The Asian Journal of Biology Education

ISSN 1447-0209

Volume 10: July 2018

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Practical Report

Antifungal Activity of Capsicum frutescens and Allium cepa against Aspergillus spp.: An Application of Scientific Process Skills by High School Students

Lewis Kedrick L. Ong, John Elisha D. Dela Cruz, Jed Benedict M. Lim, Joseph Vincent D. San Pedro and Emmanuel D. Delocado ........................................... 2

Country Report

Biology Education in Upper Secondary Schools at Present in Japan

Teiko Nakamichi and Nobuyasu Katayama ................................................................. 7

Archives

Contents of the Proceedings of Past Biennial Conferences of the AABE ................. 17

Publications ..................................................................................................... 40

From the Editor-in-Chief ............................................................................................... 40
Antifungal Activity of Capsicum frutescens and Allium cepa against Aspergillus spp.: An Application of Scientific Process Skills by High School Students

Lewis Kedrick L. Ong*, John Elisha D. Dela Cruz, Jed Benedict M. Lim, Joseph Vincent D. San Pedro, Emmanuel D. Delocado*

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(Received: 23 December 2014; accepted: 27 May 2016)

Aspergillus niger and Aspergillus flavus are known to induce risks including aspergillosis in humans and common crop drought to plants. Allium cepa (white onion) and Capsicum frutescens (cayenne pepper) have been reported as having some antifungal potential. Thus, to practice scientific process skills, high school biology students investigated whether A. cepa and C. frutescens extracts are effective antifungal agents against these two pathogens. Sensitivity testing using Kirby-Bauer assay revealed that C. frutescens was more effective against A. niger and A. flavus. C. frutescens extract alone produced an inhibition zone of 19.29 mm for A. niger and 10.47 mm for A. flavus. Using t-test and repeated measures ANOVA (95% level of confidence), the results were comparable to an antifungal drug miconazole. It is therefore concluded that C. frutescens or the mixture of C. frutescens and A. cepa extracts (50-50 v/v) can be effectively used as antifungal agent against A. niger. This study possibly serves as a model for students to learn the scientific method practically and to experience different process skills essential in biological research tangibly.

Keywords: Allium cepa, antifungal activity, Aspergillus niger, Aspergillus flavus, Capsicum frutescens, laboratory model for high school biology

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INTRODUCTION

Aspergillus spp. are fungi commonly present in the air that people breathe (Yassin and Almouqatea, 2010; cf. CDC Website). There are more than 185 known species of Aspergillus, and at least 20 of them have been reported to cause human diseases, such as aspergillosis, pneumonia and fungus ball which attacks the lungs (Yassin and Almouqatea, 2010; Choudhury et al., 2011).

The most common species of Aspergillus are A. niger and A. flavus – both species cause diseases in human beings and plants. Moreover, A. niger is one of the top three causatives of human fungal diseases (Barker and Carrington, 1953; Choudhury et al., 2011). A. niger is very versatile and not fastidious, allowing itself to grow in different environments and microhabitats where the other fungi cannot. A. niger also infects plants, specifically ginger, onion, peanut, grapes and mangoes. It produces toxins that induce crop or fruit rotting rendering them unsafe for human consumption (U. S. EPA Website, 1997; Choudhury et al., 2011).

On the other hand, A. flavus produces aflatoxins that cause rotting in plants. It usually infects the seeds of plants like corn and peanut. However, manifestation of the infection becomes apparent
only in the post-harvest and storage stages. As a result, the infected seeds would then not be useful any more (Montes-Belmont and Carvajal, 1998).

Corn is the second most important crop in the Philippines after rice. An annual report generated by the Bureau of Agriculture Statistics (BAS) in 2012 indicated that the crop sector grossed a total of 375.1 billion Philippine pesos, which is 51.79% of the total production of the agriculture sector, and 60% of which comes from the crop sector and 6% is attributable to corn (PSA Website, 2014). If an outbreak of these species of *Aspergillus* arises in the Philippines or even in neighboring Asian countries, the agricultural industry would certainly be paralyzed. With such unfortunate event, the poultry and livestock industries would also be affected because corn is one of the main sources of feeds in the country (Montes-Belmont and Carvajal, 1998). Sixty percent of corn produced in the Philippines (the average is around 3.21 metric tons per hectare) is used for feeds in the livestock and poultry sectors, while the remaining is used for human consumption. This would ultimately have a negative effect on the economy since the combined corn, livestock, and poultry industries are approximately 30% of the total agriculture sector of the Philippines (PSA Website, 2014).

Fortunately, natural products have been proven to inhibit the growth of these fungal species. For example, *Allium cepa* (white onion) has been reported to have some useful medicinal properties: It has anti-inflammatory, anti-asthmatic and antimicrobial properties and was even found to have a good effect on the cardiovascular system (Santas et al., 2010). Moreover, onion has the potential to be a fungicidal agent. Species of *Aspergillus* and *Candida* were not able to reproduce when onion extracts were applied (Benkeblia, 2004). Also, Lanzotti et al. (2012) reported that three saponins in onion had a high antifungal activity. *Capsicum frutescens* (cayenne pepper) or locally known as “siling labuyo” in the Philippines, is usually used in food preparation and for homemade remedies (Cichewicz and Thorpe, 1996). De Lucca et al. (2006a) reported that it had an antifungal property due to a certain saponin called CAY-1.

In these studies, the antifungal properties of both *A. cepa* and *C. frutescens* were characterized. However, the effectiveness mainly was examined on the dosage of each sample. Since *A. cepa* and *C. frutescens* have different types of saponins (De Lucca et al., 2006a; Lanzotti et al., 2012), it is presumed that the two would complement each other and become a stronger fungicidal agent. Therefore, in the present study, the combined antifungal effects of the extracts of *A. cepa* and *C. frutescens* on the growth of *A. niger* and *A. flavus* were investigated.

It should be noted that the present study came about after high school students were immersed to the different concerns about the safety of food crops and the development of antimicrobial agents through the works of Pandey et al. (1982) and De Lucca et al. (2006b) with further reinforcements from local news articles and stories in Philippine provinces. The students also learned from Yassin and Almouqatea (2010) that scientists had been producing much safer antifungal agents from plants in comparison to synthetic or artificial fungicides, which might be harmful to both plants and humans.

**MATERIALS AND METHODS**

**Preparation of Test Organisms**

Pure cultures of *A. niger* and *A. flavus* were obtained from the Microbial Culture Collection and Testing Laboratory of Department of Biological Sciences, Central Luzon State University, Philippines. All apparatus used were sterilised with heat. The pure cultures of *A. niger* and *A. flavus* were inoculated from a heated wire loop on the potato-dextrose-agar (PDA) slants and were kept in the refrigerator at 5°C until needed.

**Acquisition and Extraction of Plant Materials**

Plant materials, *A. cepa* bulbs and *C. frutescens* fruits, were purchased from a local market. They were identified by an agronomist at the Central Luzon State University.
Extraction procedure was carried out as adapted and modified from Abdou *et al.* (1972) and Benkeblia (2004). The cayenne pepper, alongside with onions were washed with clean water and allowed to air dry for 4 days. The outer coverings (tunic) of onion’s bulb were manually peeled off. They were then separately cut into small pieces and underwent the process of maceration in which 20 g of each of the dried plants were soaked in 20 ml of 20% ethanol for 48 hours. They were then filtered using a filter paper.

**Antimicrobial Sensitivity Testing**

Sensitivity testing was carried out for *A. niger* and *A. flavus* using the Kirby-Bauer technique (Bauer *et al.*, 1966). A sterile cotton swab was used to spread the microorganisms all over the surface of the PDA plates. The plates were allowed to dry for about 5 minutes.

Whatman filter paper No. 2 disks, 6 mm in diameter, were immersed in the extracts of *A. cepa*, or *C. frutescens*, a 50-50 v/v mixture of *C. frutescens* and *A. cepa* extracts, chloramphenicol (30 mg/ml), or miconazole (30 mg/ml). The disks were placed on respective plates of test organisms which then were incubated at 37°C for 72 hours. Three replicates were made.

**RESULTS**

All the treatments showed positive results (Figure 1). The extracts inhibited the growth of *A. niger*. Chloramphenicol exhibited the widest zone of growth inhibition for *A. niger* (26.97 mm). The zones of growth inhibition of *C. frutescens* extract (19.29 mm) and the mixture of *A. cepa* and *C. frutescens* extracts (19.20 mm) were statistically comparable to that of miconazole (20.33 mm) using *t*-test and repeated measures ANOVA (95% level of confidence).

For *A. flavus*, *C. frutescens* extract inhibited the growth to a certain extent (10.47 mm) which was not comparable to chloramphenicol (25.50 mm) and miconazole (16.25 mm). However, this value was significant compared to the other treatments which did not significantly inhibit the growth of *A. flavus*.

**DISCUSSION**

Phytochemical testing in previous studies, such as Benkeblia (2004) and Kamilla *et al.* (2009), revealed that secondary metabolites present in cer-
tain plant extracts and commercially available medicines are responsible for antifungal activities against fungi from genera *Aspergillus* and *Candida* among others. The tested plants contain tannins, polyphenols, alkaloids and glycosides, which have natural antimicrobial properties (De Lucca et al., 2006a; Lanzotti et al., 2012).

Saponins are also a group of these secondary metabolites. They serve as important components in a wide range of plant species, for they function as a defending agent against microbial infections (Lanzotti et al., 2012). They have detergent-like properties that are lethal to fungi due to their ability to combine with membrane sterols, which cause a loss of membrane integrity. Some plant species show compromised resistance to different fungal pathogens because of a deficiency in saponins (De Lucca et al., 2006a). Two saponins found in *C. frutescens* were tested amongst many strains of fungi, including some strains of *Aspergillus*, and were shown to be effective antifungal agents against most strains of fungi (De Lucca et al. 2006b). On the other hand, Ceposide A, B, and C are the saponins found in *A. cepa*, which have also been tested positively against different strains of fungi (Lanzotti et al., 2012).

In the present study, *C. frutescens* extract showed the highest activity in all experiments. Antifungal results of *C. frutescens* were in line with that of Kamilla et al. (2009) who got 19.89 mm as the average zone of inhibition of *Clitoria ternatea* on *A. niger*. *A. cepa* extract showed an inhibitory activity against *A. niger*, but significantly less activity against *A. flavus*. Using repeated measures ANOVA, there was a significant difference between the results for *A. flavus* and for *A. niger*. This suggests that *A. flavus* might be resistant to *A. cepa* as affirmed by De Lucca et al. (2006a). The 50-50 v/v mixture of *A. cepa* and *C. frutescens* extracts showed an exemplary result for *A. niger*. On the other hand, the result for *A. flavus* was significantly lower. The result gap may be due to the synergism of the resistance of *A. flavus* to *A. cepa* and uncertain factors.

The description of methods and the presentation of findings in the present paper are derived from an attempt of high school students to apply the “scientific process research skills” necessary in biology. Onorato (2014) noted that one of the reasons why students have difficulty appreciating these research skills is the use of conceptual approach in teaching them, rather than the use of practical approach in the context of an actual scientific investigation problem. Thus, through the study they conducted as a class requirement, the first year high school students (grade 9, ages 14 - 15) were exposed to meaningful experiences to make theory meet practice while triggering curiosity to higher-level science for their age.

**ACKNOWLEDGEMENT**

The authors thank the Science Subject Area of Ateneo de Manila High School and the Department of Biological Science of the Central Luzon State University for all the support throughout the study.

