OPTIMAL PREDICTION MODEL OF MOSQUITO LARVAL ABUNDANCE IN BENTHIC MACROINVERTEBRATE COMMUNITIES OF NATURAL AND ARTIFICIAL HABITATS, KOREAN PENINSULA (2016-2017)

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Abstract. Mosquitos are the most prolific invasive insect species contributing to spread of endemic or exotic diseases, which exert a large burden on human health. Humans continue to develop and use physical, chemical, and biological methods for control of mosquito-borne infections. However, mosquito control methods are usually more effective when they target larvae, density of which is closely associated with biological factors, such as the community index and the predator population. Predictive models of mosquito larval abundance were developed based on species richness and on diversity and richness index ratio of individual Odonata, Coleoptera and Hemiptera group (OCH index) to evaluate suitability of the models using r^2 value and Akaike information criterion (AIC) score. The most suitable model had an r^2 value of 0.532 and an AIC score of 2.659 and was employed to estimate mosquito larval abundance. The prediction model developed should help explain correlation between benthic macroinvertebrate communities and mosquito larval abundance.

Keywords: *Coleoptera*, *Hemiptera*, *Odonata*, abundance prediction model, benthic community, macroinvertebrate, mosquito larva

INTRODUCTION

Various physical and biological systems are undergoing worldwide transformation owing to climate change, particularly humidity, temperature, and precipitation, which are fueling spatial expansion of disease vectors, including mosquitoes (Gage *et al*, 2008; Shuman, 2010). For example, malaria is spreading from alpine zones to east of the African continent, and Brazil is now facing issues regarding distribution and expansion of mosquito vectors *Aedes aegypti* and *Aedes albopictus* following the 2015 Zika virus epidemic (Shuman, 2010; Bogoch *et al*, 2016). Under such circumstances, humans have to develop and adopt a variety of physical, chemical and biological methods to control spread of mosquito-borne diseases (Troyo *et al,* 2008). An ability to predict sites of outbreaks and sizes of

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target mosquito population will help to optimize mosquito population preventive measures. To achieve this goal various studies have been conducted to predict emergence of vector mosquitoes and their spread of disease (Shaman *et al*, 2002).

The most common prediction method relies on remote sensing of soil moisture level to determine emergence of vectors and vector-borne diseases (Linthicum *et al*, 1987; Rogers and Randolph, 1991; Wood *et al*, 1991; Wood *et al*, 1992; Beck *et al*, 1994; Washino and Wood, 1994; Hay *et al*, 1996; Beck *et al*, 1997). Other studies using climatic variables have been conducted to predict emergence of mosquito populations (Hacker *et al*, 1973a; Hacker *et al*, 1973b; Tranwinski and Mackey, 2008). In Korea, correlations among temperature, humidity, precipitation, and number of adult mosquitos were studied based on data generated by a digital mosquito monitoring system (KIHASA, 2012). A prediction model has been developed and a mosquito warning system is currently in operation via a Seoul Metropolitan Government web site (http://health.seoul.go.kr/mosquito).

Early mosquito prediction models focused on analyzing correlations among environmental factors, including temperature, humidity and precipitation with emergence of adult mosquitos or their blood-feeding activity (KIHASA, 2012). However, emergence of mosquitoes in natural ecosystems is closely associated not only with environmental factors but also with biological environment in larval habitats, such as benthic communities and natural enemy populations (Rejmankova *et al*, 1991; Minakawa *et al*, 1999; Sunahara *et al*, 2002). In practice, in order to prevent a rise in mosquito population larval control is more effective than that of adult stage (Service, 1983, Service, 1992; Becker *et al*, 2010). Moreover, recently developed environment-friendly biological mosquito prevention methods target mosquito larvae (Chandra *et al*, 2008; Baek *et al*, 2014; Benelli, 2015; Chala *et al*, 2016).

Here the relationship among biological environments of natural and artificial habitats of mosquito larvae and emergence of such populations were investigated and a prediction model was developed to identify optimal sites for application of biological mosquito prevention measures, best time of application and optimal quantity to be applied.

