. 2006. 'Code' is the label used in the figures

| Taxa                                 | Code | Site |      |      |      |      |      |      |
|--------------------------------------|------|------|------|------|------|------|------|------|
|                                      |      | 1    | 2    | 3    | 4    | 5    | 6    | 7    |
| Ephemeroptera                        |      |      |      |      |      |      |      |      |
| <i>Afronurus floreus</i>             | EAF  | 2.4  | -    | 0.8  | 0.4  | 0.4  | 1.7  | -    |
| <i>Baetis</i> spp.                   | EBa  | 5.4  | 27.1 | 38.9 | 34.5 | 30.2 | 46.2 | 33.3 |
| <i>Caenis</i> sp.                    | ECa  | -    | 0.0  | 0.3  | 0.7  | 0.1  | 0.5  | -    |
| <i>Electrogena fracta</i>            | EEl  | -    | 0.6  | -    | -    | -    | -    | -    |
| <i>Epeorus erratus</i>               | EEp  | 5.8  | 0.6  | 0.1  | 0.1  | 0.1  | -    | -    |
| <i>Ephemera sauteri</i> <sup>a</sup> |      | 0.1  | -    | -    | -    | -    | -    | -    |
| <i>Pseudocloeon bispinosa</i>        | EPa  | 6.2  | 5.3  | 4.7  | 1.8  | 10.2 | 1.4  | -    |
| <i>Pseudocloeon</i> sp.              | EPb  | 2.9  | 8.9  | 2.9  | 14.2 | 3.1  | 1.4  | -    |
| <i>Rhithrogena ampla</i>             | ERh  | 7.3  | -    | 0.1  | -    | -    | -    | -    |
| Plecoptera                           |      |      |      |      |      |      |      |      |
| <i>Amphinemura</i> sp. <sup>a</sup>  |      | 0.1  | -    | -    | -    | -    | -    | -    |
| <i>Cryptoperla</i> sp. <sup>a</sup>  |      | 0.1  | -    | -    | -    | -    | -    | -    |
| <i>Kamimuria</i> sp. <sup>a</sup>    |      | 0.1  | -    | -    | -    | -    | -    | -    |
| <i>Neoperla</i> sp.                  | PNe  | 10.3 | 0.3  | -    | -    | -    | -    | -    |
| <i>Protonemura</i> sp.               | PPr  | 0.8  | -    | -    | -    | -    | -    | -    |
| Odonata                              |      |      |      |      |      |      |      |      |
| <i>Euphaea formosa</i>               | OEu  | 0.4  | -    | -    | -    | -    | -    | -    |
| Megaloptera                          |      |      |      |      |      |      |      |      |
| <i>Protohermes grandis</i>           | MPr  | 0.9  | -    | -    | -    | -    | -    | -    |
| Trichoptera                          |      |      |      |      |      |      |      |      |
| <i>Agapetus</i> sp.                  | TAg  | 0.4  | -    | -    | -    | -    | -    | -    |
| <i>Apsilochorema</i> sp.             | TAp  | 0.5  | -    | -    | -    | -    | -    | -    |
| <i>Cheumatopsyche</i> sp.            | TCh  | -    | 3.4  | 28.2 | 19.6 | 7.1  | 4.9  | -    |
| <i>Chimarra</i> sp.                  | TCm  | 0.2  | -    | 0.1  | 0.1  | -    | -    | -    |
| <i>Glossosoma</i> sp.                | TGI  | 34.8 | 1.6  | -    | -    | -    | -    | -    |
| <i>Goera</i> sp.                     | TGo  | 0.1  | -    | -    | -    | -    | -    | -    |
| <i>Goerodes</i> sp. <sup>a</sup>     |      | 0.1  | -    | -    | -    | -    | -    | -    |
| <i>Hydropsyche</i> spp.              | THa  | 6.0  | 30.7 | 16.9 | 10.2 | 23.5 | 11.3 | -    |
| <i>Hydroptila</i> sp.                | THt  | -    | -    | 0.2  | -    | 8.0  | 0.2  | -    |
| <i>Psychomyia</i> sp.                | TPs  | 0.1  | 0.2  | -    | -    | -    | -    | -    |
| <i>Rhyacophila</i> spp.              | TRh  | 1.8  | 1.4  | -    | -    | -    | -    | -    |
| <i>Stenopsyche</i> sp.               | TSt  | 0.5  | -    | -    | -    | -    | -    | -    |
| Lepidoptera                          |      |      |      |      |      |      |      |      |
| <i>Parapoynx</i> sp. <sup>a</sup>    |      | -    | -    | -    | -    | 0.1  | -    | -    |
| Coleoptera                           |      |      |      |      |      |      |      |      |
| Dytiscidae <sup>a</sup>              |      | -    | -    | -    | -    | -    | -    | 16.7 |
| <i>Grouvellinus</i> sp. <sup>a</sup> |      | 0.1  | -    | -    | -    | -    | -    | -    |
| Hydrophilidae <sup>a</sup>           |      | -    | 0.2  | -    | -    | -    | -    | -    |
| <i>Stenelmis</i> sp. <sup>a</sup>    |      | -    | -    | -    | 0.1  | -    | -    | -    |
| <i>Zaitzevia</i> sp.                 | CZa  | 1.2  | -    | -    | -    | -    | -    | -    |
| Diptera                              |      |      |      |      |      |      |      |      |
| <i>Amika</i> sp.                     | DAm  | 0.3  | -    | -    | -    | -    | -    | -    |
| <i>Antocha</i> sp.                   | DAn  | 0.1  | 2.7  | 0.1  | 0.4  | -    | -    | -    |
| Ceratopogonidae <sup>a</sup>         |      | 0.1  | -    | -    | -    | -    | -    | -    |
| Chironomidae                         | DCh  | 9.2  | 16.8 | 6.8  | 17.7 | 17.1 | 32.1 | 50.0 |
| <i>Clinocera</i> sp. <sup>a</sup>    |      | -    | 0.2  | -    | -    | -    | -    | -    |
| <i>Hexatoma</i> sp.                  | DHe  | 0.4  | -    | 0.1  | 0.1  | -    | 0.2  | -    |
| Psychodidae <sup>a</sup>             |      | -    | -    | -    | -    | 0.1  | -    | -    |
| <i>Simulium</i> spp.                 | DSi  | 1.0  | -    | -    | -    | -    | -    | -    |
| <i>Wiedemannia</i> sp.               | DWi  | 0.2  | -    | -    | -    | -    | -    | -    |