**REFERENCES**


Antifungal Activity of Cayenne Pepper and Onion

Cayenne pepper (Capsicum species) and garlic (Allium sativum) have been studied for their antifungal properties. Cichewicz, R. H. and Thorpe, P. A. (1996) in their study on the antimicrobial properties of chili peppers and their uses in Mayan medicine, highlighted the effectiveness of cayenne pepper extracts against fungal pathogens. Lanzotti, V., Romano, A., Lanzuise, S., Bonanomi, G. and Scala, F. (2012) investigated the antifungal saponins from bulbs of white onion, Allium cepa L., and their impact on fungal growth.


Onorato, T. M. (2014) emphasized the importance of connecting students and microbiology through the lived experiences. Community College Journal of Research and Practice 38:625-637.


WEBSITES
Philippine Statistical Authority (PSA)

Center for Disease Control and Prevention (CDC)

U. S. Environmental Protection Agency (EPA)
Country Report

Biology Education in Upper Secondary Schools at Present in Japan

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(Received: 31 October 2017; accepted: 14 March 2018)

The present Japanese national curriculum standard, the Course of Study (CS), for upper secondary schools was announced in 2009 by the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT). The mathematics and science curricula are being enforced in upper secondary schools from 2012. In the present CS, biology-related subjects for upper secondary schools are “Basic Biology” (biology for all, 2-credit) and “Advanced Biology” (biology for interested students, 4-credit). In 2017, about 95% of students are taking Basic Biology, and 22% of students are taking Advanced Biology. “Basic Biology” is composed of three units: (1) Organisms and Genes, (2) Maintenance of Internal Environment, and (3) Biodiversity and Ecosystems. The key words for Basic Biology are DNA, Health and Environment. In addition, “Basic Biology” emphasizes concepts of Unity and Diversity with relation to Evolution. “Advanced Biology” is composed of five units: (1) Life Phenomena and Substances, (2) Reproduction and Development, (3) Environmental Response, (4) Ecosystems and Environment, and (5) Evolution and Phylogeny. Both subjects have inquiry activities at the end of each unit. These biology-related subjects have been modernized by reflecting the rapid progress in life science research in recent years. As a result, a lot of newest topics in biological sciences and new biological terms have appeared in biology textbooks. Some new modern experiments have also been introduced. In 2014, the action of revising the present CS was started. MEXT will announce the new CS for upper secondary schools by March, 2018, and will enforce it from 2022. The guiding concept of the CS revision is to enable students to cope with the changes in Japanese society when they become adults. Therefore, the strategy of school education must be improved. The new CS will shift from the traditional content-based teaching to competency-based learning by introducing some innovative methods such as active learning.

Key words: biology education in Japan, contents modernization, Course of Study, upper secondary school.

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INTRODUCTION

Since 1947 when a new mandate for education in Japan began, the Japanese national curriculum standard, the Course of Study (CS), has been revised by the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT) about every ten years (Table 1, Katayama et al. 2004, Nakamichi 2011). The present CS for elementary schools is being enforced from 2011, and that for lower secondary schools from 2012. The present CS for upper secondary schools was announced in 2009 and the revised curricula of mathematics and science are being enforced from 2012, one year earlier than those of the other subjects (Table
1). As mentioned in Table 1, the CS for both elementary schools and lower secondary schools has been revised and announced in 2017; the newly revised CS for elementary schools will be enforced from 2020 and that for lower secondary schools will be enforced from 2021. The CS for upper secondary schools will be revised and announced by March, 2018 and the revised CS will be enforced from 2022. The present CS has three principles, “Solid academic prowess,” “To be rich in humanity,” and “Health and fitness,” all of which support the fundamental philosophy of the CS, "Zest for life" (Nakamichi 2011). The fundamental philosophy is carried on by the newly revised CS.

In the present CS for Science for upper secondary schools, there are ten science subjects (Table 2, Nakamichi 2011). Among them there are two new subjects, “Science and Our Daily Life” and “Science Project Study.” The former subject, which is correspondent to Basic Science in the last CS, aims to raise students’ interests in nature, science and technology. The latter subject aims to enrich students’ inquiry abilities. In the latest revision

### Table 1  Year of Announcement and Enforcement of the Course of Study in Japan

<table>
<thead>
<tr>
<th>Elementary Schools</th>
<th>Lower Secondary Schools</th>
<th>Upper Secondary Schools</th>
</tr>
</thead>
<tbody>
<tr>
<td>Announcement</td>
<td>Enforcement</td>
<td>Announcement</td>
</tr>
<tr>
<td>1951</td>
<td>1951</td>
<td>1951</td>
</tr>
<tr>
<td>2017</td>
<td>2020</td>
<td>2017</td>
</tr>
</tbody>
</table>

The years on the second line from the bottom indicate the present Course of Study.
*Only the curricula of Math and Science of the present CS are to be enforced from 2012. The years on the last line indicate the next Course of Study.

### Table 2  Science subjects in the present Course of Study (2009) in comparison with those in the last one (1999)

<table>
<thead>
<tr>
<th>1999*</th>
<th>Subject</th>
<th>Basic Science</th>
<th>Comp. Sci.** A / B</th>
<th>P / C / B / E*** I</th>
<th>P / C / B / E*** II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Credit</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2009*</th>
<th>Subject</th>
<th>Science and Our Daily Life</th>
<th>Basic P / C / B / E***</th>
<th>Advanced P / C / B / E***</th>
<th>Science Project Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Credit</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* The year of announcement of the Course of Study.
** Comp. Sci. A / B: Comprehensive Science A (Physics and Chemistry areas) and Comprehensive Science B (Biology and Earth Science areas).
*** P / C / B / E: Physics, Chemistry, Biology and Earth Science.
of the CS, Comprehensive Science A and B have been deleted and four 2-credit basic subjects, *i.e.*, Basic Physics (P), Basic Chemistry (C), Basic Biology (B) and Basic Earth Science (E) which correspond to respective traditional subject areas, have been established. These basic subjects are prepared for almost all students. Students have to take at least three 2-credit subjects (Basic P / C / B / E) or two 2-credit subjects including Science and Our Daily Life; it is supposed that most students will choose the former pattern because the contents of Science and Our Daily Life are not considered to be included in university entrance examinations. This credit requirement enables students to take many more subjects in different fields of science. There also are four 4-credit advanced subjects for respective science fields. These advanced subjects are provided for the students who are interested in a particular field of science. Every advanced subject is designed to let students study the corresponding field of science in a systematic way.

**BIOLOGY-RELATED SUBJECTS IN THE PRESENT CS AND THEIR CONTENTS**

In this chapter, the contents of biology-related subjects for upper secondary school students in the present CS, *i.e.*, “Basic Biology” and “Advanced Biology,” are reviewed.

**Contents of Basic Biology (2-credit)**

Basic Biology (Biology for All) is composed of the following three units: Unit 1 Organisms and Genes, Unit 2 Maintenance of Internal Environment (Homeostasis), and Unit 3 Biodiversity and Ecosystems. Key words for this subject are DNA, Health and Environment (Table 3).

Although Basic Biology is a subject which corresponds to Biology I in the last CS, its contents have changed and been modernized significantly (Table 4). In the first unit, the contents are mainly related to cellular and molecular biology. In the second unit, students study the mechanism of maintenance of the internal environment and immunity in multicellular organisms. The contents can give basic knowledge to understand human health and illness. In the third unit, students study biodiversity and a variety of ecosystems; they are expected to realize the importance of environmental conservation through understanding the structure and function of ecosystems. In each unit, there are some observations and experiments. In addition, on completing the study of each unit, students are required to carry out some inquiry activities whose topics are related to the contents of the unit. These are the same as for Biology I in the last CS.

A noticeable characteristic of Basic Biology is to emphasize the concepts of “Unity” and “Diversity” which are related to evolution, though there is no heading of “Evolution.”

**Table 3 Composition of Basic Biology**

<table>
<thead>
<tr>
<th>Unit</th>
<th>Hierarchy Level</th>
<th>Aspects of Scientific Literacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organisms and Genes</td>
<td>Cellular and Molecular Level</td>
<td>Basis of Molecular Biology (DNA)</td>
</tr>
<tr>
<td>Maintenance of Internal Environment</td>
<td>Individual Level</td>
<td>Health</td>
</tr>
<tr>
<td>Biodiversity and Ecosystems</td>
<td>Community and Ecosystem Level</td>
<td>Environment</td>
</tr>
</tbody>
</table>
Table 4 Comparison of major headings of Biology I and Basic Biology

<table>
<thead>
<tr>
<th>Biology I in the last CS (1999)</th>
<th>Basic Biology in the present CS (2009)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unit 1. Continuity of Life</strong></td>
<td><strong>Unit 1. Organisms and Genes</strong></td>
</tr>
<tr>
<td>(1) Cells</td>
<td>(1) The characteristics of organisms</td>
</tr>
<tr>
<td>• Structures and functions of cells</td>
<td>• Unity and diversity of organisms</td>
</tr>
<tr>
<td>• Reproduction of cells and the structure of organisms</td>
<td>• Cells and energy</td>
</tr>
<tr>
<td>(2) Reproduction and development</td>
<td>(2) Genes and their function</td>
</tr>
<tr>
<td>• Formation of germ cells and fertilization</td>
<td>• Genetic information and DNA</td>
</tr>
<tr>
<td>• Mechanisms of development</td>
<td>• Distribution of genetic information</td>
</tr>
<tr>
<td>(3) Heredity</td>
<td>• Genetic information and synthesis of protein</td>
</tr>
<tr>
<td>• Laws of heredity</td>
<td>(3) Inquiries into organisms and genes</td>
</tr>
<tr>
<td>(4) Inquiries into Continuity of life</td>
<td></td>
</tr>
<tr>
<td><strong>Unit 2. Responses of Organisms to Their Environment</strong></td>
<td><strong>Unit 2. Maintenance of Internal Environment</strong></td>
</tr>
<tr>
<td>(1) Responses of animals to their environment</td>
<td>(1) Internal environment of the organisms</td>
</tr>
<tr>
<td>• Body fluid and Homeostasis</td>
<td>• Internal environment</td>
</tr>
<tr>
<td>• Stimuli reception and reaction</td>
<td>• Mechanism of maintenance of internal environment</td>
</tr>
<tr>
<td>(2) Responses of plants to their environment</td>
<td>• Immunity</td>
</tr>
<tr>
<td>• Plant life and environment</td>
<td>(2) Inquiries into maintenance of internal environment</td>
</tr>
<tr>
<td>• Plant responses and regulation</td>
<td></td>
</tr>
<tr>
<td>(3) Inquiries into Responses of organisms to their environment</td>
<td></td>
</tr>
<tr>
<td><strong>Unit 3. Biodiversity and Ecosystems</strong></td>
<td><strong>Unit 3. Biodiversity and Ecosystems</strong></td>
</tr>
<tr>
<td>(1) Vegetation diversity and distribution</td>
<td>(1) Vegetation diversity and distribution</td>
</tr>
<tr>
<td>• Vegetation and succession</td>
<td>• Vegetation and succession</td>
</tr>
<tr>
<td>• Climate and biomes</td>
<td>• Climate and biomes</td>
</tr>
<tr>
<td>(2) Ecosystems and their conservation</td>
<td>(2) Ecosystems and their conservation</td>
</tr>
<tr>
<td>• Ecosystem and the circulation of matter</td>
<td>• Ecosystem and the circulation of matter</td>
</tr>
<tr>
<td>• Ecological balance and conservation</td>
<td>• Ecological balance and conservation</td>
</tr>
<tr>
<td>(3) Inquiries into biodiversity and ecosystems</td>
<td>(3) Inquiries into biodiversity and ecosystems</td>
</tr>
</tbody>
</table>

It had been pointed out by many biology educators and biologists that biology-related subjects in the last CS for both lower secondary schools and upper secondary schools were lacking in the concept of evolution. However, in the present CS, evolution is regarded as the most important concept in biology and is treated as a superior concept among biological concepts. Therefore, in Basic Biology, teachers are asked to teach “the unity and diversity of organisms” prior to the other topics to let students understand that the phenomena are the results of evolution.