MATERIALS AND METHODS

Study sites, sampling method and invertebrate identification

Thirteen different natural and artificial wetlands in the Korean Peninsula were investigated from 2016 to 2017 (Fig 1; details shown in Table 1). Benthic macroinvertebrates were collected using a 40 × 20 cm dredge sampler (500 μm mesh size), a collection tool normally used in quantitative wetland surveys. Taking into account characteristics of the physical environment, season of sampling and hatching period, three sites, one m distant from each other, were chosen for each location and samples were collected twice at each target site. Benthic macroinvertebrates (72 insect species and 25 non-insect species) were identified in 24 data sets. Identification was conducted to species level for all the taxa (Yoon, 1995; Merritt *et al*, 2008) except for certain Diptera and non-insect species, which were identified to family or genus level (McCafferty, 1981; Song, 1995; Merritt *et al*, 2008). Mosquito species were categorized into three different genera, namely, *Aedes* spp, *Anopheles* spp and *Culex* spp. Abundance of each species is

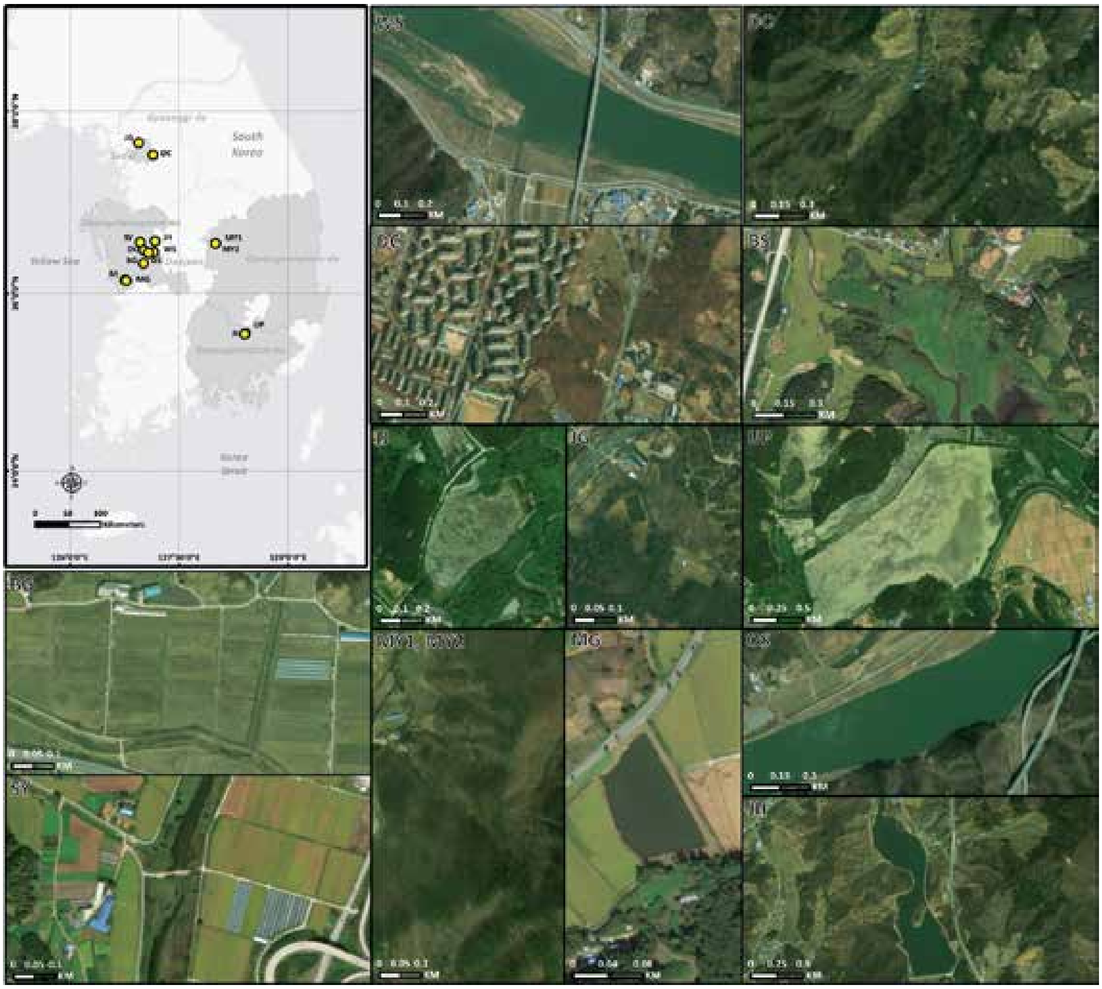


Fig 1-Location of study sites in South Korea. (Abbreviations as appeared in Table 1).

expressed as number/m^2.