<sup>a</sup>Taxa were not used in the statistical analyses.

distributions along the 1st axis of the RLQ from pollution-sensitive to pollution-tolerant taxa (Fig. 5). Although the variance in the data structure of functional traits was relatively low, it was still strong enough to reflect clear gradients occurring in several functional traits (maximum body size, attachment to soil or substrate, body flexibility, and feeding habit) along the 1st axis of the RLQ (Fig. 4). This result suggested that the functional approach was more sensitive to the gradients of the 1st RLQ axis than the taxon approach. When the 1st 2 CA and FCA axes were compared, the functional approach had greater variability than the taxonomic approach. The relative variances of the 1st 2 FAC and CA axes were 48.7% and 41.3%, respectively (Table 4). Therefore, functional traits should be more appropriate for biomonitoring than taxon abundances in the form of a 2-dimensional diagram. The relationship between functional traits and taxonomic grouping could be determined by superimposing figure 4 onto figure 5. Taxa in the right part of figure 5 consisted mainly of large-sized predators that feed on macroinvertebrates, like *Protohermes grandis*, *Neoperla* sp., *Euphaea formosa*, *Apsilochorema* sp., and *Rhyacophila* spp.; scrapers that possess a flattened body form and feed on microphytes, like *Amika* sp., *Epeorus erratus*, *Rhithrogena ampla*, *Electrogena fracta*,

and *Afronurus floreus*; and collector-gatherers like *Zaitzevia* sp. which possesses an aquatic adult stage, plastron respiration, and a spherical body



**Fig. 2.** RLQ diagram as defined by the 1st 2 axes with projection of environmental variables. The d value in the upper right corner is the scale of the graph given by a grid (see Table 2 for environmental codes).