Contents of Advanced Biology (4-credit)

As shown in Table 5, Advanced Biology (Biology for Interested Students) is composed of the following five units: Unit 1 Biotic Phena nomena and Substances, Unit 2 Reproduction and Development, Unit 3 Responses to the Environment, Unit 4 Ecosystems and Environment, and Unit 5 Evolution and Phylogeny. Unit 1 is related mainly to the phenomena at molecular, subcellular and cellular levels. Unit 2 and Unit 3 are the phenomena at organ and individual levels. Unit 4 is the phenomena at the level of population and above. The final unit treats evolitional phenomena and the theory of evolution.

The following are three key characteristics of this subject: (1) Contents correspond to rapid progress in life science research in recent years, (2) Viewpoint of “unity and diversity” is continually emphasized from Basic Biology onward through other biology contents, and (3)
Table 5 Comparison of major headings of Biology II and Advanced Biology

<table>
<thead>
<tr>
<th>Biology II in the last CS (1999)</th>
<th>Advanced Biology in the present CS (2009)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unit 1. Life phenomena and Organic Substances</strong></td>
<td><strong>Unit 1. Biotic Phenomena and Substances</strong></td>
</tr>
<tr>
<td>(1) Proteins and their functions</td>
<td>(1) Cells and molecules</td>
</tr>
<tr>
<td>• Enzymes and chemical reactions within organisms</td>
<td>• Living substances and cells</td>
</tr>
<tr>
<td>• Anabolism and catabolism</td>
<td>• Life phenomena and protein</td>
</tr>
<tr>
<td>• Functions of proteins</td>
<td>(2) Metabolism</td>
</tr>
<tr>
<td>(2) Genetic information and its expression</td>
<td>• Respiration</td>
</tr>
<tr>
<td>• Genetic information and synthesis of proteins</td>
<td>• Photosynthesis</td>
</tr>
<tr>
<td>• Regulation of phenotypic expressions and morphogenesis</td>
<td>• Nitrogen assimilation</td>
</tr>
<tr>
<td>• Biotechnology</td>
<td>(3) Expression of genetic information</td>
</tr>
<tr>
<td></td>
<td>• Genetic information and its expression</td>
</tr>
<tr>
<td></td>
<td>• Control of gene expression</td>
</tr>
<tr>
<td></td>
<td>• Biotechnology</td>
</tr>
<tr>
<td></td>
<td>(4) Inquiries into life phenomena and substances</td>
</tr>
<tr>
<td><strong>Unit 2. The Classification and Evolution of Organisms</strong></td>
<td><strong>Unit 2. Reproduction and Development</strong></td>
</tr>
<tr>
<td>(1) The classification and phylogeny of organisms</td>
<td>(1) Sexual reproduction</td>
</tr>
<tr>
<td>• Classification</td>
<td>• Reduction division and fertilization</td>
</tr>
<tr>
<td>• Phylogeny</td>
<td>• Genes and chromosomes</td>
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<tr>
<td>(2) The evolution of organisms</td>
<td>(2) Development of animals</td>
</tr>
<tr>
<td>• Changes in organisms</td>
<td>• Gametogenesis and fertilization</td>
</tr>
<tr>
<td>• Mechanisms of evolution</td>
<td>• Process of early development</td>
</tr>
<tr>
<td></td>
<td>• Cell Differentiation and morphogenesis</td>
</tr>
<tr>
<td></td>
<td>(3) Development of plants</td>
</tr>
<tr>
<td></td>
<td>• Gametogenesis, fertilization and embryogenesis</td>
</tr>
<tr>
<td></td>
<td>• Differentiation of organs in plants</td>
</tr>
<tr>
<td></td>
<td>(4) Inquiries into reproduction and development</td>
</tr>
<tr>
<td><strong>Unit 3. Biocoenose</strong></td>
<td><strong>Unit 3. Responses to the Environment</strong></td>
</tr>
<tr>
<td>(1) Populations, their structures and maintenance</td>
<td>(1) Responses and behavior of animals</td>
</tr>
<tr>
<td>• Population maintenance and adaptation</td>
<td>• Stimuli reception and reaction</td>
</tr>
<tr>
<td>• Matter production and plant lives</td>
<td>• Behavior of animals</td>
</tr>
<tr>
<td>(2) Biocoenose and ecosystems</td>
<td>(2) Responses of plants</td>
</tr>
<tr>
<td>• Biocoenose, their maintenance and changes</td>
<td>• Plant response to the environment</td>
</tr>
<tr>
<td>• Ecosystems and their balance</td>
<td>(3) Inquiries into responses to the environment</td>
</tr>
<tr>
<td><strong>Unit 4. Research Activities</strong></td>
<td><strong>Unit 4. Ecosystems and Environment</strong></td>
</tr>
<tr>
<td>(1) Research on particular organisms or life phenomena</td>
<td>(1) Population and biotic community</td>
</tr>
<tr>
<td></td>
<td>• Population</td>
</tr>
<tr>
<td></td>
<td>• Biotic community</td>
</tr>
<tr>
<td>(2) Investigation of the natural environment</td>
<td>(2) Ecosystems</td>
</tr>
<tr>
<td></td>
<td>• Matter production in an ecosystem</td>
</tr>
<tr>
<td></td>
<td>• Ecosystems and biodiversity</td>
</tr>
<tr>
<td></td>
<td>(3) Inquiries into ecosystems and environment</td>
</tr>
<tr>
<td><strong>Unit 5. Evolution and Phylogeny</strong></td>
<td><strong>Unit 5. Evolution and Phylogeny</strong></td>
</tr>
<tr>
<td>(1) Mechanism of evolution</td>
<td>(1) Mechanism of evolution</td>
</tr>
<tr>
<td>• Origin of life and transition of organisms</td>
<td>• Origin of life and transition of organisms</td>
</tr>
<tr>
<td>• Mechanism of evolution</td>
<td>(2) Phylogeny</td>
</tr>
<tr>
<td></td>
<td>• Phylogeny</td>
</tr>
<tr>
<td></td>
<td>(3) Inquiries into evolution and phylogeny</td>
</tr>
</tbody>
</table>
Various fields from the micro level to the macro level are covered. In each unit, some new topics are included, e.g., biomembrane and cell skeleton in Unit 1, processes of organogenesis in plants in Unit 2, neuro-ethology and photo-receptors in Unit 3, diversity of organisms at various levels in Unit 4, and neutral theory of molecular evolution and three domains in phylogeny in Unit 5.

NUMBER OF STUDENTS WHO TOOK BIOLOGY-RELATED SUBJECTS

Figure 1 shows the changes in the number of textbooks of each basic subject (left) and each advanced subject (right) sold. The figures are compiled by using the data obtained from The Jiji Press (2014, 2015, 2016 and 2017) which were based on MEXT data. These numerical values are considered to roughly correspond to the number of students who took each subject. In the school year 2017, nearly 1.1 million students (about 95% of senior secondary students) take Basic Biology; it is the highest rate of course registration in basic subjects. About 261 thousand students (about 22% of senior secondary students) take Advanced Biology. In the school year 2013, the number of students who took one of these subjects appears to be smaller than in the later school years because all schools did not necessarily start all science subjects in this school year. The number of students who take Advanced Biology has been decreasing continuously after the school year 2015.

OBSTACLES TO ENFORCING THE LATEST BIOLOGY CURRICULUM FOR UPPER SECONDARY SCHOOLS

On enforcing the present CS, MEXT encourages science teachers to introduce various students’ activities into their teaching to nurture students’ abilities of thinking, decision-making and expression. The ministry requests them to avoid the traditional chalk and talk teaching style (a teaching style of only the teacher’s explanation and his/her writing on the blackboard). The following student activities are recommended:

- Peer discussion using a tablet, a whiteboard and/or post-its,
- Explanation including a poster presentation and a debate between the students, and
- Making a handout, a report or a poster,
using Information and Communication Technology (ICT).

Thus, science teachers in both lower and upper secondary schools are expected to shift their teaching style from teacher-centered to student-centered. But, many science teachers feel some difficulties in implementing the above-mentioned student activities in their classes, and they consider the time allotted to science is insufficient for such time-consuming activities. Not only because of such time limitation, but also because of their inexperience in student-centered teaching style, many teachers still prefer to adopt the teacher-centered teaching style. They are apt to require students to memorize the items and terms of the textbook for term examinations. At the upper secondary level, science teachers still consider it necessary to give a lot of information to students in preparation for university entrance examinations instead of allotting time for inquiry activities.

In spite of the rapid progress in biological and life science research and the accumulation of new biological knowledge, the contents of upper secondary school biology had remained rather static until the last revision of the CS. But, by the latest reform, biology education at the upper secondary level has been modernized by reflecting the rapid progress in biological research in recent years, bringing it closer to biology education at the tertiary level. Especially in the case of Advanced Biology, the quantity of topics has increased and the contents have become more challenging. As a result, some new problems have arisen. For example: (1) Although a lot of the newest topics in biological sciences and new biological terms appear in biology textbooks, many biology teachers, especially older ones, in upper secondary schools lack up-to-date knowledge of biology; (2) Some new modern experiments have been introduced, but most of the older biology teachers are lacking the skills for instructing students in new modern experiments. In addition, most schools, particularly public schools, do not have enough equipment for new modern experiments.

It is desirable that some effective measures for surmounting these obstacles be promptly implemented. However, both national and local governments still have not adopted enough measures for in-service teacher training. The budget for improving school science equipment also does not seem to be enough.

Another problem is related to the recent trend in the “Textbook Authorization” process (see Appendix 1). Recently, “Textbook Authorization” for biology textbooks, particularly for Advanced Biology textbooks, has become relaxed. As a result, a wider range of topics including up-to-date contents have been introduced in Advanced Biology textbooks. Then, there seems to be a big gap in the degree of difficulty in understanding the contents between Basic Biology and Advanced Biology. Many biology teachers feel they are forced to teach students all of the contents in the Advanced Biology textbook within the school hours allotted to this subject. Students think that they have to memorize lots of contents when they take Advanced Biology. This must be one of the reasons why the number of students who take this subject has been decreasing (Fig. 1).

In addition to the increase in the amount of contents mentioned above, there also is an increase in the number of biological terms which include some latest ones in the present upper secondary biology textbooks (Matsu-ura 2013, Nakamichi 2018, see Appendix 2). Among these terms, there are quite a few synonyms. So far, it has been pointed out that too many biological terms used in biology textbooks possibly make students lose interest in learning biology, and the use of synonymous terms makes both biology teachers and students confused. Students are required to memorize all
of these terms including synonyms for term examinations and for university entrance examinations if they choose biology as one of the examination subjects. Unfortunately, only a few attempts at reduction and standardization of these terms have been carried out until now. Furthermore, no reflection of the results of these few attempts has appeared in biology textbooks, yet.