Data analysis

Basic community indexes, namely, dominance (McNaughton, 1967), diversity (Shannon, 1949), richness (Margalef, 1958), and evenness (Pielou, 1966), were calculated using the survey data. In addition, an OCH-group index, representing community ratio of the major predators of mosquito larvae, namely, Odonata, Coleoptera and Hemiptera, was determined, employing two OCH indexes (individual ratio and species ratio) to indicate number of individuals and number of species of the OCH group existing in each community. Nonmetric multidimensional scaling (NMS) analysis was conducted using a PC-ORD Multivariate Analysis of Ecological Data, v. 5.10 (MJM Software Design, Gleneden Beach, OR) to denote number of factors determining complexity of the benthic macroinvertebrate community and differences in emergence of mosquito larvae (Clarke, 1993). Correlation analysis (Pearson's correlation) and regression analysis were performed using an IBM

Table 1
Location, area and type of study wetlands, Korean Peninsula.

Name of wetland	Abbreviation	GPS coordinate	Area (m^2)	Wetland type*
Wolsong wetland	WS	36°27′14″N, 127°09′11″E	55,400	M
Bungang wetland	BG	36°20′00″N, 127°00′35″E	23,200	M
Sinyeong wetland	SY	36°34′03″N, 126°57′52″E	221,505	M
Dongcheon wetland	DO	36°28′43″N, 127°00′45″E	176,800	M
Okseong-li wetland	OS	36°26′57″N, 127°04′58″E	19,300	Tp
Jungheung wetland	JH	36°34′42″N, 127°10′09″E	273,371	O
Dunchon-dong wetland	DC	37°31′21″N, 127°08′36″E	36,958	Va
Mungyeong wetland	MY1, MY2	36°33′08″N, 128°00′15″E	1,361	Tp /3
Myeonggok wetland	MG	36°08′52″N, 126°45′40″E	6,069	2
Bongseon wetland	BS	36°08′22″N, 126°46′35″E	373,100	O
Upo wetland	UP	35°33′7″N, 128°24′52″E	1,728,285	O/Tp
Jingwannae-dong wetland	JG	37°39′21″N, 126°56′36″E	16,693	Va
Jjokjibeol marsh	JJ	35°32′45″N, 128°23′58″E	139,626	O/Tp

*Wetland type classification followed that of Ramsar (2013); 2: pond; 3: irrigated land; M: permanent creek/river/stream; O: permanent freshwater lake (>8 hectare); Tp: permanent freshwater marsh/pool; Va: alpine wetland

Statistical Package for the Social Science (SPSS) Version 21 (IBM Corp, Boston, MA) to determine the relationship between benthic macroinvertebrate community and mosquito abundance.

Prediction model selection

Owing to an absence of previous data on the correlation between benthic macroinvertebrate community and the mosquito larval abundance, various linear models associated with mosquito larval abundance were evaluated. Selection was based on the model with the most effective composite variables through conducting experiments with the highest number of variables possible, including interaction terms and quadratic terms of each variable. In addition, a natural log of mosquito larva individual ratio for extending the limit of simple linear regression was tested. Although it is challenging to associate a biological or ecological meaning to each variable in a model, the most meaningful variable was finally selected. Linear regression analysis of each model was performed to derive equations using selected meaningful composite variables to determine each model goodness of fit. An r^2 value was employed as representing statistical significance and Akaike information criterion (AIC) value as an indicator of a model goodness of fit (Akaike, 1974). The model with the highest r^2 and the lowest AIC values was selected.

RESULTS

Benthic macroinvertebrate community analysis and mosquito emergence probability in study wetlands of the Korean Peninsula

Species of the family Chironomidae were either dominant or subdominant in the majority of the wetlands, and the diversity of *Cloeon dipterum*, Culicidae and Zygoptera was high (Table 2). Individual mosquito ratio ranged 0.005-0.433 and in comparison to the entire benthic macroinvertebrate community, individual mosquito ratio was relatively low and richness index and OCH-group individual ratio in certain sites.

In order to identify possible correlations, the values with relatively high mosquito larval abundance were categorized into three different ranges (Fig 2). Larval abundance ≤0.1 was associated with richness index ≥2.5 and individual OCH-group ratio ≥0.4, while larval abundance >0.1 was associated with richness index ≤2.5 and individual OCH-group ratio of ≤0.4. Subsequently, for ease of assessment each study site was categorized with respect to a richness index of 2.5 and OCH-group individual ratio of 0.4, and the probability of large-scale mosquito outbreak was divided into three grades, namely, high, low and almost impossible (Fig 2).