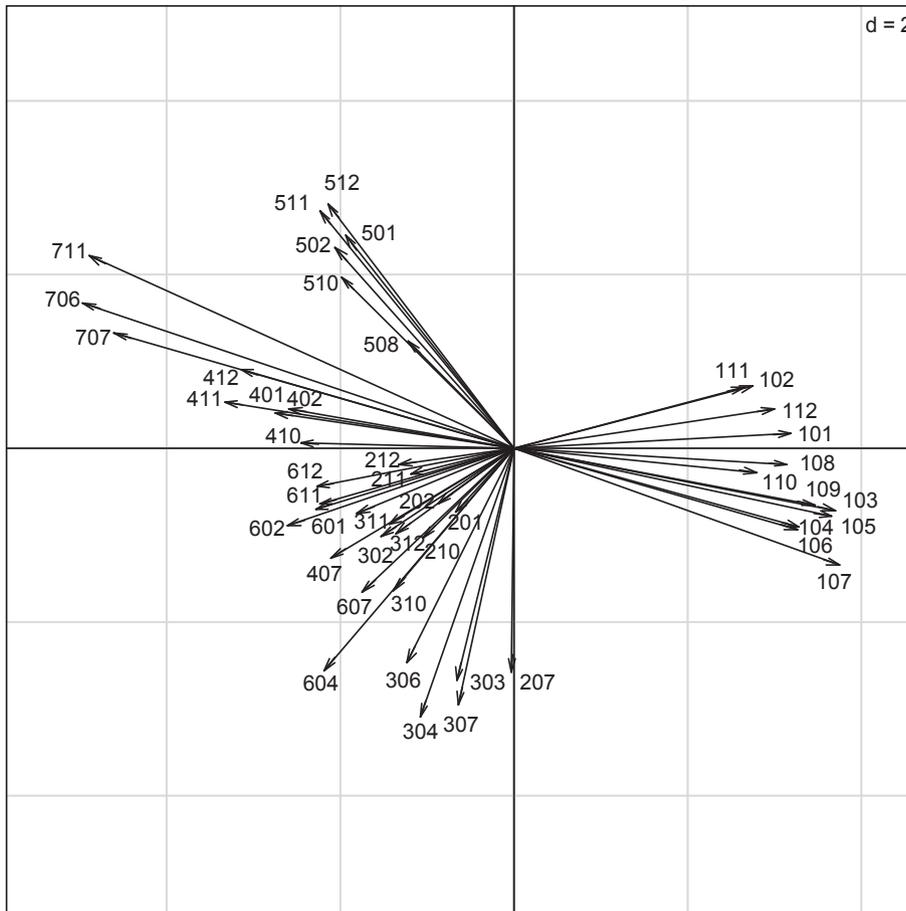
**Table 4.** Results of the multivariate analyses. (A) Separate analyses: eigenvalues and percentages of the total inertia accounted for by axes 1 and 2. Ordinations were a principal component analysis (PCA) of table R (environmental variables), a correspondence analysis (CA) of table L (taxon composition), and a fuzzy correspondence analysis (FCA) of table Q (functional trait). (B) Summary of the RLQ analysis: eigenvalues and percentages of the total co-inertia accounted for by RLQ axes 1 and 2, covariance and correlation between the sample scores constrained by environmental variables in R and species scores constrained by traits in Q, projected inertia of the R, L, and Q tables onto the 1st 2 RLQ axes, and percentage of the inertia obtained in separate analyses of R and Q along the same axes. For example, RLQ axis 1 accounted for 95.9% of the projected inertial of R along the PCA axis 1 [(6.311 / 6.584) × 100% = 95.9%]

| (A) Separate analysis | Variance axis 1 | Variance axis 2 |
|-----------------------|-----------------|-----------------|
| R/PCA                 | 6.584 (54.9%)   | 1.749 (14.6%)   |
| L/CA                  | 0.501 (31.8%)   | 0.150 (9.5%)    |
| Q/FCA                 | 0.346 (22.5%)   | 0.248 (16.2%)   |
| (B) RLQ analysis      | RLQ axis 1      | RLQ axis 2      |
| Eigenvalue            | 0.404 (96.5%)   | 0.010 (2.3%)    |
| Covariance            | 0.636           | 0.098           |
| Correlation           | 0.533           | 0.182           |
| R/RLQ                 | 6.311 (95.9%)   | 1.481 (84.7%)   |
| L/RLQ                 | 0.533 (75.3%)   | 0.182 (47.1%)   |
| Q/RLQ                 | 0.226 (65.3%)   | 0.196 (79.2%)   |