**PREPARATION AND ENFORCEMENT OF THE NEXT CS**

**Schedule of CS revision**

In 2014, the Minister of MEXT requested the Central Council for Education (CCE) to commence discussion for revising the CS (MEXT 2014). In 2015, the CCE submitted an Interim Discussion Report to the Minister of MEXT (MEXT 2015) and, in 2016, the CCE submitted the Final Discussion Report to the Minister (MEXT 2016). After this, as shown in Table 1, MEXT announced the new CS for elementary schools and lower secondary schools in 2017 (MEXT 2017). MEXT will announce the new CS for upper secondary schools by March, 2018. Now, textbook publishers are preparing new textbooks. The textbook authorization process for new textbooks by MEXT will be started from 2018 onward. The new CS for elementary schools, lower secondary schools and upper secondary schools will be enforced from 2020, 2021 and 2022, respectively.

**Conception and realization of the CS revision**

The guiding concept of the CS revision is to enable students to cope with the changes in Japanese society when they become adults. At that time, in Japan, there will be a reduction in the working-age population, a progression of globalization, and technological innovation. It will be more important for students to be aware of the connection between their learning and the changing society than ever before. Students will be expected to foster the following qualities and abilities which will be required for a new era.

1. Practical knowledge and skills;
2. Abilities of thinking, decision-making and expression in responding to unexpected situations;
3. Individual characteristics enabling them to use acquired knowledge and abilities to live a better life and engage in regional and international societies.

Therefore, the strategy of Japanese school education in the future must be improved in order to satisfy these students’ needs. From now on, the quality of contents is more strongly emphasized. Thus, it is necessary to consider “what knowledge students acquire” and “what they can do using the knowledge they have acquired.” In addition, the quality of learning method, *i.e.*, “how to learn,” should be considered to deepen students’ understanding; it is strongly recommended that the teaching style be shifted from teacher-centered to student-centered. It is also important to evaluate “what kinds of abilities students have acquired” as an outcome of student learning. Therefore, in the new CS, competency-based education, by introducing some innovative methods, such as active learning, will be incorporated with the traditional content-based education.

For realization of the new CS, in general, the following must be considered:

* Improvement of the quality of learning process;
* Pre- and in-service teacher training for student-centered teaching style, such as active-learning;
* Promotion of ICT use;
* Reform of the university entrance examination system;
* Improvement of the working environment for teachers.

In science, in particular, laboratory equipment and teaching materials must be updated
to cope with the modernization of the subject matter. In-service training to give science teachers up-to-date knowledge of biology and skills for teaching with new modern experiments is also essential.

**Note:** This paper is based on the country report presented at the 26th Biennial Conference of AABE.

**REFERENCES**


**WEBSITES**


APPENDIXES

Appendix 1: Textbook Authorization

In Japan, there is a “Textbook Authorization” system. Books to be used as textbooks for Japanese elementary and secondary schools are compiled and edited by private publishers. Textbook Authorization is the process of screening these books. Through the careful deliberations of the Textbook Authorization Council under the control of MEXT, these books are examined to decide whether they are appropriate for use as textbooks. Science textbook authorization has been carried out by certain examiners in the Elementary and Secondary Education Bureau, MEXT, who have scientific and educational backgrounds. The purpose of textbook screening might be the correction and improvement of the material in textbooks. In practice, however, the textbook screening has come to be a severe check to see if they deviate from the CS and its guidelines.

So far, regarding biology textbooks for upper secondary students, some important biology concepts and the concepts in other science areas which are required for understanding biological phenomena were requested to be deleted from biology textbooks, because they were not included in the biology section of the CS and its guidelines. As a result, biology textbook authorization made it more difficult for teachers to teach biology because these textbooks restricted biology education within narrow limits. On the other hand, scientific terms appearing in biology textbooks do not seem to have been examined thoughtfully in the textbook authorization process. This resulted in the number of scientific terms being very large compared to the textbooks of other science areas. Moreover, there were many synonymous terms (see below).

The process of textbook “authorization” by the officers of MEXT continues, but the screening of textbooks at present is not as severe as before.

Appendix 2: Scientific terms used for biology education in Japan

In Japan, science subjects are taught in Japanese, and therefore only Japanese scientific terms are used in teaching science and in science textbooks. Among science subjects for upper secondary students, the number of scientific terms appearing in biology textbooks is larger than that in textbooks of other science subjects. Biology learners in upper secondary schools are forced to memorize a lot of scientific terms. This has been considered to be a burden to upper secondary school students who choose biology and it leads to many students losing interest in learning biology.

Furthermore, there are a lot of Japanese synonymous terms in biology. Synonymous scientific terms appearing in biology textbooks for Japanese upper secondary students are much larger in number than those appearing in biology textbooks for English upper secondary students. Sometimes three or more synonymous Japanese scientific terms are used for one English term. Usually, in a biology textbook, one of these synonyms is used according to the authors’ decision. Frequently, one of these synonyms is used at random in university entrance examinations. Therefore, students must memorize a lot of biological terms including all of these synonyms if they choose biology as a university entrance examination subject. This has been an issue for a long time, but only a few attempts at reduction and standardization of biological terms have been carried out. Details of this matter will be described elsewhere.
Contents of the Proceedings of Past Biennial Conferences of the AABE

The AABE published the proceedings of each biennial conference, from the first conference to the seventeenth one, until 1998. Since then, the association has been publishing AJBE as its bulletin which includes the conference reports and the abstracts of the papers presented at each conference. At present, it is difficult to obtain not only a copy of these proceedings, but even the information on the contents of them. Here is the table of contents and author index of the proceedings (Volume 1 – 17). As for further information on these proceedings, please inquire of the AABE Website master.

A TABLE OF CONTENTS

< I > First Conference held in Manila, Philippines (December, 1966)

First Asian Regional Conference on School Biology

Foreword: Soriano, L. B. v

The Conference: Background and Arrangements

The Leading Papers

The Aims of School Biology Teaching and the Criteria for the Section of Course Content

Peterson, G. E. 5

Teaching Methods and Teacher Training

Poljacoff-Mayber, A. and Jungwirth, E. 17

Evaluation of Biology Curricula

Grobman, H. 24

The Relation of School Biology to Post-School Biology and Everyday Life

Basnayake, V. and Crusz, H. 48

The Role of Universities and Other Agencies in School Biology

Madan, D. R. 58

The Symposium Papers (Project Papers)

The Nuffield O-Level Biology Project

Dowdeswell, W. H. and Kelly, P. J. 65

The CAAS School Biology Project

Crusz, H. 76

Attempts in Adaptation of the BSCS Program for Use in Secondary Schools in Israel

Poljacoff-Mayber, A. 100

The Adaptation of the BSCS Yellow Version in Taiwan, China

Koh, T.-P. 106

The Progress of Biological Education in Vietnam

Ngan, P.-T. 113

The Philippine Adaptation Project: A Report

Hernandez, D. F. and Sangalang, L. 117

School Biology in the United States

Grobman, A. B. 136

The Survey Papers

Biology Teaching in the High Schools of Afghanistan

Fakoor, S. R. 145

Evaluating the Teaching of Biology in Ceylon

Gunaratne, M. M. 155

Biology in Indian High Schools

Johri, B. M. and Lal, M. 164

Biology Teaching and the Educational System in the State of Israel

Poljacoff-Mayber, A. 180

The Teaching of Biology in Secondary Schools in Iraq

Al-Julili, A. R. 192

The Teaching of Biology in Hong Kong

Harris, H. 199

Survey of High School Biology Teaching in Korea

Kim, C. M. 202

Biology Teaching in Japan at the Secondary Level

Nakayama, K. 206

A Survey of Secondary School Biology in Malaysia

Yoong, C. S. 254

Survey of High School Biology Teaching in Taiwan

Yang, J.-H. 285

The Teaching of Biology for High School Teachers in Taiwan

Koh, T.-P. 288

High School Biology in Vietnam

Duong, D.-N. 293

Summary of Discussions
Report of Discussion Group I – The Aims of School Biology Teaching in Asia 304
Report of Discussion Group II – Teaching Methods and Teacher Training 306
Report of Discussion Group IV – On the Relation of School Biology to Post School Biology and Everyday Life 310
Report of Discussion Group V – The Role of the Universities and Other Agencies in School Biology 313

Recommendations and Resolutions

The Conference Program
The Conference Program 327
Welcome Address: Soriano, L. B. 333
Introduction of Dr. Carlos P. Romulo: Morales, A. T. 335
Address: Romulo, C. P. 337
Better Biology Education in Asia: Salcedo, J. Jr. 340

Appendix
Participants and Observes 345

< II > Second Conference held in Tokyo, Japan (August, 1968)
Second Asian Regional Conference on School Biology

The Leading Papers
Role of Basic Researches in School Biology and New Trends in Biology Teaching Johri, B. M. and Tandon, S. L. 1
Terrestrial Ecology of Tropical Asia – Implications for Biological Education Mayer, W. V. and Larsen, V. C. 15
Ecology in Asia – Its Marine Resources Takasugi, S. 24
Mass Media Techniques in the Teaching of School Biology Nishimoto, M. 33
Conservation of Natural Resources Sakai, T. 40

The Activity Reports (Project Papers)
BSCS – International Cooperation Peterson, G. E. 42
The Role Played by an Association of Biology Teachers in the General Movement to Improve Secondary School Biology in the Philippines Alfons, P. J. 47
Ehime Prefectural Education Center in Science Training Programs Yoshida, T. 50
Science Education and the Explosion of Scientific Knowledge Glass, B. 56

The Nuffield Biology Project Dowdeswell, W. H. 64
Elements for Presentation of the Biology Teaching Pilot Project in Africa Hunwald, A. 69
Recent Development in Biological Education in the United States Mayer, W. V. 75
Continuing Education for Biology Teachers Morikawa, H. 87
Science Education Centers in Japan Morikawa, H. 99

The Assessment Papers
Afghanistan Biology Project Fakoor, S. R. 111
Improvement Achieved in Biology Teaching by the Republic of China Yang, J.-H. 117
Hong Kong’s Biology Project Harris, H. and Madan, D. R. 121
Some Reflections on the Instruction of Biology in Schools in Indonesia Prawirosudirdja, G. 122
Progress in Biology Teaching in Israel Poljacoff-Mayber, A. 129
Assessment of Development in Biology Education in Japan 1966 - 1968 Nakajima, Y. 132
Recent Progress and Achievement on High School Biology in the Republic of Korea Oh, K. C. 135
An Experimental Study in Four High Schools in Korea on the Effectiveness of Teaching English and Biology by Television Mitchell, J. L., S. J. 137
Progress Report on the Sogang College’s CCTV Experiment Conducted in Four High Schools of Seoul Mitchell, J. L., S. J. 149
Biological Education in Malaysia Vohra, F. C. 155
An Assessment of Development in Biology Education in the Philippines 1966 - 1968 Zamora, R. I. 181
Biology Education in Thailand Pavanarit, S. 199

School Systems
The School System of Ceylon 214
The Educational System of China 218
The Educational System of Hong Kong 223
Education Pattern in India 242
Israel System of Education. 262
The Educational System in Korea 266
Malaysian System of Education 273
The Educational System in the Philippines 280
School System in the Republic of Singapore 297
The School System of Thailand 301
The System of Education in the United States 305

**Group Reports, Recommendations and Resolutions**

Group I The Role of Basic Research in School Biology 308
Group II Ecology in Asia – Land Resources 311
Group III Ecology in Asia – Marine Resources 313
Group IV Mass Media Technique in the Teaching of School Biology 315
Group V On the Teaching of Conservation of Natural Resources 319

