Journal of Hazardous Materials 219–220 (2012) 95–102

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Utilization of multiple organisms in a proposed early-warning biomonitoring system for real-time detection of contaminants: preliminary results and modeling

Aryo Maradona[a], Gillianne Marshall[a], Mehrab Mehrvar[b,*], Ronald Pushchak[c], Andrew E. Laursen[d], Lynda H. McCarthy[d], Vadim Bostan[d], Kimberley A. Gilbride[d]

[a] Environmental Applied Science and Management Graduate Program, Ryerson University, 350 Victoria Street, Toronto, Ontario M5B 2K3, Canada
[b] Department of Chemical Engineering, Ryerson University, 350 Victoria Street, Toronto, Ontario M5B 2K3, Canada
[c] School of Occupational and Public Health, Ryerson University, 350 Victoria Street, Toronto, Ontario M5B 2K3, Canada
[d] Department of Chemistry and Biology, Ryerson University, 350 Victoria Street, Toronto, Ontario M5B 2K3, Canada

ARTICLE INFO

Article history:
Received 21 October 2011
Received in revised form 5 March 2012
Accepted 22 March 2012
Available online 2 April 2012

Keywords:
Early-warning biomonitoring system
EWBS
Water quality assessment
Rapid biotests
Principal component analysis
Discriminant analysis

ABSTRACT

During past decades, biomonitors were deployed in lakes and rivers to rapidly detect hazardous chemicals by measuring the endpoints of a single aquatic species at defined short intervals. Most biomonitors, however, are only capable of indicating a departure from baseline water conditions without identifying the cause. In order to provide a more comprehensive assessment, a biomonitoring system which features a library of stereotyped responses of multiple aquatic species in various water conditions is proposed. A preliminary library was constructed by characterizing the behavioural and physiological responses of *Daphnia magna*, *Hyalella azteca*, *Lumbriculus variegatus*, and *Pseudokirchneriella subcapitata* to various concentrations of atrazine and tributyltin. By employing multivariate statistical tools such as principal component analysis (PCA) and discriminant analysis, this library (which contained responses after 6 h of exposure to contaminants) was used as a template to classify and to model other sets of earlier measurements at 2 and 4 h, resulting in an accuracy of 73 and 97%, respectively. These findings demonstrated the potential capability of the proposed early-warning biomonitoring system to provide real-time water quality assessment and early-warning contaminant detection.

1. Introduction

The establishment of municipalities, farmlands, and industrial sites near freshwater bodies has increased the risk of chemical contamination in North American lakes and rivers. Based on a survey conducted among 153 water providers in the United States and Canada, transportation accidents, pipeline and storage tank leaks, pesticides from agricultural runoffs, and pathogenic microbes from untreated sewage were the most common causes of water contamination [1]. In Ontario, Canada, alone, 1162 spills into the aquatic systems were reported in 2009, of which, 595 cases were confirmed to have caused a significant environmental impact [2]. Although far less likely, another threat to freshwater supplies includes the deliberate release of harmful chemicals, microbes, and radioactive materials into the aquatic system. To mitigate the above risks, the United States Environmental Protection Agency [3] recommended installing early-warning systems equipped with online sensors to rapidly detect any harmful agents including chemical contaminants. Current chemical sensors, however, are still facing a number of technological and logistical limitations which include the inability to detect a broad range of contaminants [4,5], and thus water safety assessments often must be conducted in off-site facilities and not in real-time.

To supplement the shortcomings of current chemical-sensing technologies, aquatic organisms can be employed as components of an online monitoring system to detect the presence of harmful contaminants. Similar to a miner's canary, these organisms display rapid changes in their physiological and behavioural endpoints when exposed to sub-acute, fast-acting toxicants [6]. When the endpoints exhibit statistically-significant departures from baseline conditions, a warning alarm is raised to notify water safety operators of the situation, and appropriate corrective actions can be carried out. Many endpoint-monitoring technologies have been recently developed, and comprehensive review of these technologies is published elsewhere [4]. Various studies have also evaluated the use of fish [5,7,8], cladocerans [9–12], bivalves [13–16], and algae [17–20] for implementation in various biomonitoring systems. While these studies demonstrated the sensitivities of the

* Corresponding author. Tel.: +1 416 979 5000x6555.
E-mail address: mmehrvar@ryerson.ca (M. Mehrvar).

0304-3894/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jhazmat.2012.03.064

organisms and their quick response times to different contaminants, the aforementioned experiments only tested one type of organism in their respective biomonitoring system. An effective early-warning system cannot rely solely on one species because different organisms may exhibit varying levels of sensitivity to the same contaminant [21,22]. A particular species may also be overly sensitive to natural variations in water quality, as previously reported, where a heavy rainfall prompted an alarm in a cladocerans-based biomonitoring system [12]. Based on the above remarks, a single-species biomonitoring system could potentially underestimate or overestimate the risks of certain toxicants.

To address the problems associated with single-species biomonitors, some water-monitoring stations in Germany and the Netherlands have installed several biomonitors covering multiple aquatic species of various trophic levels [23]. By relying on numerous species, a more robust system which minimizes the chance of false positives or false negatives could be established. Different contaminants may also elicit a unique response from each biomonitoring organism, and a study analyzing the various patterns of responses could potentially allow the identification of unknown contaminants. Despite the system redundancy at these monitoring stations, however, there has been no scholarly-reviewed publication which thoroughly describes the management of several biomonitors (e.g. if a spill alarm is registered by one biomonitor but not the others) or any work which seeks to characterize the responses of these biomonitoring organisms to different contaminants. As a result, there is a lack of understanding on managing complex response data from multiple biomonitors, and current biomonitoring technologies are still limited by the inability to identify any detected contaminants.

In the present study, a model for integrative, multi-species, early-warning biomonitoring system (EWBS) was developed. An essential feature of this proposed system was the establishment of a library database which contained the stereotyped responses of multiple aquatic organisms to several contaminants. A preliminary library of responses was created by tabulating the responses of *Pseudokirchneriella subcapitata*, *Daphnia magna*, *Hyalella azteca*, and *Lumbriculus variegatus* to multiple concentrations of the herbicide atrazine and the pesticide tributyltin. Atrazine is typically applied in North American croplands to control the growth of unwanted weeds, but some improper agricultural practices and run-offs have caused the contamination of various surface and well waters across Canada and the United States [24,25]. Conversely, tributyltin is a biocide and anti-fouling component found in the paints of many boats, cargo ships and other floating structures, and the widespread use of this chemical had resulted in contamination of various ecosystems at concentrations exceeding chronic and even acute toxicity levels [26,27]. By characterizing the responses of selected organisms to atrazine and tributyltin and creating a library of responses, the proposed EWBS could provide a method for managing multiple biomonitors as well as a water-surveillance system for detecting and identifying various environmental contaminants.