form. All of these organisms are pollution-sensitive taxa and occurred only at site 1 and/or site 2 (Table 3). Taxa in the left part of figure 5 were composed of piercers that feed on macrophytes, like *Hydroptila* sp.; collector-gatherers that feed on fine deposited detritus, like *Baetis* spp. which are swimmers, and chironomids, and *Antocha* sp. which are burrowers; and collector-filterers that feed on fine suspended detritus, like *Simulium* spp., *Cheumatopsyche* sp., *Hydropsyche* spp., and *Stenopsyche* sp.

The fourth-corner analysis extracted 93 significant relationships between the 12 environmental variables and the 40 trait categories at  $\alpha = 0.05$  (Fig. 6). Environmental variables most significantly related to the trait structure of aquatic insects included the gravel (with 11 significant relationships), slope (10), sand (10), conductivity (9), alkalinity (9), boulder (9), rubble (9), water temperature (8), and elevation (8). In contrast, pH

(6), sulfate (3) and seasonality (1) and were less significantly related to the trait structure of aquatic insects. Inversely, trait categories of aquatic insects most significantly related to environmental variables were body form (with 22 significant relationships among 48 relationships at 45.8%), attachment to soil or substrate (17/60 at 28.3%), food type (20/72 at 27.8%) and body flexibility (10/36 at 27.8%). Trait categories of feeding habit (13/72 at 18.1%), maximum body size (9/60 at 15.0%), body armor (1/36 at 2.8%) and respiration (1/48 at 2.1%) showed low relationships with environmental variables. However, trait categories of aquatic stages and body armor showed no significant correlations with any environmental variables. These results were in accordance with results of the RLQ analysis that sample units were principally identified by the functional traits such as body form, attachment to soil or substrate, food type and body flexibility (Fig. 4).



**Fig. 3.** RLQ diagram as defined by the 1st 2 axes with projection of the sample unit. The 1st digit represents the study site, and the 2nd and 3rd digits represent the sampling month. For example, sample unit '101' indicates a sample collected at site 1 in Jan. The d value in the upper right corner is the scale of the graph given by a grid.

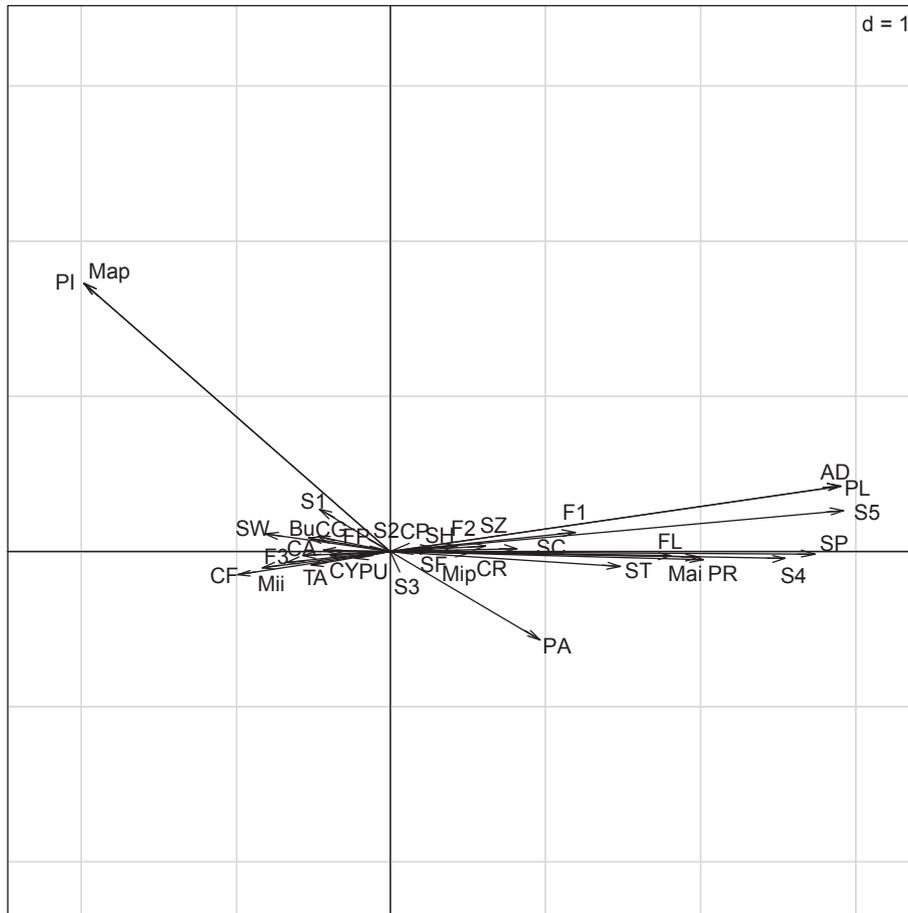
On the basis of trait categories, environmental variables could be divided into 2 groups. The 1st group, including slope, boulder, rubble, and elevation, had higher values at upstream sites. The 2nd group included water temperature, pH, conductivity, alkalinity, gravel, and sand which generally showed higher values at downstream sites. These 2 groups of environmental variables showed contrasting relationships with functional trait categories. Therefore, trait categories that showed a general positive relationship with environmental variables in the 1st group were negatively correlated with environmental variables in the 2nd group, and vice versa (Fig. 6). Environmental variables in the 1st group were related to high current velocities and had a positive relationship with traits such as lower body flexibility ( $< 10^\circ$ ), crawlers, and a flattened body form, but were harmful to traits such as feeding on fine particulate organic matter (FPOM) and microinvertebrates, a collector-filterer feeding