Explanatory variables

Correlation analysis of individual mosquito ratio indicated no correlation with dominance, diversity or richness index (data not shown). However, when OCH-group individual ratio was high, Culicidae individual ratio tended to be reduced, although no linear relationship was observed. Correlation analysis of mosquito population and benthic community indices did not yield any

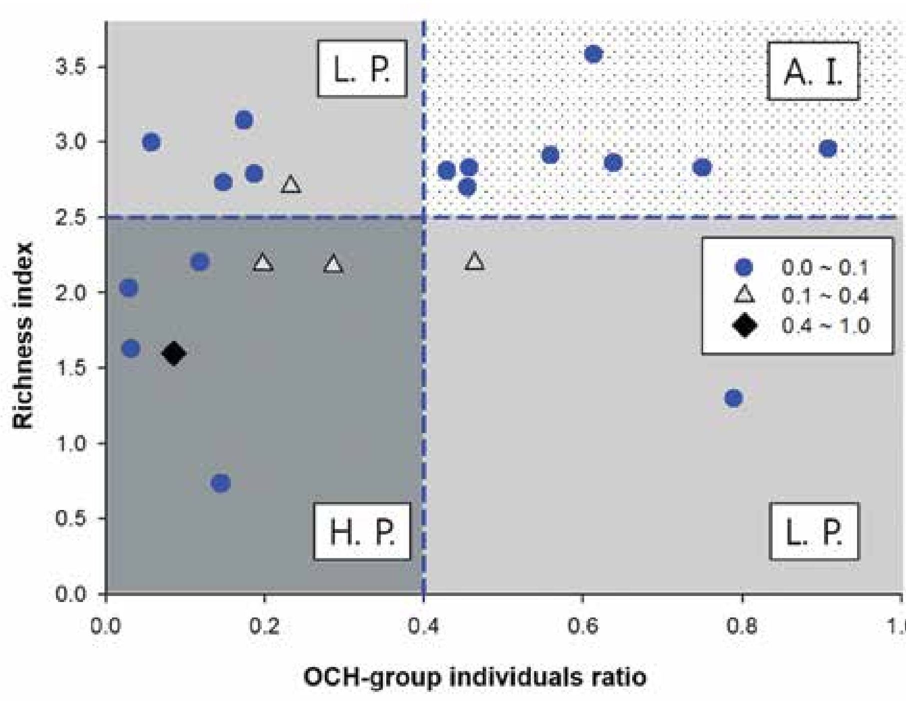


Fig 2-Relationship between richness index and Odonata, Coleoptera and Hemiptera (OCH)-group individuals ratio of wetland benthic community, Korean Peninsula (2016-2017).
Numbers refer to mosquito abundance expressed as number/m^2; A.I.: almost impossible probability of mosquito emergence; H.P.: high probability of mosquito emergence; L.P.: low probability of mosquito emergence.

Table 2
Benthic macroinvertebrate communities in thirteen wetland study sites, Korean Peninsula (2016 -2017).