2. Materials and methods

2.1. Aquatic organisms

2.1.1. Daphnia magna, Hyalella azteca and Lumbriculus variegatus cultures and bioassays

The procedures for culturing and testing *D. magna*, *H. azteca* and *L. variegatus* were outlined in greater detail in the previous study [22]. The procedures for culturing and conducting *D. magna* and *H. azteca* bioassays were conducted following Environment Canada's protocol and will not be reiterated here [22,28,29]. The water temperature was maintained between 18–20 °C throughout the entire culturing and experiments.

For the bioassay, the organisms were placed in a beaker containing 150 mL of test solution, and silica sand for *H. azteca* and *L. variegatus*, and a digital video recorder (Canon S515) documented the entire experiment. The recording was then manually reviewed to count the percentage of organisms exhibiting stressed endpoints such as (i) a change in swimming height, (ii) looping or twirling behaviour, (iii) a change in body orientation, (iv) immobilization, (v) the use of secondary antennae, and (vi) a change in swimming style for *D. magna*; (vii) a change in swimming height, (viii) immobilization, (ix) burrowing, (x) grouping, (xi) shortening in body length, and (xii) a change in body orientation during swimming for *H. azteca*; and (xiii) abnormal behaviours, (xiv) immobilization, (xv) shortened body length, (xvi) a change in body orientation, and (xvii) abnormal group movements for *L. variegatus*. These 17 behavioural stress responses were evaluated in a previous trial and found to be the most sensitive as well as most reliable in indicating atrazine and tributyltin contaminations [22]. Endpoint measurements at 2, 4, and 6 h were then used for the present study. The age of the daphnids was less than 24 h old, where all ecotoxicological assays use neonates.

2.1.2. Pseudokirchneriella subcapitata cultures and bioassays

The procedures for growing and conducting *P. subcapitata* bioassays are outlined in a previous study [30]. Algal cultures were cultured aseptically in a medium modified from Environment Canada [31]. For the algal bioassay, test solutions were prepared in 250-mL Erlenmeyer flasks, each containing 50-mL aliquots of *P. subcapitata* cultures undergoing an exponential growth phase and at a concentration of $\sim 1.25 \times 10^6$ cells/mL. The water temperature was maintained between 18 to 20 °C throughout the entire culturing and experiments. Using a peristaltic pump, the test solution was fed into a pulse amplitude modulation fluorometer (Algae Online Monitor AOM 2800, Photon Systems Instruments, Czech Republic) to measure the effective photosynthetic yield at 2, 4, and 6 h. This endpoint supplemented the previously described endpoints, bringing the total number of endpoints to 18.

2.2. Test contaminants

To simulate in situ contamination by either tributyltin or atrazine, a series of test solutions were prepared for the bioassays. Tributyltin and atrazine solutions were prepared at concentrations as outlined in Table 1. These concentrations were selected based on their sensitivities, where atrazine at concentration <0.005 mg/L and tributyltin at <0.010 mg/L had no rapid observable effects on organisms, as well as based on environmental relevant levels. For atrazine, the highest values in the US are recorded in waters from the "corn belt" and they can reach 20 μg/L [24] and for tributyltin,

Table 1
Three distinct water conditions according to the type of treatment applied to each replicate.

Condition	Basis for establishing condition
Reference condition	Endpoints measured in dechlorinated tap water only Endpoints measured in dechlorinated tap water with the addition of 0.1% (v/v) dimethyl sulfoxide
Contamination by tributyltin	Endpoints measured in tributyltin solutions with the addition of 0.1% (v/v) dimethyl sulfoxide. Test solutions were prepared at concentrations of 0.010, 0.050 and 0.100 mg tributyltin per litre of dechlorinated tap water.
Contamination by atrazine	Endpoints measured in atrazine solutions with the addition of 0.1% (v/v) dimethyl sulfoxide. Test solutions were prepared at concentrations of 0.005, 0.050 and 0.100 mg atrazine per litre of dechlorinated tap water.