**Resolutions** 321

**The Conference Program** 322

**Addresses**
- Welcome Address: Hisatake 333
- Words of Welcome: Miyake, A. 335
- Message: Itoh, R. 337
- Opening Remarks: Soriano, L. B. 337
- Greetings: Glass, B. 335
- Closing Remarks: Nakayama, K. 340

**Participants**
- Officers of the Conference 337
- Japanese Participants 335
- International Participants 340

< III > Third Conference held in Manila, Philippines (December, 1970)

Third Asian Regional Conference on School Biology

**Research Project Papers**

“Green” Bean and “Butter” Bean – Varieties of Species?
  Marandawala, P. 1

Supporting Function of Collenchyma as Seen in the Petiole of Typhonium roxburghii
  Eriyagama, I. 9

“Lipase” Activity in Seeds
  Eriyagama, I. 13

The Amount of Water Given out from Leaves of Different Ages
  Weerasinghe, A. 20

Colour Change in the Petals of Hibiscus mutabilis
  Hoole, G. J. 25

Some Observations on the Breeding Habits of the Ceylon House Sparrow
  Daniel, C. J. S. 32

Age of Menarche in School Girls in Kandy, Ceylon
  Dissanayake, P. 37

Rejuvenation of Mandarin
  Lee Liu, H. C. 42

Metabolism of Silkworm Population
  Kawasaki, T. 46

Study of Pollution as a Student Research Project
  Kim, C. M. 53

Life History of the Jute Hairy Caterpillar, Diacrisia oblique Walker
  Kahn, S. M. H. 59

Response of the Rice Plant to Added Nutrients
  Vergara, B. S., Asis, C. V., Hernandez, D. F. and Ramirez, L. B. 64

Projects on Talahib, Saccharum spontaneum L. and Cogon, Imperata cylindrica (L.) Beauv.

Ecological and Physiological Approach
  Vergara, B. S. 82

The Incidence of Myopia among the School Population in the Republic of Singapore
  Paran, T. P. 90

Propagation of Plants from Leaves
  Natarajan, S. 98

Dormancy of Rice Grains
  Mai-Tran-Ngoc-Tieng 107

Investigation on Earthworm and Seed Germination
  Goyal, K. C. and Swami, P. 111

**Papers on General Topics**

The Social Responsibilities of Biological Educators
  Grobman, A. 115

Quantitative Biology: Statistical Evaluation and Analysis of Data
  Goldwin, A., Lev, H. and Strauss, G. 122

Making a Model of Tracheal Gills with Yumicron
  Nakajima, Y. 165

Simple Investigational Work in the Practical Classroom in Human Physiology
  Basnayake, V. 167

Biology Education in Malaysia with Particular Reference to Biology Projects in the School Curriculum
  Rajendram, K. H. 186

Biology Teaching through an Integrated Approach
  Prawirosudirdja, G. 189

Biology Education in Secondary Schools in Japan
  Nakajima, Y. 207

**Concluding Report** 210

**The Conference Program** 214

**Addresses**
- Welcome Address: Manuel, J. L. 218
- Keynote Address: Medina, G. F. 220
- Orientation and Overview: Soriano, L. B. 224

**The Participants and Officers of the Conference**
< IV > Fourth Conference held in Jerusalem, Israel (August, 1972)

Fourth Asian Regional Conference on School Biology

Evaluation in Science Education

Evaluation of Curriculum

Content Analysis in Formative and Summative Evaluation of Curriculum

Grobman, H. 1

Curriculum Evaluation with Some Reference to Nuffield Advanced Level Biological Science

Lister, R. E. 25

Evaluation Strategy of the Nuffield A-Level Biological Science Project

Kelly, P. J. 35

The Practice of Curriculum Evaluation

Lewy, A. 51

Evaluation of Achievement of Objectives

Evaluation of the Achievement of Objectives in Nuffield Advanced Level Biological Science

Lister, R. E. 81

Feasibility – Means What for Whom?

Jungwirth, E. 90

Evaluation of Teachers and Teaching

Evaluation of Teachers and Instruction

Jungwirth, E. 99

Evaluation of Teachers and Learning Environments with Respect to Elementary School Science

Harlen, W. 107

The Evaluation of Teachers and Teaching

Mayer, W. V. 118

Teachers’ Cognitive Style in Evaluation Studies

Eggleston, J. F., Galton, G. and Jones, M. 122

An Approach to “Formative” Evaluation of a Concept Oriented Science Program at the Elementary Level

Nussbaum, J. 135

Evaluation of Student Performance in the Classroom

Evaluation of Children’s Progress by Teachers in the Classroom

W. Harlen 147

Domains of Evaluation in the Inquiry Role Approach

Bingman, R. M., Koutnik, P. G., Seymour, L. A., Padberg, L. F., Chan, J. Y. and Bingman, K. J. 155

Laboratory and Practical Examinations

The Development and Standardization of Inquiry-oriented Laboratory Examination

Tarmir, P. and Glassman, B.-G. 178

English Translation of Practical Examination in Biology 1971 (Bagrut Ha Ma’asit) for Classes Using BSCS Curriculum

The Amos de Shalit Science teaching Center, The Hebrew University 187

Attitudes of Students and Teachers towards the Practical Matriculation in Biology

Tarmir, P. 205

Evaluation of Attitudes and Interests

Students’ Attitudes towards a School Subject as Affected by Curriculum Reform

Blum, A. 215

Assessing “Understanding of the nature of Science”

Jungwirth, E. 220

Problems in Evaluation

Evaluation of Student’s Attitudes towards Drawings in a Student’s Text

Blum, A. 234

Attitudes of Junior High School Students towards the Study of Plants and Animals

Mayer, M. and Tamir, P. 240

Assessment of Children’s Ability to Observe

Zuzovsky, R. 253

The Uses of Educational Technology in Science Education

General Considerations in the Uses of Technologies in Education

The New Bio-Technology – Potential Applications to the Educational Environment

Beal, J. B. 263

Educational Consideration in the Use of Technology in Education

Salomon, G. 277

Closed Circuit Television in the Teaching and Learning Concept in Higher Education

Elton, L. R. 285

Uses of Technological Means in Teacher Training

The Use of Technological Means in Teacher Training and Retraining

Allen, D. 291

The Uses of Technological Means in Teacher Training and Retraining

Lee, A. and Lewis, M. 295

The Use of Microteaching Techniques to Train Student-Teachers in Stimulating Learners’ Questions

Perlberg, A. and Kremer, L. 314

The Facet Approach in Developing a Theory of Instruction and Teacher Training

Bar-on, E. and Perlberg, A. 323

The Use of the Technion Diagnostic System (T.D.S) and Microteaching Techniques in Modi-
Modifying Instructional Strategies of Teachers in Service through the Use of Microteaching Techniques and Video Recordings
Perlberg, A., Shimron, D., Rot, S. and Libaee, Y. 329

Patterns and Styles in the Supervision of Teachers in Individual Conferences Following Classroom Observations
Theodor, E. and Perlberg, A. 332

A Different Approach to the Use of Microteaching in Teacher Training
Tamir, P. 335

Uses of Technological Means in Classroom Instruction
The Use of Film and Television in Science Education
Smith, J. 341

Uses of Educational Technology: Computer Literacy Course
Peless, Y. 347

The Use of Film to Modify Mental Skills
Salomon, G. 353

Report of Members: Outcomes of the Third AABE Conference
An Integrated Program for Teaching Biology and Agriculture
Lev, C. and Adler, J. H. 376

Performance in a Biology Examination of School before and during Participation in a Curriculum Revision Project in Sri Lanka (Ceylon)
Eriagama, L. 380

Consistency of Performance in In-Course Tests and in a Public Examination of Classrooms in a Biology Curriculum Trial
James, S. L. 388

Open University – An Educational Tool
The Organization of the Open University
Haynes, L. J. 399

Integrated Science and Integrated Teaching Methods
Stannard, F. 401

Practical Work and Home Experimental Kits
Haynes, L. J. 409

Evaluation of Science Courses at the Open University
Moss, G. D. 412

Individualized Instruction
Considerations Regarding Individualized Instructions
Mayer, W. 415

Individualized Instruction – Theory and Practice
Novak, J. 420

Towards Independent Study
Elton, L. R. 430

A Modular Approach to Biology Curricula
Dowdeswell, W. 434

Individualized Instruction: A Proper Context for It
Edling, J. V. 434

Use of Technological Means in General Classroom Work and Individualized Instruction
Can Students of Mixed Abilities Successfully Study Biology in the Same Classroom?
Sabar, N. 448

Instructional Television Centre: Facts and Figures
Ben-Shaul and Prener, J. 455

Teaching Abstract Concepts in High School Physics Especially to Disadvantaged Students
Weiss, M. 459

Science Teaching Kits as the Material Base for Improvement of Science Education in Indian Schools
Care, R. A. and Galakhov, V. J. 465

Diffusion and Implementation of Use of Educational Technologies
Technology and Evaluation in Biology Education
Johri, B. M. and Sinha, U. K. 470

Recent State of Uses of Educational Technology in the Senior High School Biology Education in Japan
Nisizawa, K. 478

Utilization of Technological Means in Teaching Science in General and Biology in Particular
Ben-Chanan, M. and Sharomi, S. 483

Using Animals of Economic Importance in Schools
Blum, A. 492

Country Reports
A Review of “Scientific Research Projects in Schools in Singapore”
Paran, T. P. 497

A Follow-Up of the Two Philippine Papers Presented during the Third AABE Conference
Garcia, F. C. 506

School Biology Research in Sri Lanka
Crusz, H. and Weerasinghe, A. 509

The Role of Marine Microbes in the Nutrification Process
Zamora, R. 522

Biology Education in Japan 1970 - 1972
Nakajima, Y. 531

Biology Education in Singapore
Paran, T. P. and Natarajan, S. 533

Education in Israel and Science Teaching
Gottlieb, S. 552

Diffusion and Dissemination of New Curricula
and Improved Teaching through the Science Education Project

Hernandez, D. F. 563

School Biology Education in India – A Survey Report

Doraiswami, S. and Guru, G. 573

The Conference

Conference Programme 579
Addresses, Lecture & Remarks

Soriano, L. B. 589
Harman, A. 592
Allon, Y. 593
Elkana, Y. 595
Care, R. A. 606

List of Invited Lecturers 607
List of Participants 608

< V > Fifth Conference held in Singapore (June, 1974)

Fifth Asian Regional Conference on School Biology

Biology Teaching

Aims and New Directions

Man and Society: Redirection in Biological Education

Chye, Y. O. 1

The Role of Social Biology and Its Implications for Secondary School Science Teaching


Curriculum

Applied Biology Teaching in Singapore

Johnson, A. 31

Some Problems in Teaching Biology

Rao, A. N. 35

Some Problems in Biological Education in Urban Singapore

Elliot, A. B. 43

A Proposed Syllabus for Teaching Environmental Pollution at Secondary III and IV in Singapore Schools

Hong, L. C. 51

Teaching of Ecology in Singapore Schools: A Sample Study

Tan, J. 57

Some Problems of Biology and Nature Conservation in Thailand

Sirjaraya, P. 61

Ecology of Naturalized Plants: An Analytical Method for the Study of Vegetation Dynamics as Influenced by Human Impact

Odaki, K. and Iwase, T. 64

Current Changes in Microbiology: A Personal Point of View

Leong, T. Y. 74

The Teaching of Plant Physiology – Problems and Solutions

Avadhani, P. N. 83

Methods and Materials

Towards Independence in Learning – an Appraisal of Different Learning Modes to Ideas and Concepts in Biology