habit, a high body flexibility ( $> 45^\circ$ ), a cylindrical body form, and swimmers.

## DISCUSSION

### Longitudinal patterns of habitat variables

The 1st RLQ ordination axis accounted for a large fraction of the total variance explained, indicating a strong longitudinal environmental gradient structuring the characteristics of sites and aquatic insect taxa. The main longitudinal patterns of water quality were increases in organic pollution and eutrophication downstream shown by greater concentrations of ammonia, total nitrogen, total phosphorus, and coliform groups (Table 2). The increased nutrient concentrations in streams were related to agricultural land use (Omernik 1977, Hunsaker and Levine 1995), fertilizer use (Goolsby et al. 2001), and urban runoff (Osborn and Wiley



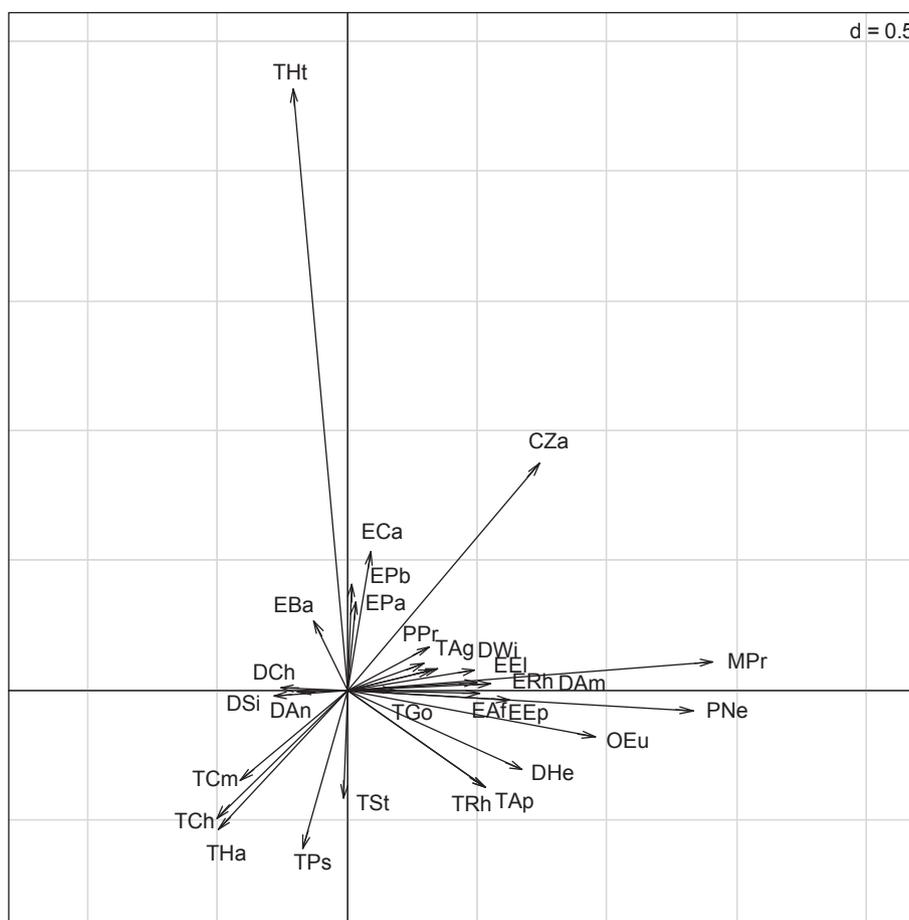
**Fig. 4.** RLQ diagram as defined by the 1st 2 axes with projection of trait categories. The d value in the upper right corner is the scale of the graph given by a grid (see Table 1 for trait codes).