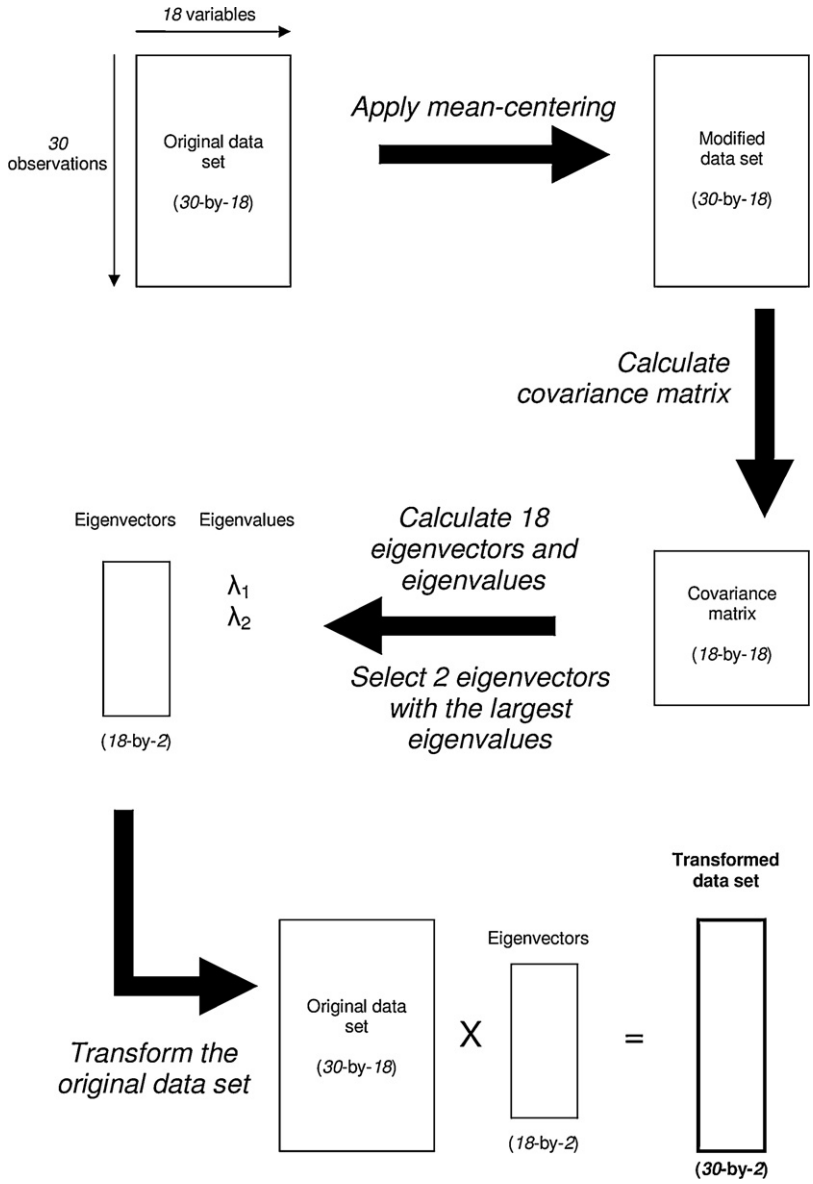

Fig. 1. Principal component analysis was applied on the data set in the present study to reduce data-dimensionality and reveal underlying patterns.

highest values in water are about 0.5 μg/L [32]. In order to increase the solubility of both chemicals in the tap water, dimethyl sulfoxide (DMSO), as a carrier, at a concentration of 0.1% (v/v) was added into each test solution without significantly affecting the organisms as previously reported [22,33–35]. Based on the above procedures, three distinct water conditions were established for the current study as highlighted in Table 1.

2.3. Statistical methods to establish and test the library of responses

In summary, a total of 18 endpoints were measured in the reference and two stressed conditions. The selected organisms displayed statistically significant departures from reference condition after 6 h of exposure to the contaminants [30]. Based on this finding, the measurements at 6 h could be designated as a library of responses, or a template against which other measurements were assessed. Prior to establishing the library of responses, however, the data must be transformed to process experimental noise and manage large variances in some endpoints. A statistical method known as principal component analysis (PCA) reduces a large number of possibly-correlated variables into a smaller set of independent variables, or principal components [36]. By applying PCA to all measurements, the 18 variables (endpoints) were transformed into 2 new principal components which minimized any variable interdependencies and highlighted the greatest variance in the dataset. All calculations were performed using a MATLAB package (Version 7.7.0.471 (R2008b), MathWorks), and the following steps describe the procedure as also illustrated in Fig. 1:

1. The endpoint data measured at $t = 6$ h were arranged into a 30-by-18 matrix where each column represented an endpoint and each row corresponded to a replicate (12 total replicates for reference condition, 9 for atrazine experiments, and 9 for tributyltin experiments).
2. To reduce any bias introduced by variables of larger magnitudes, mean-centering was applied by subtracting each data point by

Table 2
Mean and standard deviation of the percentages of *D. magna* exhibiting various stressed endpoints after 6 h in the test solutions. Experiments were conducted in triplicates, and five organisms were used for each replicate.

Treatment	Changing swimming height	Spinning	Changing body orientation	Immobilized	Using secondary antennae	Changing swimming style
Dechlorinated tap water	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Dechlorinated tap water with 0.1% (v/v) DMSO	0 ± 0	[a]6 ± 10	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Tributyltin (0.010 mg/L)	87 ± 12	40 ± 20	[a]27 ± 31	47 ± 12	47 ± 12	60 ± 20
Tributyltin (0.050 mg/L)	100 ± 0	[a]13 ± 23	20 ± 20	53 ± 12	73 ± 12	80 ± 20
Tributyltin (0.100 mg/L)	100 ± 0	[a]47 ± 50	40 ± 20	60 ± 0	73 ± 12	80 ± 20
Atrazine (0.005 mg/L)	[b]87 ± 23	27 ± 23	33 ± 30	[a]20 ± 35	[a]20 ± 35	60 ± 0
Atrazine (0.050 mg/L)	87 ± 12	33 ± 23	53 ± 12	73 ± 12	67 ± 12	100 ± 0
Atrazine (0.100 mg/L)	100 ± 0	13 ± 23	73 ± 12	80 ± 20	80 ± 20	100 ± 0

[a] Range extended below 0% indicating statistically-insignificant deviation from reference conditions.
[b] Range extended above 100% signifying large variance among replicates.

Table 3
Mean and standard deviation of the percentages of *H. azteca* exhibiting various stressed endpoints after 6 h in the test solutions. Experiments were conducted in triplicates, and five organisms were used for each replicate.

Treatment	Changing swimming height	Immobilized	Burrowing	Changing grouping behaviour	Shortening body length	Changing body orientation
Dechlorinated tap water	[a]3 ± 8	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Dechlorinated tap water with 0.1% (v/v) DMSO	[a]3 ± 8	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Tributyltin (0.010 mg/L)	[b]93 ± 12	73 ± 23	27 ± 23	13 ± 23	60 ± 20	20 ± 0
Tributyltin (0.050 mg/L)	100 ± 0	80 ± 0	0 ± 0	33 ± 31	73 ± 12	[a]13 ± 23
Tributyltin (0.100 mg/L)	100 ± 0	100 ± 0	67 ± 31	60 ± 20	100 ± 0	20 ± 20
Atrazine (0.005 mg/L)	[b]93 ± 12	[b]93 ± 12	60 ± 20	87 ± 12	[b]93 ± 12	0 ± 0
Atrazine (0.050 mg/L)	[b]93 ± 12	[b]93 ± 12	67 ± 31	80 ± 20	100 ± 0	[a]7 ± 12
Atrazine (0.100 mg/L)	100 ± 0	100 ± 0	80 ± 20	[b]93 ± 12	[b]93 ± 12	[a]13 ± 23

[a] Range extended below 0% indicating statistically-insignificant deviation from reference conditions.
[b] Range extended above 100% signifying large variance among replicates.

the mean across each dimension, resulting in a modified matrix with a mean of zero for each endpoint.
3. An 18-by-18 covariance matrix was calculated based on the mean-centered matrix in Step 2. This covariance matrix described the linear relationships among all 18 variables.
4. Using the covariance matrix, unit eigenvectors and eigenvalues were calculated. In order to reduce the data set from 18 variables to 2 variables, two eigenvectors with the largest eigenvalues were selected.
5. A matrix multiplication was applied between the original data set and the 2 eigenvectors in Step 4, resulting in a newly transformed library of responses.