Tribe, A. A. 90

A Study in Teaching of Environmental Education

Nakajima, Y. 104

Biology and Biological Materials

Rajendram, K. A. 110

Inquiry and Integrated Approaches in Physiology Classes in Secondary Schools

Prawirodirdja, G. 114

Training Teachers to Teach Science as Inquiry

Tamir, P. 119

Population Education

Growth of Population in the Philippines and the Need for Functional Population Education

Kapili, P. H. 133

The Teaching of Population Biology

Dwidjoseputro, D. 146

Integration of Population Education in a College Biology Course

Garcia, F. C. 153

The Age of Menarche in Girls in Singapore

Nalliah, C. 161

Rural Education

Biology Education for Rural Areas

Yoong, C. S. 164

The Biology Curriculum for the Rural School: A Case for Integration

Jungwirth, E. 172

A Study of the Biotic Relationship between Root Nodule Bacteria and Mimosa pudica: A Suggested Project in for Rural-Urban Schools in Tropical Asia

Kwan, L. P. and Nah, C. K. 195

Biology Education in Rural and Urban India: Problems and Prospects

Johri, B. M. and Sinha, U. 207

Evaluation

Evaluating Curriculum Development in Asia

Wong, R. H. K. 210

Evaluation of the Investigatory Project Work Done by a School Biology Group in Sri Lanka

Weerasinghe, A. 217

Country Reports

RECSAM’S Contribution in Upgrading Biology Education in SEAMEO

Ponniah, W. D. 223

New Approaches to Biology Teaching in Thailand

Hormchong, T. 237

The Science Education Project of the
The Conference
Opening Address 301
Conference Programme 307
Summary of Discussions 313
Summary of Recommendations 329
Participants and Committees
Foreign delegates 331
Local delegates 333
AABE Executive Committee, 1974 335
Organizing Committees 336
Coordinators and Rapporteurs 338
Workshop Sessions 340

< VI > Sixth Conference held in Bangkok, Thailand (July - August, 1976)
Sixth Asian Regional Conference on School Biology
Foreword 1

Leading Papers
Preparation of Teachers for Biology Teaching
Vohra, F. C. 1

Educating Teachers as Researchers and Curriculum Developers
Kelly, P. J. 18

How Are the Teacher Curriculum and Training Strategies Made Relevant for Biology Teaching?
Yoong, C. S. 26

The Concept of Competency in Teacher Training Courses
Hernandez, D. F. 38

Participants’ Papers
Curriculum and Instruction
Restructuring School Biology - Relevance and Consequences of New Approaches for Biology Teaching and Teacher Training
Kattmann, U. and Schaefer, G. 47

IPN Unit Bank Biology - A New Type of Teacher Training
Schaefer, G. and Kattmann, U. 60

Cell Biology in Secondary Schools in Relation to the New Trends in Indian Education
Mishra, A. K. 72

Modern Biology Course in Malaysia
Ghani, Z. 78

Teaching Biology in the Context of Culture and Socioeconomic Values of the Country
Zamora, R. I. 84

Development of a Textbook in Biology for Secondary Schools in the Philippines
Villavicencio, R. R. 89

The Philippine Science High School Curriculum and its Relevance to the Needs of the Country
Reyes, V. F. 102

The Increasing importance of the Biological Sciences in Today’s Society
Lee, A. E. 107

Science Education and the Ecology of Thailand
Carter, J. 113

Teacher Education
Associations and Regional Training Centres - How They Make Biology Training More Effective and Relevant
Ramsey, G. A. 116

Training of Biology Teachers of Indian Schools
Jain, S. C. 124

Preparation of Teachers for Biology Teaching in Israel
Tamir, P. 131

The Prospective Biology Teacher and the Philosophy of Science
Jungwirth, E. 141

Teacher Retraining and Curriculum Evaluation: Function of the Science Education Center
Nakajima, Y. 154

The Influence of a National Association of Biology Teachers in the Philippines
Garcia, F. C. 164

Quality versus Quantity in Preservice and Inservice Teacher Education Programmes with Special Emphasis on Biology: A Point of View
Singham, J. K. 173

Evaluation
The Development of Instruments to Determine the Teacher’s and Student’s Classroom Activities and Attitudes toward the IPST Biology Program
Soydharum, P. 196

Evaluation of IPST Biology Curriculum
Padungratana, J. 203
The Conference
Welcome Address: Krishnamra, T. 207
Opening Address: Vangsayanha, C. 209
Conference Director’s Report: Hormchong, T. 211
The Conference Programme 214
Summary of Discussions 220
Summary of the Conference 242
Organizing Committee 244
Participants 246

< VII > Seventh Conference held in Kuala Lumpur, Malaysia (December, 1978)
Multidisciplinary Biology Education Relevant to Community Development

Preface vii
Opening Ceremony
Welcoming Address: Hamiddon, F. xi
Opening Address: Jafaruddin, T. H. S. xiii
Message: Vohra, F. C. xvii
Address: Yoong, C. S. xxiii

Conference Papers
Developments in Multidisciplinary Biology Education
Biology in Community Education: Philippine Scenario for Lifelong Education
Hernandez, D. F. 5
Problems on Biological Curricula Relevant to Inter-, Uni-, and Multi-Disciplines
Imahori, K. 27
Moral Education: Implications for Biology Teachings
Kanagasabai, S. 33
Interdisciplinary Science: Pros and Cons, Trends and Examples
Lee, A. E. 41
Population Education and Birth Planning Studies: A Project Design for Hong Kong and the Southeast Asian Regions
Marsh, A. R. 57
Curricular Innovations and Priorities in Biology Education: A case for the Developing Countries
Sood, J. K. 71
Biology in Environmental Education
Stokes, D. M. 79
Concepts in Social Biology
Ghani, Z. 87

Multidisciplinary Biology Education for Schools and Tertiary Institutions
A Preliminary Study of Fouling Organisms of Johore Straits and the Extent of Pollution in the Region
Charles, S. 97
Curricular Aspects in Multidisciplinary Biology Education in Malaysia Relevant to Community Development
Prabhakar, M. P. 105
A Nature Study Centre in Taman Negara (West Malaysia) for Use by Student Groups
Rubeli, K. 121
A Child-Centred Approach to Biology Education in Indonesia
Wayan Seregeg, G. 137
Recognising the Need for a Multidisciplinary Biology Education
Chou, L. M. 153
Biology in Adult Education
Cocude, M. 157
Integrated Science Education for College Students in Japan
Koshida, Y. 163
Multidisciplinary Biology at the Tertiary Level with Special Reference to the University of Malaya
Kuthebatheen, A. J. 171
Biology Education in the Open University of Thailand
Phettongkam, M. 187
A Proposed Biology Syllabus and Scheme of Work for Integrating Diverse Disciplines of Pure, Applied and Philosophical Biology at First Year Level in Post Secondary Educational Streams
Santiago, A. 191
The Role of the Teacher in Multidisciplinary Biology Education with Special Reference to Environmental Approach
Perrott, E. 207
Community Based Resource Materials for Improving Competence of Biology Teachers
Savellano, J. M. 223

Environmental Issues and Education
Environmental Education: A Strategy in Development
Chelliah, T. 233
Ecological Education for Community Development
Furtado, J. I. 243
Aspects of Food, Health and Nutrition Problems and their Relevance to Biology Education
Gnanamuthu, E. 255
Education and Environmental Needs in Malaysia
Singh, G. 271
Education against Race Prejudices as a Topic of Biology Education
Kattmann, U. 277
Natural Environmental Educational through Japanese Monkeys
Kawasaki, T. 295
Conversation Education in Malaysia
Heang, K. B. 313
The Environmental Crisis and the Potential Role of Biology Education in Combating It

Feng, K. K. 317

The Conference
Asian Association for Biology Education
Executive Committee 325
Organising Committees 326
Conference Programme 329
List of Participants 333
Acknowledgements 343

< VIII > Eighth Conference held in Osaka and Gifu, Japan
(October - November, 1980)

Biology Education for the Next Decade

Linking Biology to Social Studies

Biological Education in a Changing World
Kelly, P. J. 1

Coordination between Biological Tents and Sociobiology
Rao, A. N. 7

Some Aspects of the Socialization of Teachers and Students of Biology
Jungwirth, E. 17

Integrating Some Topics in Biology to Social Studies
Villavicencio, R. R. 26

Thought of an Educational Module on Biology and Human Values in Sri Lanka
Basnayake, V. 41

Some Thoughts on Biological Education for Community Development
Atchia, M. 47

Utilization of Zoological Museum and Marine Aquarium for Instructional Purposes and for Laboratory Works
Hormchong, T. 53

Adaptation of the Individual and the Species to the Environment: A Principle Common Both to Biology and Social Sciences
Morimasa, S. 55

The Study of Environmental Education in Seventies
Satofuka, F. 61

Using Living Organisms for Field Study and Laboratory Work

Living Organisms in Biology Education
Vohra, F. C. 63

Introduction of the “living Materials Study Group (LMSG)” and Its Activities
Yamagiwa, T. 75

Attitudes of Secondary School Students in Israel towards the Use of Living Organisms in the Study of Biology

Tamir, P. 81

Use of Living Organisms for Laboratory Work in Biology Education
Kanagasabai, S. 95

A Survey Project to Search for Potentially Teachable Biological Materials from the Regions of Thailand
Chantharasakul, V. 105

The House Gecko as a Useful Specimen for Field and Laboratory Work
Chou, L. M. 113

Use of Gibasis Geniculata as Live Teaching Material
Shigenobu, Y. 117

A Simple Analysis of Laboratory Microecosystems by BCP Agar Method
Ueda, H. 125

Green- and Yellow- Euglena as an Educational Biomaterial
Shihira-Ishikawa, I. 131

Bring Nature into Your Classroom – Simple Way to Culture Organisms
Yamada, T. and Yamagiwa, T. 135

Ecological Adaptation of Rhodeus ocellatus and Their Use as a Teaching Material
Kawasaki, T. 145

Observation of Mitotic Division and DNA in Root Tip Cells
Chouhdry, A. S., Tanaka, R. and Yonezawa, Y. 155

Simplification of Gasmetry for Measuring Respiration and Photosynthesis
Yokohama, Y., Katayama, N. and Furuya, K. 159

Environmental Education

Environmental Education in Japanese Schools
Numata, M. 167

On the Way Wild and Domesticated Plants and Animals are Treated in an Elementary Science Textbook Authorized in Japan - An Issue from the Standpoint or Environmental Education
Hiroki, M. 173

A Field Study on Biological Education in Elementary School
Taniguchi, H., Shimizu, J. and Sato, K. 179

Environmental Problems and Orientations – A Malaysian Case Study
Yoong, C. S. 189

A General Comparative Method for the Development of Field Project in Contrasted Habitats
Meyer, R. G. 199