1988). The main physical variables along the stream were the slope and mineral substrate size, indicating dominant overall downstream trends (Figs. 2, 3). Giller and Malmqvist (1998) indicated that substrate stability increases with the mean mineral substrate size, and sandy substrates are the most fluctuating habitats because hydraulic variations are more likely to act as disturbances in such substrates. So the sandy substrate was poorest due to its instability. Our results, therefore, suggested that longitudinal patterns of environmental variables in the stream mainly exhibited both substrate stability and human stress. On the contrary, only a small fraction of the total variance was explained by the 2nd RLQ ordination axis, indicating that the functional approach was less sensitive to temporal variability. Statzner et al. (2004) and Hausner et al. (2003) also indicated that functional traits were less affected by temporal scales.

### Longitudinal patterns of aquatic insect assemblages

Changes in aquatic insect assemblages along the stream gradient reflected the ability of aquatic insects to utilize habitats. In this study, there was a trend of taxon richness and abundances of aquatic insects decreasing downstream (Beisel et al. 1998, Giller and Malmqvist 1998). This corresponded to the habitat stability gradient and suggested that aquatic insect assemblages were functionally diverse and developed towards more-specialized (i.e., functionally less diverse) assemblages downstream. This was also partly because cumulative impacts of human activities may have degraded habitats (Waters 1995) and the taxon richness of downstream sites (Karr and Chu 1999).

It was noteworthy that declines in taxon richness between sites 1 and 2 and between sites 6 and 7 were accompanied by a substitution of



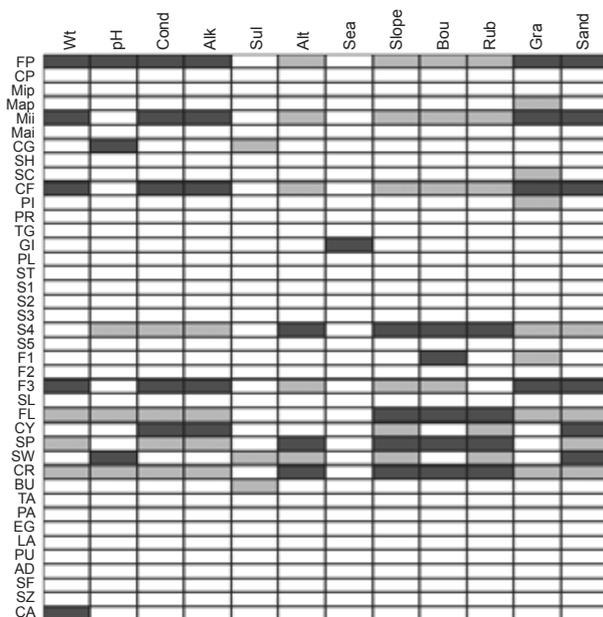
**Fig. 5.** RLQ diagram as defined by the 1st 2 axes with projection of taxa. The d value in the upper right corner is the scale of the graph given by a grid (see Table 3 for taxonomic codes).