Ideally, blind experiments would be conducted next where the suite of organisms is exposed to one of the two contaminants and the observed responses are then compared to the library of responses to deduce the unknown contaminant. Due to a number of logistical limitations, however, such experimental set up could not be conducted in the present study, and thus measurements from 2 and 4 h were analyzed instead. By applying another statistical technique called discriminant analysis (DA), each observation from 2 and 4 h was assigned into one of the three conditions outlined in Table 1 by using the library as a template. The accuracy of this set up was then determined based on the number of correctly classified data points.

3. Results and discussion

3.1. Endpoint variation in response to atrazine and tributyltin

Average endpoint measurements for the aquatic organisms after 6 h of exposure in the test solutions are shown in Tables 2–5. The invertebrates exhibited minimal stressed behaviour in dechlorinated tap water with and without DMSO. When exposed to atrazine or tributyltin, however, the invertebrates reacted by rapidly changing their behavioural endpoints with some organisms showing more sensitivity to one of the two contaminants. For example, higher percentages of *H. azteca* contracting their body length were found in test solutions containing atrazine, while more

Table 4
Mean and standard deviation of the percentages of *L. variegatus* exhibiting various stressed endpoints after 6 h in the test solutions. Experiments were conducted in triplicates, and ten organisms were used for each replicate.

Treatment	Displaying abnormal behaviour	Immobilized	Shortening body length	Changing body orientation	Moving within groups
Dechlorinated tap water	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Dechlorinated tap water with 0.1% (v/v) DMSO	0 ± 0	0 ± 0	0 ± 0	[a]2 ± 4	0 ± 0
Tributyltin (0.010 mg/L)	[b]78 ± 39	[a]11 ± 19	[a]6 ± 10	[a]6 ± 10	[a]35 ± 40
Tributyltin (0.050 mg/L)	100 ± 0	23 ± 10	35 ± 9	35 ± 9	[a]11 ± 19
Tributyltin (0.100 mg/L)	100 ± 0	51 ± 16	83 ± 14	28 ± 5	89 ± 10
Atrazine (0.005 mg/L)	67 ± 29	[a]7 ± 12	[a]10 ± 17	0 ± 0	27 ± 6
Atrazine (0.050 mg/L)	[b]83 ± 29	7 ± 6	21 ± 12	0 ± 0	10 ± 0
Atrazine (0.100 mg/L)	100 ± 0	10 ± 10	[b]53 ± 50	0 ± 0	10 ± 10

[a] Range extended below 0% indicating statistically-insignificant deviation from reference conditions.
[b] Range extended above 100% signifying large variance among replicates.

Table 5
Mean and standard deviation of the initial and final effective photosynthetic yield of *P. subcapitata*. Experiments were conducted in triplicates, and a concentration of ~1.25×10^6 cells/mL was used.

Treatment	Effective photosynthetic yield at $t = 0$ h	Effective photosynthetic yield at $t = 6$ h
Dechlorinated tap water	14 ± 1	24 ± 1
Dechlorinated tap water with 0.1% (v/v) DMSO	14 ± 1	21 ± 1
Tributyltin (0.010 mg/L)	15 ± 1	19 ± 1
Tributyltin (0.050 mg/L)	15 ± 1	20 ± 1
Tributyltin (0.100 mg/L)	16 ± 1	19 ± 1
Atrazine (0.005 mg/L)	15 ± 1	13 ± 2
Atrazine (0.050 mg/L)	13 ± 1	9 ± 1
Atrazine (0.100 mg/L)	15 ± 1	7 ± 2

L. variegatus were immobilized when placed in test solutions containing tributyltin.

The link between the mechanism of uptake for the two contaminants and the immediate sub-acute responses by the test organisms was still largely unknown, but the range in responses could be attributed to a number of factors. First, a structural difference in the outer skeletons of the organisms could potentially lead to different uptake rates for the two contaminants which then resulted in different reaction severities. Trimble and Lydy [37] noted some variation in the responses of *H. azteca* and the nematoceran fly larvae *Chironomus tentans* when subjected to atrazine and other organophosphorous insecticides, and the exoskeleton hardness of each organism was hypothesized as a possible cause. Similarly, in the current study, the highly-calcified exoskeletons of the *H. azteca* would have a different permeability than the hydrostatic skeleton of the *L. variegatus*, and this structural difference may explain the variation in stress response. Another possible factor causing the diverse reactions was the modes of action of each chemical. Ren et al. [34,35] found some variation in the behavioural responses of *D. magna* in experiments with deltamethrin, chlorothalonil, and nitrofen, where a neurotoxic stressor such as deltamethrin elicited a more prompt reaction but the cytotoxic chlorothalonil and nitrofen resulted in a more gradual impairment. It was postulated that the two test contaminants in the present study could have exerted their toxicities differently on the selected aquatic organisms as well, resulting in distinct reactions in the endpoints of *D. magna*, *H. azteca*, and *L. variegatus*.

Contrary to the varying sensitivities among the invertebrates to the two contaminants, the green algae *P. subcapitata* indicated a predominant susceptibility to atrazine than tributyltin. Atrazine, classified as a herbicide, is known to inhibit the electron transport mechanism of photosystem II in aquatic plants which would then affect the photosynthetic yield of the algae, or the ratio of absorbed light energy for chemical synthesis versus the energy released in the form of heat and radiation or fluorescence [38,39]. A decreased value in photosynthetic yield indicates that the plant cell is under stress from a contaminant which affected its physiological state. An examination of Table 5 revealed that *P. subcapitata* cultures in dechlorinated tap water exhibited an increase of ~10% in their effective photosynthetic yields over a period of 6 h as the algal cultures were in their active growing phase. Exposure to DMSO and tributyltin solutions slightly reduced the rate of increase, resulting in a rise of between 4 to 7% after 6 h. When exposed to atrazine, however, the green algae produced noticeably lower values of photosynthetic yield, with a replicate exhibiting a decrease of ~7%. An inverse relationship was also observed between the concentration of atrazine and the photosynthetic efficiency of the algae, where higher concentrations of the chemical caused a larger reduction in the effective photosynthetic yield. A one-way analysis of variance comparing the effective photosynthetic yields in reference