Biology Education, Teacher’s Education and Educational Evaluation
<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biology Education in the 1980s</td>
<td>215</td>
</tr>
<tr>
<td>Kennedy, M. H.</td>
<td></td>
</tr>
<tr>
<td>Biological Education towards the Year 2000</td>
<td>229</td>
</tr>
<tr>
<td>Tamir, P., Adler, J. H. and Poljakoff–Mayber, A.</td>
<td></td>
</tr>
<tr>
<td>Trends in Biological Science Education in Japan and the U. K.</td>
<td>239</td>
</tr>
<tr>
<td>Kille, R. A.</td>
<td></td>
</tr>
<tr>
<td>Biological Education As It Ought to Be in Future</td>
<td>251</td>
</tr>
<tr>
<td>Tate, T.</td>
<td></td>
</tr>
<tr>
<td>New Trends in Biological Education in Kuwait</td>
<td>255</td>
</tr>
<tr>
<td>Subbarini, M. S.</td>
<td></td>
</tr>
<tr>
<td>The Concepts of “Health” and “Environment” in Future Biology Teaching</td>
<td>259</td>
</tr>
<tr>
<td>Schaefer, G.</td>
<td></td>
</tr>
<tr>
<td>The Education of Biology Teachers: Retrospect and Prospect</td>
<td>277</td>
</tr>
<tr>
<td>Sood, J. K.</td>
<td></td>
</tr>
<tr>
<td>The Biology Teacher, A Moral Force</td>
<td>285</td>
</tr>
<tr>
<td>Dwijoseputro, D.</td>
<td></td>
</tr>
<tr>
<td>Study on Three Kinds of Instructive Media</td>
<td>289</td>
</tr>
<tr>
<td>Nakajima, Y.</td>
<td></td>
</tr>
<tr>
<td>The Curriculum of Biological Education in the National Teacher’s Colleges and Educational</td>
<td>295</td>
</tr>
<tr>
<td>Departments of Universities in Japan</td>
<td></td>
</tr>
<tr>
<td>Tara, M.</td>
<td></td>
</tr>
<tr>
<td>Towards Achieving the Central Objectives of School Science Practical Work</td>
<td>323</td>
</tr>
<tr>
<td>Leong, T. Y.</td>
<td></td>
</tr>
<tr>
<td>Biological Subjects taken up in the Interdisciplinary Courses at Chiba University</td>
<td>337</td>
</tr>
<tr>
<td>Tamanoi, I., Yoshida, O., Fukuda, Y. and Kobayashi, K.</td>
<td></td>
</tr>
<tr>
<td>Illustrated Stamps As a Teaching Aid for Field and Laboratory Studies</td>
<td>345</td>
</tr>
<tr>
<td>Katayama, N., Kitano, H. and Kobayashi, H.</td>
<td></td>
</tr>
<tr>
<td>Marine Biology Courses of College Education in Japan</td>
<td>363</td>
</tr>
<tr>
<td>Koshida, Y.</td>
<td></td>
</tr>
<tr>
<td>Educational Technology and Biological Technology</td>
<td></td>
</tr>
<tr>
<td>Introducing Educational Technology into the Classroom –Towards Biology Education in 1980s</td>
<td>373</td>
</tr>
<tr>
<td>Nakayama, K.</td>
<td></td>
</tr>
<tr>
<td>Role and Problems of Educational Technology in an Open University in Thailand</td>
<td>391</td>
</tr>
<tr>
<td>Puriveth, S.</td>
<td></td>
</tr>
<tr>
<td>VTR Student Practice for Nerve Impulse Conduction with an Electronic Neuron Model</td>
<td>397</td>
</tr>
<tr>
<td>Homma, S. and Mizota, M.</td>
<td></td>
</tr>
<tr>
<td>Biological Technology</td>
<td>405</td>
</tr>
<tr>
<td>Vohra, F. C.</td>
<td></td>
</tr>
<tr>
<td>Biological Technology</td>
<td></td>
</tr>
<tr>
<td>Rao, A. N.</td>
<td>419</td>
</tr>
<tr>
<td>Study on Recognition System for Handwritten Letters</td>
<td></td>
</tr>
<tr>
<td>Fujii, K. and Morita, T.</td>
<td>425</td>
</tr>
<tr>
<td>Conference</td>
<td></td>
</tr>
<tr>
<td>Opening Address: Imahori, K.</td>
<td>433</td>
</tr>
<tr>
<td>Welcoming Address: Kelly, P. J.</td>
<td>435</td>
</tr>
<tr>
<td>Greetings: Yoshiki, M.</td>
<td>437</td>
</tr>
<tr>
<td>Greetings: Ootsuka, H.</td>
<td>439</td>
</tr>
<tr>
<td>Closing Address: Nakayama, K.</td>
<td>441</td>
</tr>
<tr>
<td>Proposed Recommendations of the Conference</td>
<td>442</td>
</tr>
<tr>
<td>List of Participants</td>
<td>444</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>451</td>
</tr>
<tr>
<td>&lt; IX &gt; Ninth Conference held in Melbourne, Australia (December, 1982)</td>
<td></td>
</tr>
<tr>
<td>The Role of Biology Education in Enhancing the Quality of Life</td>
<td></td>
</tr>
<tr>
<td>Information Provided for Conference Participants</td>
<td>1</td>
</tr>
<tr>
<td>Presented Papers</td>
<td></td>
</tr>
<tr>
<td>Biological Conservation</td>
<td>8</td>
</tr>
<tr>
<td>Sirijaraya, P.</td>
<td></td>
</tr>
<tr>
<td>Fieldwork on Invertebrate Zoology in the Gulf of Thailand</td>
<td>14</td>
</tr>
<tr>
<td>Chullasorn, S.</td>
<td></td>
</tr>
<tr>
<td>The Biology Program at a Japanese Women’s University</td>
<td>24</td>
</tr>
<tr>
<td>Kimura, I.</td>
<td></td>
</tr>
<tr>
<td>Genetics and Life in Thailand</td>
<td>26</td>
</tr>
<tr>
<td>Saksoong, P.</td>
<td></td>
</tr>
<tr>
<td>Biology Courses at National Universities in Japan</td>
<td>27</td>
</tr>
<tr>
<td>Koshida, Y.</td>
<td></td>
</tr>
<tr>
<td>Biology Education and Quality of Life: A Malaysian Case Study in Teacher Preparation</td>
<td>34</td>
</tr>
<tr>
<td>Chelliah, T.</td>
<td></td>
</tr>
<tr>
<td>Teaching Health through Biology Education</td>
<td>49</td>
</tr>
<tr>
<td>Hernandez D. F.</td>
<td></td>
</tr>
<tr>
<td>Biology Education in a Developing Country</td>
<td>69</td>
</tr>
<tr>
<td>Dwijoseputro, D.</td>
<td></td>
</tr>
<tr>
<td>Health Education in Biology and the Quality of Life</td>
<td>77</td>
</tr>
<tr>
<td>Imahori, K.</td>
<td></td>
</tr>
<tr>
<td>Ecological Training on Secondary Succession at the School Campus</td>
<td>92</td>
</tr>
<tr>
<td>Odaki, K.</td>
<td></td>
</tr>
<tr>
<td>Biological Investigations and Field Work on Boso Peninsula in an Interdisciplinary Course</td>
<td>106</td>
</tr>
<tr>
<td>Yoshida, O. Tamanoi, I., Fukuda, Y. and Kobayashi, K., Nishino, E., Nagawa, H. and Asai, N.</td>
<td></td>
</tr>
<tr>
<td>Teachers’ Group Activities for Promotion of Teaching Genetics at the Senior High School</td>
<td></td>
</tr>
</tbody>
</table>
Hatakeyama, T. 118
How to Cultivate Efficient Ways of Laboratory Exercises in Biology
Tamanoi, I., Yoshida, O., Fukuda, Y. 119
Kobayashi, K., Nishino, E., Nagowa, H., and Asai, N.
Educational Uses of Wild Flowers with Special Reference to Out-door Biology at Elementary School Level in Japan
Katayama, N. 130
New Systematic Structure of Biology Education
Tate, T. 164
The Educational Uses of a Braconid Wasp, Aponteles glomeratus L. with Special Reference to Ethology Teaching
Kitano, H. and Kawahara, H. 169
List of Participants 183

< X > Tenth Conference held in Chang Mai, Thailand (December, 1984)

AABE Executive Committee 1982-1984
Acknowledgements 3
Opening Ceremony 5
Welcome Address: Thitasut, P. 7

Conference Papers
Biology Education and Technology
Soydhurm, P. 11
Biology Education at Pre-medical and Pre-dental School in Japan
Koshida, Y. and Horiuchi, S. 24
Teaching and Laboratory Exercises of Biology in Pre-medical and Pre-dental Courses in Colleges and University of Japan
Tamanoi, I. and Koshida, Y. 34
Biology for Upper Secondary School in Thailand Chantharasakul, V. and Soydhurm, P. 47
Strategies for Improving Biology Education: A Philippine Experience
Gregorio, L. C. 58
Integration of Different Teaching Strategies in a Biology Course
Poljakoff-Mayber, A. 70
Duckweeds as Biomaterial for Teaching Population Ecology
Prakobvitayakit Beaver, O. 75
The Educational Uses of the Domesticated Silk-worm Adult, Bombyx mori, with Special Reference to Ethology Teaching
Kitano, H. and Yamazaki, S. 90
Natural Dyes for Animal Tissue Staining
Patinawin, S. 103
Culture of Setae and Induction of Polyploid Mutant – A Simple But Useful Tissue Culture of Plant for High School Students
Yonezawa, Y. 117
Vorticella sp., an Example of Cell Motility
Phanichyakarn, V. and Cherdshewasart, W. 128
Study on Inhabitable Place of Mammals in Gifu Prefecture (Central Part of Japan) and Mammal’s Life
Kawasaki, T. 137
Thinking Logically – A Prerequisite for Pupils’ Research Project
Jungwirth, E. 148
The Importance of Biological Research on Traditional Culture
Tate, T. 161
Research on Australian Mammals – A Low Technology Approach
Wallis, R. L. and Brunner, H. 166
Biology Research Project for High School Students
Prakobvitayakit Beaver, O. 183
Cultivation of Seaweed and Measurement of Its Photosynthetic Activity Using the Improved Productmeter, As a Laboratory Exercise for Upper Secondary School Biology
Katayama, N., Tokunaga, Y., Furuya, K., and Yokohama, Y. 187
A Project for Study on Bird Biology through Nature Observation
Wilasdachanont, W., Isarankura, K. and Sirijaraya, P. 207
The Biology in a Women’s University
Kimura, I. 219
Biology in the Future – Some Trends in Biology Research and Their Implications for Biology Educator
Wallis, R. L. 225
Final Report on the 10th AABE Conference
Chiowanich, P. 233
Organizing Committees 237
Conference Programmes 241
List of Participants 251