less- to more-pollution-tolerant taxa (Table 3). Both taxon richness and relative abundances of Ephemeroptera, Plecoptera, and Trichoptera (EPT) decreased, but the relative abundance of chironomids increased at downstream sites. These results were in accordance with suggestions of Karr and Chu (1999) that diversity and/or richness decrease and chironomid dominance increases with increasing human impacts. These effects were due to domestic effluents and agricultural and industrial pollution. This combination of factors contributed to a drastic decrease in pollution-sensitive taxa in aquatic insect assemblages. Moreover, aquatic insect assemblages at sites 1 and 7 clearly differed from assemblages at other sites according to environmental characteristics and functional traits. Site 1 showed the highest habitat heterogeneity and supported the most-diverse assemblage, both in terms of taxon distribution and trait combinations (Townsend and Hildrew 1994). The separation of site sample units agreed with the results of Richards et al. (1997) that habitat conditions at the reach scale may act as selective forces in determining adaptive traits. At site 7; however, the fine sediment cover had direct relevance as a stressor for some aquatic

insects (Waters 1995) and led to the low substrate heterogeneity and stability which made functional organization simpler but less stable.

### Longitudinal patterns of functional traits

Much effort has been concentrated on examining variations in aquatic insect traits along environmental gradients (Lods-Crozet et al. 2001, Usseglio-Polatera and Beisel 2002, Gayraud et al. 2003, Statzner et al. 2005, Díaz et al. 2008, Dolédec et al. 2011). Patterns of trait variations along these gradients are generally viewed as indirect evidence that habitat characteristics are a major factor determining aquatic insect assemblages. In this study, we found a highly significant relationship between functional traits of aquatic insects and environmental variables along the longitudinal gradient of this subtropical stream. Eighteen of 40 trait categories were significantly correlated with environmental variables. In particular, traits relating to flow, such as body form, attachment to substrate, body flexibility, and food type showed the strongest correlations with environmental variables. The potential maximum size, feeding habit, body armor and respiration showed weaker but significant correlations. This was because flow-related variables influence attachment modes (by reducing swimmers), body flexibility (by reducing flexible body forms), food types (by reducing FPOM and microinvertebrate consumption), and body size (by reducing small body sizes) (Fig. 6). These results were in accordance with the prediction of the habitat templet hypothesis (Townsend and Hildrew 1994) with the exception of body flexibility which should increase with fluctuating flow. This was possibly because downstream sites of this study were dominated by chironomids which possess flexible body forms. In contrast, several studies also found similar results. For instance, Gayraud et al. (2003) found that body form, feeding habit, and food type all responded to a range of human activities, including water diversion, and organic and toxic pollution in European rivers. Dolédec et al. (2006) found that traits related to feeding habits, body form, and respiration were related to agricultural development in New Zealand streams. Tomanova et al. (2008) found that traits relating to body form and respiration were susceptible to flow modifications in Neotropical streams with different levels of anthropogenic disturbance. A possible explanation for the results could be that our study stream was affected by several different



**Fig. 6.** Results of the fourth-corner analysis performed using taxon abundances, taxon functional traits, and environmental variables collected in the Wu Stream, Taiwan. White indicates non-significant relationships, light-gray negative significant relationships, and dark gray positive significant relationships. See tables 1 and 2 for trait codes and environmental codes, respectively.

human impacts at different sites thereby making different trends in these functional responses possible. However, Tomanova et al. (2008) found no correlations between feeding habits and environmental variables. This was probably because they only identified organisms to the family level. They pointed out that there might be within-family variations in feeding habits and thus the use of a family-level analysis could lead to the loss of ecological information. Karr and Chu (1999) also indicated that functional feeding groups of aquatic insects are generally poor indicators of human disturbance of rivers. In addition, the few relationships between feeding habits and environmental variables did not agree with the river continuum concept that predicts a shift in functional feeding groups along a stream order gradient (Vannote et al. 1980). This is probably because our studied stream is highly impacted by human activities, but the river continuum concept assumes that the stream is relatively pristine.