Site[a]	Data set number	Mosquito individuals ratio	Mosquito species ratio	Dominance	Diversity	Richness index	Evenness	OCH-group[b] individual ratio	OCH-group[b] species ratio	Dominance 1	Dominance 2
WS	1	0.013	0.111	0.932	0.816	1.296	0.371	0.789	0.556	*Micronecta sedula*	*Cloeon dipterum*
BG	1	0.068	0.077	0.591	1.964	2.788	0.766	0.186	0.462	*Chironomidae* spp	*Radix auricularia*
SY	1	0.061	0.077	0.727	1.594	2.498	0.622	0.122	0.615	*Chironomidae* spp	*Ephydridae* spp
DO	1	0.049	0.056	0.433	2.256	2.829	0.781	0.457	0.389	*Chironomidae* spp	*Cloeon dipterum*
OS	1	0.179	0.077	0.485	1.906	2.195	0.743	0.463	0.769	*Paraplea japonica*	*Cloeon dipterum*
JH	1	0.181	0.077	0.643	1.641	2.188	0.64	0.197	0.769	*Chironomidae* spp	*Culicidae* spp
DC	1	0.011	0.067	0.736	1.647	2.996	0.608	0.057	0.333	*Cloeon dipterum*	*Chironomidae* spp
	2	0.050	0.100	0.521	1.995	2.832	0.866	0.750	0.700	*Crocothemis servilia mariannae*	*Chironomidae* spp
MY1	1	0.017	0.083	0.507	1.935	2.201	0.779	0.119	0.417	*Limnodrilus gotoi*	*Chironomidae* spp
	2	0.020	0.050	0.812	1.099	2.954	0.367	0.908	0.600	*Zygoptera* spp	*Sigara substriata*
	3	0.005	0.053	0.609	1.795	2.698	0.609	0.454	0.579	*Cleon dipterum*	*Zygoptera* spp
MY2	1	0.006	0.063	0.532	1.899	2.808	0.685	0.429	0.625	*Chironomidae* spp	*Hippeutis cantori*
	2	0.020	0.056	0.633	1.742	2.863	0.603	0.638	0.556	*Zygoptera* spp	*Pyralidae* spp
	3	0.011	0.050	0.673	1.792	2.912	0.598	0.559	0.700	*Zygoptera* spp	*Hippeutis cantori*
MG	1	0.433	0.100	0.75	1.458	1.597	0.633	0.085	0.600	*Culicidae* spp	*Chironomidae* spp
BS	1	0.059	0.200	0.805	1.005	0.734	0.624	0.145	0.400	*Chironomidae* spp	*Hesperocorixa hokkensis*
UP	1	0.031	0.143	0.531	1.686	1.627	0.866	0.031	0.143	*Paludinellassiminea tanegashimae*	*Helobdella stagnalis*
	2	0.173	0.067	0.7	1.609	2.355	0.594	0.059	0.267	*Chironomidae* spp	*Cloeon dipterum*
	3	0.014	0.067	0.422	2.296	3.143	0.848	0.174	0.133	*Chironomidae* spp	*Cipangopaludina japonica*
JG	1	0.143	0.125	0.6	1.758	2.175	0.845	0.286	0.500	*Simuliidae* spp	*Culicidae* spp
	2	0.035	0.071	0.915	0.611	2.03	0.232	0.029	0.286	*Cloeon dipterum*	*Culicidae* spp
	3	0.047	0.056	0.645	1.736	2.73	0.601	0.148	0.500	*Cloeon dipterum*	*Pyralidae* spp
	4	0.172	0.071	0.549	1.970	2.701	0.746	0.232	0.500	*Pyralidae* spp	*Culicidae* spp
JJ	1	0.008	0.053	0.458	2.245	3.578	0.762	0.613	0.684	*Chironomidae* spp	*Paraplea indistinguenda*

[a]From Table 1. [b]Odonata, Coleoptera and Hemiptera.

Table 3
Correlation among properties of wetland benthic macroinvertebrate community, Korean Peninsula (2016 -2017).

Community structure variables		Mosquito population	Dominance	Diversity	Richness index	OCH-group# individual ratio	OCH-group# species ratio
Mosquito population	Correlation coefficient	1	0.074	-0.043	-0.347	-0.320	0.194
	Significance level*		0.730	0.840	0.097	0.127	0.363
Dominance	Correlation coefficient		1	-0.943	-0.507	-0.016	-0.006
	Significance level*			<0.001	0.011	0.941	0.977
Diversity	Correlation coefficient			1	0.647	0.024	0.076
	Significance level*				0.001	0.913	0.723
Richness index	Correlation coefficient				1	0.301	0.161
	Significance level*					0.152	0.451
OCH-group# individual ratio	Correlation coefficient					1	0.566
	Significance level*						0.004
OCH-group# species ratio	Correlation coefficient						1
	Significance level*						

#Odonata, Coleoptera and Hemiptera; *Significant at <0.05.

statistically significant result, except for an inverse correlation of dominance with diversity (strong) and richness index (weak), and a (weak) positive correlation of OCH-group individual ratio with OCH-group individual species ratio (Table 3). In order to facilitate identification of the optimal explanatory variables, natural log transformation was applied to the individual mosquito ratio and expressed as negative value [-ln (individual mosquito ratio)]. Using the converted individual mosquito ratio value, a relatively weak linear relationship was obtained with richness index and OCH-group individual ratio (Fig 3A).

Mosquito abundance prediction model and their assessments

A non-linear relationship of mosquito population was assessed by introducing four interaction (INT) terms related to numbers of mosquitoes and species, and OCH-group individual and species ratio (Table 4). Thirteen different mosquito abundance prediction models were constructed based on sums of various

Table 4
Integrated terms used in determination of wetland optimal mosquito abundance prediction model, Korean Peninsula.