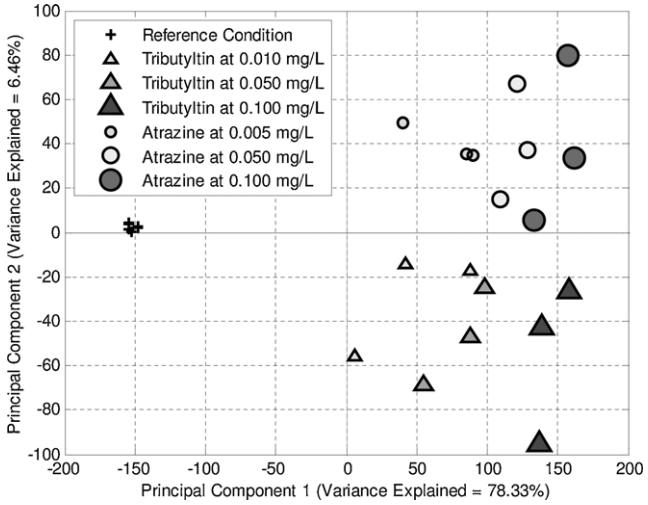

Fig. 2. Distribution of the library of responses, or transformed endpoint measurements of *D. magna*, *H. azteca*, *L. variegatus*, and *P. subcapitata* after 6 h incubation for experimental conditions outlined in Table 1. Each test condition elicited unique sets of reactions among the organisms, resulting in well-separated groups of data points.

condition and each of the test solutions at 6 h revealed *p*-values of less than 0.05, indicating a greater than 95% probability that the addition of either contaminants had significantly influenced the physiology of the algal cells. Since drinking water reservoirs are characterized by very low suspended solids, the effect of turbidity was neglected in this study.

3.2. Establishment of a library of responses using PCA

PCA was applied to the library of responses to reduce the number of variables from 18 to 2. A graphical representation of the transformed library is shown in Fig. 2. An inspection of the eigenvalues revealed that the first and second principal components accounted for 78.3 and 6.5% of the total variance, respectively, resulting in a combined sum of almost 85%. This combined sum agreed with the recommendations of Jackson [40], which suggested the total accounted variance to be at least 80%.

Fig. 2 further highlights the distinct set of endpoints exhibited by the organisms in response to different water conditions as three clusters of data points could be observed and matched to the conditions listed in Table 1. Reference condition, referring to the endpoints measured in dechlorinated tap water with and without the addition of DMSO, formed a tight cluster to the left of the origin because the organisms exhibited very small changes in their endpoints. Conversely, stressed conditions resulted in data points located to the right of the origin, with experiments conducted in solutions containing atrazine and tributyltin producing data points above and below the Principal Component 1 (PC1) axis, respectively. The data points also showed a general drift toward the positive PC1 direction as the concentration of each contaminant was increased. These findings reiterated the capability of multiple aquatic species to produce unique patterns of responses to different contaminants, and a library of responses containing this information could be used to identify other sets of measurements.

3.3. Data classification using DA

As previously elaborated, blind experiments were not available for the present study, and thus a set of measurements at 2 and 4 h were tested instead. Figs. 3 and 4 display the distribution of the measurements at 2 and 4 h, respectively, as compared to the library of responses. Since the library contained two boundary conditions

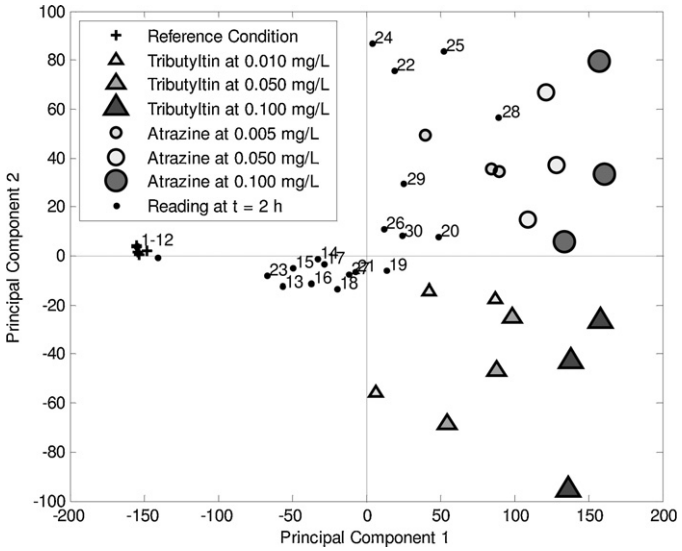

Fig. 3. Plot of the endpoint measurements at 2 h against the library of responses (see Table 6 for the description of each data point). After 2 h of exposure to atrazine or tributyltin, the organisms started to exhibit changes in their endpoints with more apparent deviations seen at higher contaminant concentrations.

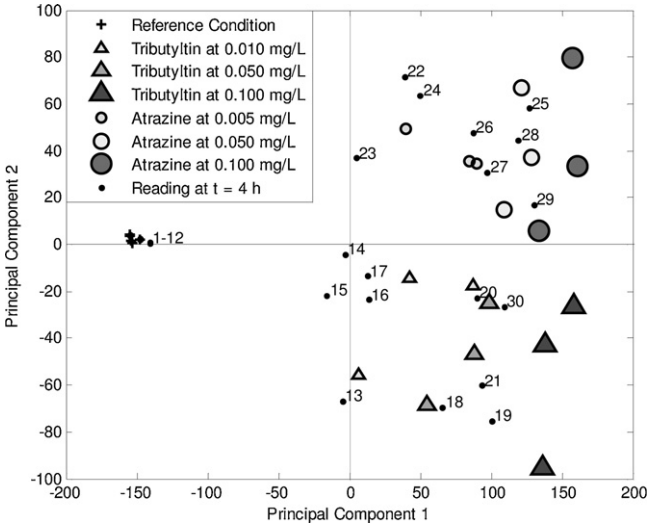

Fig. 4. Plot of the endpoint measurements at 4 h against the library of responses (see Table 7 for the description of each data point). After 4 h of exposure to atrazine or tributyltin, most organisms started to display statistically-significant changes in their endpoints with magnitudes resembling values in the library of responses.

where the endpoints were closer to 0% in non-stressed conditions (reference condition) and approaching 100% at highly-stressed conditions, any measurements conducted between 0 and 6 h would result in data points falling in between the two extremities. Many of the measurements at 2 h were situated closer to the reference condition because the organisms were still reacting to the contaminants as shown in Fig. 3. As the bioassay progressed to 4 h, however, the organisms were starting to exhibit stressed responses, resulting in a data point distribution which more closely resembled the library of responses.