Longitudinal gradients of the stream in this study reflected natural variations in stream channel morphology, such as mineral substrate compositions, fluctuating flows, and reach slopes along the study sites (Table 2, Fig. 6). Statzner et al. (2005) also found that the slope was a dominant factor affecting overall downstream trends for many trait categories of aquatic insects in European streams. These environmental variables showed positive relationships with trait categories that conferred an ability to resist high water flow (crawlers, flat and spherical body forms, and low body flexibility) (Figs. 4, 6). Similarly, Richards et al. (1997) pointed out that certain functional traits, such as scraper, clinger, and obligate erosional invertebrates, had negative relationships with the percent composition of fine sediment in east-central Michigan, USA. In our study, crawling aquatic insects were less abundant at downstream sites and became gradually more abundant with increasing mineral substrate size (Fig. 6), indicating that the type of attachment characteristics may offer adaptive advantages in habitat utilization. Townsend and Hildrew (1994) suggested that a crawling habit, through morphological and behavioral adaptations, may act to resist dislodgement in hydraulically turbulent conditions. Both swimmer and burrower habits increased at downstream sites (Figs. 4, 6). This result agreed with findings of Snook and Milner (2002) in a glacier-fed catchment in France. They suggested that the swimmer habit would potentially increase vulnerability to being swept downstream

in turbulent and high-velocity conditions. The trait of a flattened body form increased at upstream sites (Fig. 4). This result was in accordance with findings of Townsend et al. (1997) in New Zealand streams, indicating that this trait offers resistance to hydraulic disturbance. Statzner and Holm (1989) indicated that a flattened shape should reduce protrusion above the boundary layer and increase the potential area for attachment. However, our results contradicted the findings of Statzner et al. (2005) that spherical and large-sized forms increased downstream in European streams. This is probably because in this study, we considered aquatic insect taxa only, and the spherical form was dominated by elmid adults which were often found at site 1, but the study of Statzner et al. (2005) included all macroinvertebrate taxa such as bivalves which possess a spherical form.

On the other hand, the longitudinal gradients of the stream also represented stream water quality (high conductivity, alkalinity, BOD, COD, ammonia nitrogen, total nitrogen, total phosphorus, and coliform groups downstream) (Table 2, Fig. 2) and thus reflected increases in human impacts downstream. Gradients, therefore, were positively associated with trait categories of high body flexibility, a small body size, preying on microinvertebrates, and swimmers (Figs. 4, 6). These results were in accordance with assumptions of Townsend and Hildrew (1994) that the average size of organisms decreases in unstable habitats and the findings of Dolédec et al. (2006 2011) that traits associated with population resilience (a small size) would become more prevalent as agricultural land use increased in New Zealand streams. A small body size is assumed to offer an adaptive advantage in polluted habitats through an association with short life cycles (Townsend and Hildrew 1994). Short life cycles also represent an advantage for recolonization from refuges in polluted environments and therefore promote higher resilience to pollution (Snook and Milner 2002).

In spite of the low variance of the 1st FCA axis on the data structure of functional traits, the data structure reflected the applicability of functional traits for biomonitoring. Several functional traits responded to the data structure, suggesting that functional traits should be sufficiently sensitive to detect changes in environmental variables. Compared to the data structure of taxon abundances, functional traits had higher variability based on the 1st 2 FCA axes. This makes functional traits potential candidates

as applicable biomonitoring tools. Charvet et al. (2000) also found similar results and suggested that functional traits should be able to indicate a variety of pollution in a stream.

In conclusion, understanding the processes that structure aquatic insect assemblages is crucially important for establishing effective monitoring strategies. However, very few studies have assessed life history traits, and the diversity and abundances of subtropical aquatic insects, and little is known about the environmental determinants of aquatic insect assemblage compositions in Asian subtropical streams. Our results indicated that differences in abundances of various aquatic insect taxa were significantly related to their functional traits and habitat characteristics, and that the functional traits of aquatic insects might be used in biomonitoring Taiwanese streams in the future. Longitudinal gradients of aquatic insect assemblages corresponded to a gradual shift from aquatic insects that preferred living on coarse mineral substrates (rubble) to those that preferred still waters within lowlands (soft substrate). The low substrate heterogeneity and stability and high human impacts at downstream sites led to a simpler, but less-stable functional organization. However, the situation of a lack of data on life history information at the generic and/or specific levels of aquatic insects in Taiwan underlines the importance of continued studies on fundamental life-history characteristics of aquatic insects to improve our knowledge of such functional traits.

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