Integrated term	Equation
INT1	OCH-group species ratio × OCH-group individual ratio
INT2	OCH-group individual ratio / OCH-group species ratio
INT3	(Number of OCH-group species / Number of OCH-group individuals) / (Number of total species / Number of total individuals)
INT4	Number of OCH-group individuals / Number of OCH-group species

OCH, Odonata, Coleoptera and Hemiptera.

combinations of OCH-group individual ratio, (OCH-group individual ratio)^2, OCH-group species ratio, (OCH-group species ratio)^2, richness index, (richness index)^2, and INT terms (Table 5). Linear regression analysis of each model was performed to determine each model goodness of fit based on R^2 and AIC values. Model 3g produced the highest r^2 (0.532) and lowest AIC (2.639) values among the 13 models. Then, based on Model 3g an equation for calculating optimal mosquito larval abundance using domestic wetland data was derived:

Mosquito larval abundance in a community = 1.4866 × exp(–2.399 + (10.252 × OCH-group individual ratio) - (0.28 × (richness index)^2) + (4.317 × (OCH-group species ratio)^2) - (15.437 × INT1) - (0.054 × INT4) - 0.0057) × 100

This equation improved r^2 value to 0.575 (Fig 3B).

DISCUSSION

Existing studies on mosquito abundance prediction have mostly analyzed the relationship between adult mosquito population and environmental factors (humidity, precipitation and temperature) to develop predictive models (Hacker *et al*, 1973a; Hacker *et al*, 1973b). However, the present study adopted a different approach, namely, analysis of the relationship between biological factors of mosquito larval benthic habitat (community index and OCH-groups index) and the existing numbers of mosquito larvae.

Mosquito larval abundance is closely associated with presence other organisms in their natural habitat (Minakawa *et al*, 2005; Munga *et al*, 2006). Several case studies have been conducted on the relationship between mosquito larvae and predators (Bay, 1967; Service, 1973; Service, 1977; Mogi, 1981; Lacey and Orr, 1994; Washburn, 1995; Sunahara *et al*, 2002), however, there has been no attempt to develop a model based on these findings. The correlation between such factors may increase if the analysis takes into account direct predators of mosquito larvae at the species level, but in order to develop a model that has both utility and ease of use, the variables should be selected carefully based on time and ease of calculation. Hence, the present study chose to use community characteristics.

In rivers, Ephemeroptera, Plecoptera

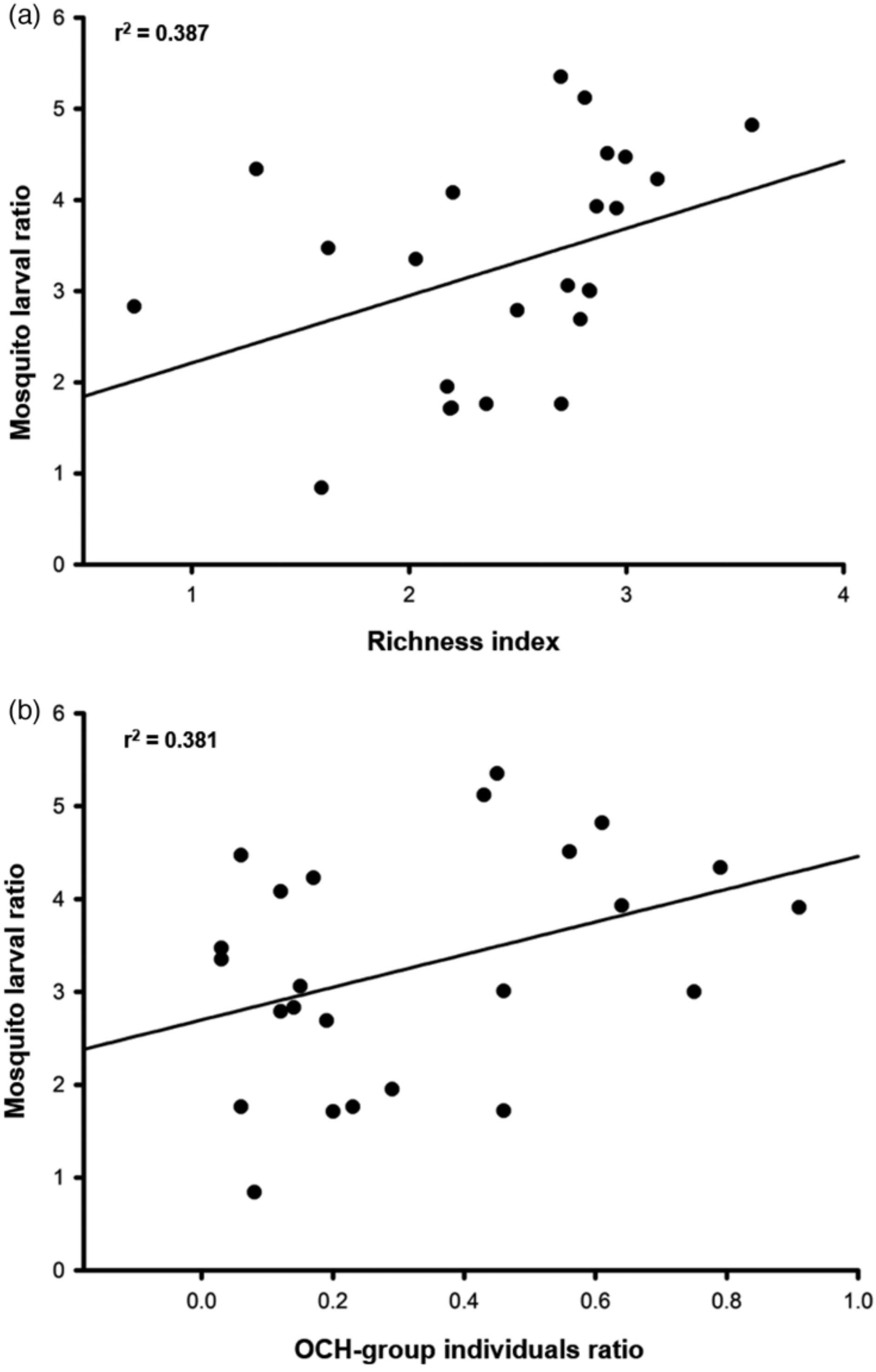
Table 5
Equations used for estimation of mosquito abundance in wetlands, Korean Peninsula.