Tables 6 and 7 summarize the results of classifying the measurements in Figs. 3 and 4, respectively, by using the library of responses as training set. For measurements at 2 h, 100% of the reference conditions were correctly identified, while only 33% of the tributyltin contaminations and 78% of the atrazine contaminations were properly classified, resulting in an overall accuracy

Table 6
Results of discriminant analysis for each data point in Fig. 3 (endpoint measurements at $t = 2$ h) by using the library of responses as template. An overall accuracy of 73%, or 22 correct classifications out of 30 observations, was achieved.

Data point	Actual condition	Classified condition
1	Dechlorinated tap water	Reference condition
2	Dechlorinated tap water	Reference condition
3	Dechlorinated tap water	Reference condition
4	Dechlorinated tap water	Reference condition
5	Dechlorinated tap water	Reference condition
6	Dechlorinated tap water	Reference condition
7	Dechlorinated tap water with DMSO	Reference condition
8	Dechlorinated tap water with DMSO	Reference condition
9	Dechlorinated tap water with DMSO	Reference condition
10	Dechlorinated tap water with DMSO	Reference condition
11	Dechlorinated tap water with DMSO	Reference condition
12	Dechlorinated tap water with DMSO	Reference condition
13	Tributyltin at 0.010 mg/L	*Reference condition
14	Tributyltin at 0.010 mg/L	*Reference condition
15	Tributyltin at 0.010 mg/L	*Reference condition
16	Tributyltin at 0.050 mg/L	*Reference condition
17	Tributyltin at 0.050 mg/L	*Reference condition
18	Tributyltin at 0.050 mg/L	Contamination by tributyltin
19	Tributyltin at 0.100 mg/L	Contamination by tributyltin
20	Tributyltin at 0.100 mg/L	*Contamination by atrazine
21	Tributyltin at 0.100 mg/L	Contamination by tributyltin
22	Atrazine at 0.005 mg/L	Contamination by atrazine
23	Atrazine at 0.005 mg/L	*Reference condition
24	Atrazine at 0.005 mg/L	Contamination by atrazine
25	Atrazine at 0.050 mg/L	Contamination by atrazine
26	Atrazine at 0.050 mg/L	Contamination by atrazine
27	Atrazine at 0.050 mg/L	*Contamination by tributyltin
28	Atrazine at 0.100 mg/L	Contamination by atrazine
29	Atrazine at 0.100 mg/L	Contamination by atrazine
30	Atrazine at 0.100 mg/L	Contamination by atrazine

* Incorrectly classified data point.

of 73%. The majority of misclassifications occurred at low contaminant concentrations, and an inspection of the raw data revealed that many of these incorrectly-assigned measurements highly resembled the reference condition because the contaminants had not yet significantly affected the organisms [22,30]. As the bioassay progressed to 4 h, the stressed responses of the organisms became more apparent, and the accuracy of the library improved as shown in Table 7. After 4 h of incubation, 29 out of 30 measurements were correctly classified, resulting in an overall accuracy of 97%. The misclassified measurement was observation number 30 where a contamination by atrazine was incorrectly designated as a contamination by tributyltin. Despite this error, observation number 30 was still classified as a non-reference condition which would nonetheless trigger a warning alarm in an actual situation and subsequently alert the water safety operators to execute any remedial action.

3.4. Future development and automation of the multi-species biomonitoring system

The use of multiple aquatic organisms enabled the characterization of various behaviours in different water conditions. The model in the present study had demonstrated the capability to detect and resolve with high accuracy 2 different contaminants within 2–4 h of exposure by using 18 behavioural and physiological endpoints from 4 aquatic organisms. Currently, no published single-species biomonitor study to the best knowledge of the authors had accomplished such feat, and thus this finding

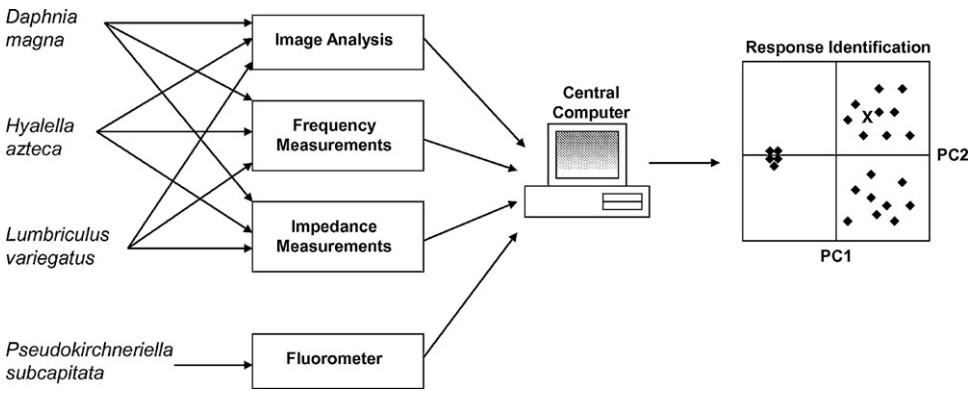

Fig. 5. Preliminary setup of the proposed early-warning biomonitoring system. An X on the Response Identification screen signified current water condition which was then evaluated against the library of responses (represented by the square data points in the same screen).

Table 7
Results of discriminant analysis for each data point in Fig. 4 (endpoint measurements at $t = 4$ h) by using the library of responses as template. An overall accuracy of 97%, or 29 correct classifications out of 30 observations, was achieved.