Model	Equation	r^2 value	AIC value
1	OCH-group individual ratio + richness index	0.227	8.714
1a	OCH-group individual ratio + richness index + diversity	0.259	9.687
1b	OCH-group individual ratio + richness index + OCH-group species ratio	0.346	6.706
1c	OCH individual ratio + richness index + OCH-group species ratio + INT1 + INT4	0.476	53.388
1d	OCH-group individual ratio + richness + (OCH-group species ratio)^2 + INT1 + INT4	0.489	4.787
2	(OCH-group individual ratio)^2 + (richness index)^2	0.265	7.502
2a	(OCH-group individual ratio)^2 + (richness index)^2 + INT4	0.421	3.775
2b	(OCH-group individual ratio)^2 + (richness index)^2 + INT1	0.278	9.077
2c	(OCH-group individual ratio)^2 + (richness index)^2 + INT1 + INT4	0.421	5.773
2d	(OCH-group individual ratio)^2 + (richness index)^2 + INT3 + INT4	0.424	5.637
2e	(OCH-group individual ratio)^2 + (richness index)^2 + (OCH-group species ratio)^2 + INT3 + INT4	0.454	6.357
3	OCH-group individual ratio + (richness index)^2	0.261	7.636
3a	OCH-group individual ratio + (richness index)^2 + INT1	0.292	8.611
3b	OCH-group individual ratio + (richness index)^2 + INT4	0.426	3.582
3c	OCH-group individual ratio + (richness index)^2 + (OCH-group species ratio)^2 + INT4	0.451	4.487
3d	OCH-group individual ratio + (richness index)^2 + INT1 + INT4	0.434	5.226
3e	OCH-group individual ratio + (richness index)^2 + OCH-group species ratio + INT4	0.457	4.256
3f	OCH-group individual ratio + (richness index)^2 + OCH-group species ratio + INT1 + INT4	0.520	3.263
3g	OCH-group individual ratio + (richness index)^2 + (OCH-group species ratio)^2 + INT1 + INT4	0.532	2.659

AIC: Akaike information criterion (Akaike, 1974); INT: integrated term (from Table 4); OCH: Odonata, Coleoptera and Hemiptera.

and Trichoptera (EPT) index was developed using diversity and richness index of EPT-group characterized by a high richness index and sensitivity to environmental changes, and was subsequently used for evaluating river ecosystems (Lenat, 1988). On the other hand, in wetlands, there are limitations to applying this index as the presence of EPT community is lower compared to that

(A)


(a)
r2 = 0.387
Mosquito larval ratio
Richness index
0
1
2
3
4
5
6
1
2
3
4
(b)
r2 = 0.381
Mosquito larval ratio
OCH-group individuals ratio
0.0
0.2
0.4
0.6
0.8
1.0

(B)

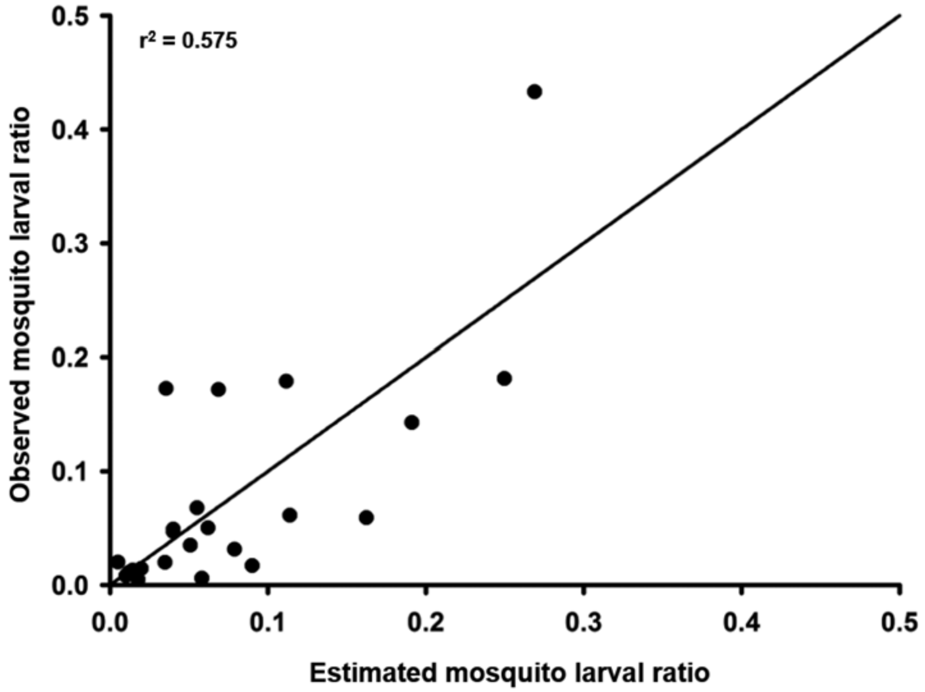


Fig 3-Relationship between (A) - (ln mosquito larval ratio) and richness index (a) and Odonata, Coleoptera and Hemiptera (OCH)-group individual ratio (b), (B) observed mosquito larval ratio and estimated mosquito larval ratio using the optimal prediction model equation.
Mosquito larval abundance in a community = 1.4866 × exp(-2.399 + (10.252 × OCH-group individual ratio) - (0.28 × (richness index)^2) + (4.317 × (OCH-group species ratio)^2) - (15.437 × INT1) - (0.054 × INT4) - 0.0057) × 100
where OCH is Odonata, Coleoptera and Hemiptera, and INT is integrated term (from Table 4).

in rivers. In order to evaluate wetland environment, the present study employed diversity and richness index of the OCH group that has a high richness index in wetland ecosystems (Kim *et al*, 2014).

No correlations among mosquito larval abundance, benthic macroinvertebrate community index (dominance, diversity, richness, evenness), and OCH-group index (OCH-group species ratio and OCH-group individual ratio) were observed. Nonetheless, it is difficult to detect any direct correlation between these factors based on a simple correlation analysis, given that various factors exert complex effects on mosquito larval abundance. Even though several additional variables were introduced to test and evaluate the model based on richness index and OCH-group individual ratio, which demonstrated a relatively high correlation, the explanatory power of the final model developed is not significantly high, but it is worth noting this model only considered benthic macroinvertebrate communities. Compared to environmental factors, measurements of biotic community characteristics are faced with the challenge of real-time measurement; however, stable wetland environments are known to undergo limited seasonal changes (Kim *et al*, 2014).

The study had several limitations. All mosquito species collected were not identified and r^2 values were low. Nevertheless, it is important that a prediction model of mosquito larval abundance is developed based on benthic macroinvertebrates communities only, and the derived equation can be further improved by using information of other communities, such as fish and vegetation, and habitat characteristics.

In conclusion, the study provides a preliminary prediction model of mosquito larval abundance in wetlands of the Korean Peninsula and can form a base for development of more efficient methods for their control using data collected from limited surveys.

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