Data point	Actual condition	Classified condition
1	Dechlorinated tap water	Reference condition
2	Dechlorinated tap water	Reference condition
3	Dechlorinated tap water	Reference condition
4	Dechlorinated tap water	Reference condition
5	Dechlorinated tap water	Reference condition
6	Dechlorinated tap water	Reference condition
7	Dechlorinated tap water with DMSO	Reference condition
8	Dechlorinated tap water with DMSO	Reference condition
9	Dechlorinated tap water with DMSO	Reference condition
10	Dechlorinated tap water with DMSO	Reference condition
11	Dechlorinated tap water with DMSO	Reference condition
12	Dechlorinated tap water with DMSO	Reference condition
13	Tributyltin at 0.010 mg/L	Contamination by tributyltin
14	Tributyltin at 0.010 mg/L	Contamination by tributyltin
15	Tributyltin at 0.010 mg/L	Contamination by tributyltin
16	Tributyltin at 0.050 mg/L	Contamination by tributyltin
17	Tributyltin at 0.050 mg/L	Contamination by tributyltin
18	Tributyltin at 0.050 mg/L	Contamination by tributyltin
19	Tributyltin at 0.100 mg/L	Contamination by tributyltin
20	Tributyltin at 0.100 mg/L	Contamination by tributyltin
21	Tributyltin at 0.100 mg/L	Contamination by tributyltin
22	Atrazine at 0.005 mg/L	Contamination by atrazine
23	Atrazine at 0.005 mg/L	Contamination by atrazine
24	Atrazine at 0.005 mg/L	Contamination by atrazine
25	Atrazine at 0.050 mg/L	Contamination by atrazine
26	Atrazine at 0.050 mg/L	Contamination by atrazine
27	Atrazine at 0.050 mg/L	Contamination by atrazine
28	Atrazine at 0.100 mg/L	Contamination by atrazine
29	Atrazine at 0.100 mg/L	Contamination by atrazine
30	Atrazine at 0.100 mg/L	* Contamination by tributyltin

* Incorrectly classified data point.

further reiterated the importance and advantages of multiple-species biomonitoring system. The library of responses in the present study was still preliminary as the model had only measured the stress responses elicited by atrazine and tributyltin, but the list of contaminants could be expanded in future studies to include other common environmental pollutants. Due to the improbability of testing *all* known chemicals, a number of contaminants could be placed in the same category according to their chemical structures or modes of action (e.g. herbicides, pesticides, hydrocarbons, etc.).

For field implementation, the proposed EWBS must be automated to provide rapid, practical, and unbiased assessments of water quality. Due to budgetary limitations, the majority of endpoints in the present study (except for effective photosynthetic yield) were measured using visual observation and manual counting, but this procedure could be automated in future trials using a number of commercially-available technologies [4]. For example, digital image analysis could be used to monitor the swimming behaviour of *D. magna* which was successfully demonstrated by Jeon et al. [11], while other non-visual based methods such as impedance and frequency measurements could be implemented for recording other endpoints. A central computer would then process the various endpoint measurements and conduct a series of statistical algorithms as outlined in the present study. Due to the relatively simple calculations, the algorithms could be rapidly executed using a program such as MATLAB, in which the processing time for the current study was under 20 s. Fig. 5 outlines a preliminary set up for the automation of the proposed EWBS.

4. Conclusions

The findings of the present study served as a groundwork where the endpoints of multiple aquatic species of different trophic levels could be monitored simultaneously to form an integrative and more advanced early-warning biomonitoring system. Based on preliminary tests, the following conclusions were obtained from this study:

1. An early-warning biomonitoring system was proposed in the current study to reduce the system reliance on a single aquatic species, and a library of responses was suggested as a method to manage the multi-variable endpoint data set.
2. Principal component analysis was effective in integrating the responses of the organisms, minimizing experimental noise, and highlighting any distinct patterns within the library of responses.
3. After establishing the library of responses, discriminant analysis was employed to evaluate the capability of the library to detect and resolve different contaminants. Using the present library of responses, sets of earlier measurements could be classified with a high accuracy within 2–4 h. These findings reflected the potential field application and capability of the early-warning biomonitoring system to assess water quality in real time and rapidly detect any hazardous aquatic contaminants.

Acknowledgement

The financial support of the National Sciences and Engineering Research Council of Canada (NSERC) through a Strategic Research Grant Program and Ryerson University is gratefully appreciated.

References

[1] R.W. Gullick, W.M. Grayman, R.A. Deininger, R.M. Males, Design of early-warning monitoring systems for source waters, J. Am. Water Works Assoc. 95 (11) (2003) 58–72.

[2] Ontario Ministry of the Environment, Spill Action Centre: 2009 Summary Report, 2010, accessed on June 28, 2011 from http://www.ene.gov.on.ca.

[3] J. Hasan, D. Goldbloom-Helzner, A. Ichida, T. Rouse, M. Gibson, Technologies and Techniques for Early Warning Systems to Monitor and Evaluate Drinking Water Quality: State-of-the-Art Review, United States Environmental Protection Agency, Storming Media, 2005.

[4] A. Gerhardt, M.K. Ingram, I.J. Kang, S. Ulitzur, In situ on-line toxicity biomonitoring in water: recent developments, Environ. Toxicol. Chem. 25 (2006) 2263–2271.

[5] W.H. Van der Schalie, R.R. James, T.P. Gargan II, Selection of a battery of rapid toxicity sensors for drinking water evaluation, Biosens. Bioelectron. 22 (2006) 18–27.

[6] C. Streb, P. Richter, M. Ntefidou, M. Lebert, D.P. Häder, ECOTOX–biomonitoring based on real time movement analysis of unicellular organisms, in: Proceedings of Life on Earth, 8th European Symposium of Life Sciences Research in Space, 23rd Annual International Gravitational Physiology Meeting, Karolinska Institute, Stockholm, Sweden, 2002.

[7] J. Cairns Jr., K.L. Dickson, R.E. Sparks, W.T. Waller, A preliminary report on rapid biological information systems for water pollution control, J. Water Pollut. C 42 (1970) 685–703.

[8] W.H. Van Der Schalie, T.R. Shedd, M.W. Widder, L.M. Brennan, Response characteristics of an aquatic biomonitor used for rapid toxicity detection, J. Appl. Toxicol. 24 (5) (2004) 387–394.

[9] Knie F J., Der Dynamische Daphnientest, Decheniana 26 (1982) 82–86.

[10] U. Green, J.H. Kremer, M. Zillmer, C. Moldaenke, Detection of chemical threat agents in drinking water by an early warning real-time biomonitor, Environ. Toxicol. 18 (2003) 368–374.

[11] J. Jeon, J.H. Kim, B.C. Lee, S.D. Kim, Development of a new biomonitoring method to detect the abnormal activity of *Daphnia magna* using automated grid counter device, Sci. Total Environ. 389 (2008) 545–556.

[12] A. Gunatilaka, P. Diehl, H. Puzicha, The evaluation of dynamic daphnia test after a decade of use, in: F.M. Butterworth, A. Gunatilaka, M.E. Gonsebatt (Eds.), Biomonitors and Biomarkers as Indicators of Environmental Change, vol. 2, Kluwer Academic/Plenum, New York, USA, 2001, pp. 29–58.

[13] G. Gunkel, B. Streit, Mechanisms of bioaccumulation of a herbicide (atrazine, s-triazine) in a freshwater mollusc (*Ancylus fluviatilis* Mull.) and a fish (*Coregonus fera* Jurine), Water Res. 14 (11) (1980) 1573–1584.

[14] K.J.M. Kramer, E.M. Foekema, The "Mussel Monitor" as a biological early warning system, in: F.M. Butterworth, A. Gunatilaka, M.E. Gonsebatt (Eds.), Biomonitors and Biomarkers as Indicators of Environmental Change, vol. 2, Kluwer Academic/Plenum, New York, USA, 2001, pp. 59–89.

[15] B. White, M. Gibert, R. Deininger, Biological Monitors, in: Awrf Crs Proaqua, E.E. Hargesheimer (Eds.), Online Monitoring for Drinking Water Utilities, American Water Works Association, Denver, USA, 2002.

[16] J. Borcherding, M. Volpers, The Dreissena-monitor – 1st results on the application of this biological early warning system in the continuous monitoring of water quality, Water Sci. Technol. 29 (1994) 199–201.

[17] P. Pandard, P. Vasseur, D.M. Rawson, Comparison of two types of sensors using eukaryotic algae to monitor pollution of aquatic systems, Water Res. 27 (3) (1993) 427–431.

[18] D. Osbild, M. Babut, P. Vasseur, Biosensors for environmental monitoring and water control, J. Water Sci. 8 (1995) 505–538.

[19] C. Durrieu, C. Tran-Minh, J.M. Chovelon, L. Barthet, C. Chouteau, C. Védrine, Algal biosensors for aquatic ecosystems monitoring, Eur. Phys. J. Appl. Phys. 36 (2006) 205–209.

[20] P.B. Fai, A. Grant, B. Reid, Chlorophyll a fluorescence as a biomarker for rapid toxicity assessment, Environ. Toxicol. Chem. 26 (7) (2007) 1520–1531.

[21] S.E. Bunn, Biological monitoring of water quality in Australia: workshop summary and future directions, Aust. J. Ecol. 20 (1995) 220–227.

[22] G.C. Marshall, Assessing behavioural and physiological responses of three aquatic invertebrates to atrazine and tributyltin in a multi-species, early-warning biomonitoring technology, M.A.Sc. Thesis, Ryerson University, Toronto, Canada, 2009.

[23] P. Diehl, T. Gerke, A. Jeuken, J. Lowis, R. Steen, J. van Steenwijk, P. Stoks, H.-G. Willemsen, Early warning strategies and practices along the river rhine, Handb. Environ. Chem. 5PartL (2006) 99–124.

[24] M. Graymore, F. Stagnitti, G. Allinson, Impacts of atrazine in aquatic ecosystems, Environ. Int. 26 (2001) 483–495.

[25] P.B. Kurt-Karakus, D.C.G. Muir, T.F. Bidleman, J. Small, S. Backus, A. Dove, Metolachlor and atrazine in the great lakes, Environ. Sci. Technol. 44 (12) (2010) 4678–4684.

[26] C. Alzieu, Tributyltin: case study of a chronic contaminant in the coastal environment, Ocean Coastal Manage. 40 (1998) 23–36.

[27] I.K. Konstantinou, T.A. Albanis, Worldwide occurrence and effects of antifouling paint booster biocides in the aquatic environment: a review, Environ. Int. 30 (2004) 235–248.

[28] Environment Canada, Biological Test Method: Acute Lethality Test using *Daphnia* spp. EPS1/RM/11, Environmental Protection Series, Environment Canada, Ottawa, Canada, 1996.

[29] Environment Canada, Biological Test Method: Test for Survival and Growth in Sediment using the Freshwater Amphipod *Hyalella azteca*, EPS1/RM/33, Environmental Protection Series, Environment Canada, Ottawa, Canada, 1997.

[30] A. Maradona, Development of a Library of Responses for an Early-Warning Biomonitoring System to Detect and Identify Various Aquatic Contaminants, M.A.Sc. Thesis, Ryerson University, Toronto, Canada, 2011.

[31] Environment Canada, Biological Test Method: Growth Inhibition Test Using a Freshwater Alga, Report EPS 1/RM/25, Environmental Protection Series, Environment Canada, Ottawa, Canada, 2007.

[32] B. Antizar-Ladislao, Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review, Environ. Int. 34 (2008) 292–308.

[33] J.C. Martins, M.L. Saker, L.F.O. Teles, V.M. Vasconcelos, Oxygen consumption by *Daphnia magna* straus as a marker of chemical stress in the aquatic environment, Environ. Toxicol. Chem. 26 (2007) 1987–1991.

[34] Z. Ren, J. Zha, M. Ma, Z. Wang, A. Gerhardt, The early warning of aquatic organophosphorus pesticide contamination by on-line monitoring behavioural changes of *Daphnia magna*, Environ. Monit. Assess. 134 (2007) 373–383.

[35] Z. Ren, Z. Li, M. Ma, Z. Wang, R. Fu, Behavioural responses of *Daphnia magna* to stresses of chemicals with different toxic characteristics, Bull. Environ. Contam. Toxicol. 82 (2009) 310–316.

[36] P. Shaw, Multivariate Statistics for the Environmental Sciences, Hodder Arnold, London, 2003.

[37] A.J. Trimble, M.J. Lydy, Effects of triazine herbicides on organophosphate insecticide toxicity in *Hyalella azteca*, Arch. Environ. Contam. Toxicol. 51 (2006) 29–34.

[38] N. Cedergreen, J. Streibig, The toxicity of herbicides to non-target aquatic plants and algae: assessment of predictive factors and hazard, Pest Manage. Sci. 61 (12) (2005) 1152–1160.

[39] R.J. Strasser, A. Srivastava, M. Tsimilli-Michael, The fluorescence transient as a tool to characterize and screen photosynthetic samples, in: P. Mohanty, M. Yunus, U. Pathre (Eds.), Probing Photosynthesis: Mechanism, Regulation and Adaptation, Taylor and Francis, London, UK, 2000, pp. 443–480.

[40] D.A. Jackson, Stopping rules in principal component analysis: a comparison of heuristical and statistical approaches, Ecology 74 (1993) 2204–2214.