< XI >Eleventh Conference held in Quezon City, Philippines (December, 1986)
Research and Evaluation in Biology Education and Its Implication for the Teachers
Foreword ix
Biology Research and Implications to Teaching
Free Radical Biology and Xenobiotic Biotransformation: A Possible Mechanism of Pesticide Toxicity
Andaya, A. A. 3
<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiments of “Functional Response” of Some Fish Species for Practical Application Population Ecology</td>
<td>11</td>
</tr>
<tr>
<td>Research Developments in Cell Biology – Implications to Applied Fields and to Biology Education</td>
<td>15</td>
</tr>
<tr>
<td>Mammalian Field Studies Using Indirect Methods</td>
<td>23</td>
</tr>
<tr>
<td>Application of Biotechnology and Genetic Engineering in the Control of Tropical Diseases</td>
<td>29</td>
</tr>
<tr>
<td>Taxonomy of the Phytoplankton Flora in Northwestern Luzon, Philippines with Notes on Their Ecology</td>
<td>41</td>
</tr>
<tr>
<td>Studies on the Cellular Defense Reaction of Insects for a General Understanding of “Homeostasis” with Special Reference to Secondary School Biology Education</td>
<td>62</td>
</tr>
<tr>
<td>The Implications of Genetics Theme in the Korean Upper Secondary School Biology Curriculum</td>
<td>73</td>
</tr>
<tr>
<td>General Research in College</td>
<td>111</td>
</tr>
<tr>
<td>Introducing the Pineal Gland – A Possible Integrator of the Biological Clock</td>
<td>116</td>
</tr>
<tr>
<td>Studies on the Teaching of Biology and Science</td>
<td></td>
</tr>
<tr>
<td>The Teaching of Modern Biology</td>
<td>131</td>
</tr>
<tr>
<td>Can the Average Secondary School Students Benefit from Sophisticated Biology Research Findings?</td>
<td>138</td>
</tr>
<tr>
<td>Survey of the Knowledge of Common Plans among Students in the Teacher Training Course</td>
<td>151</td>
</tr>
<tr>
<td>A Study in the Inclusion of Traditional Culture in the Teaching Material “Science of Plants” with Emphasis on Charcoal Making</td>
<td>178</td>
</tr>
<tr>
<td>Biology Terms in the Textbooks for Elementary and Secondary Schools</td>
<td>185</td>
</tr>
<tr>
<td>Science Education and Biology Teaching</td>
<td>192</td>
</tr>
<tr>
<td>New Programs, Courses and Materials</td>
<td></td>
</tr>
<tr>
<td>Postgraduate Programs in Biology for Teachers in Thailand</td>
<td>207</td>
</tr>
<tr>
<td>The Research Program of the PSHS Curriculum</td>
<td>215</td>
</tr>
<tr>
<td>Trends in Biology for Teacher Training Courses in India</td>
<td>219</td>
</tr>
<tr>
<td>Educational Aspects of Japan and a Proposed Undergraduate Biology Curriculum in the College of General Education</td>
<td>226</td>
</tr>
<tr>
<td>Teaching and Laboratory Exercises in Biology for the First Year Course in Khon Kaen University</td>
<td>230</td>
</tr>
<tr>
<td>Biology Enrichment Program for Science Talented Students in Thailand</td>
<td>237</td>
</tr>
<tr>
<td>Biology Curriculum in Chiang Mai University</td>
<td>243</td>
</tr>
<tr>
<td>An Approach to Biology Education (Tertiary) for Non-Science Majors</td>
<td>251</td>
</tr>
<tr>
<td>Approaches to Biology Education</td>
<td>257</td>
</tr>
<tr>
<td>Working Committees for the Eleventh AABE Biennial Conference</td>
<td>259</td>
</tr>
<tr>
<td>Participants</td>
<td>261</td>
</tr>
</tbody>
</table>

< XII > Twelfth Conference held in New Delhi, India (December, 1988)

- Explosion of Biological Knowledge and the Challenges for Secondary Education and Teacher Preparation
- Biology Education in Asian Countries
  - Trends in Australian Biology Education
  - Status of Biology Education in India
  - Science Education in the Republic of Korea

Papers Submitted
- Sex Education in Korean Middle Schools
- Recent Trends in Research into Biology Education by Japanese School Teachers
- Development of a Laboratory Exercise Using Gasmetry in Upper Secondary School Biology
- An Exercise in Practical Observation of Insects, with 4th Year Undergraduate Students
- A database of Threatened Species in Australia – An Aid in Teaching
Simple Sulfur-Dioxide Fumigation Methods as Aids for Teaching about Air Pollution
Takaoki, T. 85

Conference Report 94
Delegates at the Conference 97

< XIII > Thirteenth Conference held in Seoul, Korea (August - September, 1990)
Environmental Education in the Curriculum of Biological Education
Opening Address i
Welcoming Address ii
Provisional Program 6
Itinerary 7
Social Program 12

Environmental Education in the Curriculum of Biological Education: in Elementary, Middle, and High School
Environmental Education in Thailand Sukchotiratana, M. 13
Environmental Education in Biology Education Yoon, I. B. 22
Biology Education in Australia Wallis, R. L. 30
Quality of Life and Human Biology Education in Secondary School in Hong Kong Tang, P. L. 38

Environmental Issues in the Revised National Curriculum in Japan Umeno, K. 56
Implication of Environmental Issues in Korean School Curricula Chung, Y. J. and Yun, M. Y. 72

Environmental Education in Some Universities in Thailand Sukchotiratana, M. 100

Case Studies of Environmental Education
A Case Study in Environmental Education in Australia Wallis, R. L. 112
Field Experience on Natural History Education for Science and Non-science Students in Teachers Training College with Special Reference to Environmental Education in Japan Kitano, H. 153
Detection of Photosynthetic Oxygen Production Using Animal Blood, an Experiment Suitable for Environmental Education at the Secondary Level Katayama, N. 166

On Primates (Japanese Monkey) As the Materials of Nature Education and Environmental Education Kawasaki, T. 182
Children's Thinking about Their Surrounding Nature and Today's Environmental Problem Hirata, A. 209

Implication of Environmental Education for Social Life and Culture
The Socioeconomic and Cultural Implications of Environmental Education for the Preservation and Conservation of Natural Environment Han, S. B. 226
A Study of Taking Traditional Culture of the Nation into the Teaching Material Tate, T. 244
Science Clubbing for Environmental Education - Its Implication in Social Life and Culture de la Torre, R. U. 259

Fishpondification, a Major Controversial Environmental Issue in Mangrove Ecosystem Conservation Zamora, P. M. 263
Pollution and Environmental Destruction Arcilla, J. G. 286
Rice Field for Observing Microorganisms Mikami, K. 296

The Effect of Gamma Radiation on Some Algae of Economic Importance Pictures on Scenedesmus, Chlorella, and Nostoc Aranez, A. T., Antonio, B. and Tagliano, T. 304
Conservation of Fimbristylis globulosa (Retz.) Kunth: Effects of Nitrogen and Gibberellic Acid on the Growth and Development Escarlos, J. A. and Mino, S. 310
Analysis of Mercury Content of Selected Species of Macrobenthic Algae Relon, M. L. 320
Mycoflora of the Rhizosphere and Rhizoplane of Selected Crop Plants Saniel, L. S. 337

A Simple Gas-Volumeter for Measuring Photosynthesis and Respiration Rates Available As Teaching Aid Takaoki, T. 345

Scheme of Environmental Orientation to School Education Mohta, R. K. 353
Environmental Education in Elementary and Secondary Schools of Korea Chung, W. H. 361

Environmental Education in Elementary Schools of Korea Yu, W. I. 375
Internalization of the Awareness in Preservation of Nature through Efficient Environmental Education  
  *Kim, Y. S.*  
  398

Environmental Education in High School of Korea  
  *Park, H. S.*  
  424

The Contents of Environmental Education in the High School Curriculum in Korea  
  *Surh, K. H.*  
  433

Environment around King Sejong Station  
  *Kim, Y. S.*  
  450

Author Index  
  466

Participants  
  467

< XIV > Fourteenth Conference held in Melbourne, Australia (December, 1992)

Environmental Management in Asia – Training, Education and Research

**List of Conference Delegates**  
  iii

**Conference Theme Papers**

UNESCO-SEAMEO Biotrop Training Courses on Environmental Management  
  *Umaly, R. C.*  
  4

Environmental Education in Hong Kong: Past, Present and Future  
  *Tang, P. L.*  
  22

The Present Aspects of Environmental Education in Japan  
  *Koshida, Y.*  
  44

Pre-service Teacher Training Program on Environmental Education, a Research Based and Community-service Oriented Approach  
  *Hafalla, J. R.*  
  47

Seminar-workshops on Environmental Protection  
  *Joaquin, J. C.*  
  54

Survey of Mangrove Ecosystem for Environmental Education  
  *Attachoo, C. et al.*  
  57

Dry-lab Showing the Procedure for Evaluation of River Water Quality Using Diatoms  
  *Kabayasi, H. and Ueyama, S.*  
  63

Algae Appearing in Japanese Science Textbooks at the Compulsory Level for the Last 40 Years  
  *Katayama, N.*  
  75

Eco-watch and Eco-act: A Bridge Over the Walls  
  *Kanapi, C. G. and Amansu, W. B.*  
  86

Correlates of Household Greenhouse Emissions  
  *Lindsay A., Marinopoulos, J., Treloar, A., Stokes, D. and Wescott, G.*  
  91

Hong Kong Airport 1997: An Environmental Issue  
  *Tang, P. L.*  
  103

Turnip Sawfly, *Athalia rosae* (insecta) As an Indicator of Pesticide Contamination  
  *Kitano, H. and Kaji, A.*  
  135

**General Papers**

Permian Marine Provinciaity, a Theoretical Model and an Empirical Comparison  
  *Shi, G. R. and Archbold, N. W.*  
  155

The Garden Lizard of Singapore, *Calotes versicolor*: a Model Organism for Field and Laboratory Study  
  *Diong, C. H.*  
  183

Acid and Its Environment in Education  
  *Tate, T.*  
  189

Distribution of Meiofauna Inside and Outside Seagrass Patches, Khung Kraben Bay, Eastern Coast of Thailand  
  *Chullasorn, S.*  
  197

**Dileptus**: a Microorganism As a Live Teaching Material  
  *Mikami, K.*  
  215

The Air-borne Pollen Grains Investigated by the Senior High School Students  
  *Oka, K.*  
  222

Effects of Methyl Parathion-containing Pesticide on Chromosomes Based on the *Allium* Test  
  *Aranaz, A. T. and Rubio, R. O.*  
  228

Effects of Applied Nitrogen and Phosphorus on Nodulation of Winged Bean  
  *Escarlos, J. A.*  
  237

On the teaching of “Reproduction” and “Heredity” in Japanese Junior High School Science for the Past 40 Years  
  *Kanaizuka, Y.*  
  243

“Appreciation Lesson”, An Innovation in Science Teaching  
  *de la Torré, R. U.*  
  251

In-contest Support Programs for Non-traditional Students of Biology  
  *Goodall, M. H., Dixon, J. and Chambers, P. J.*  
  253

New Methods for the Teaching of Bioscience  
  *Wallis, A. M. and Gargett, C.*  
  263

< XV > Fifteenth Conference held in Tokyo, Japan (August, 1994)

Biology Education for Non-Biology Majors

**Opening Address**: *Koshida, Y.*  
  1

**Welcome Address**: *Imahori, K.*  
  2

**Welcome Address**: *Hasumi, O.*  
  4

**Plenary Lectures**

Crisis in Biological Science: Biology for All -  
  *Hornchong, T.*  
  5

Teaching Life Science to Non-science Majors  
  *Takahasi, K.*  
  11
Country Reports
Biology Education Report - Australia: A Case Study in the Use of Biology in a Multidisciplinary Tertiary Education Course
Wallis, R. L. and Baskaran, K. 12
Biology Education at the Secondary and Pre-university Level in Hong Kong - A Brief Report
Tang, P. L. 20
The Current Status of Biology Education in Korea
Biology Education in the Philippines: An Update
Joaquin, C. C. 44
Biology Teaching to Non-biology Majors in Japan: Before and after the Recent Curricular Innovation of Colleges and Universities
Koshida, Y. 54

Contribution Papers
A Long Term Experiment in Ecology: The Effects of Logging and Fire on Mammals in an Australian Forest
Wallis, R. L. 60
The Population Changes of the Japanese Black Bear in Gifu Prefecture and the Opinions of Area Residents concerning Them
Kawasaki, T. 67
Schwettmann, K. D. 75
Practice of Biology Education in the Experimental Plantation
Tara, M. 80
Interpreting Our Natural Heritage in the Malaysian Tropical Rainforest and Coral Reef through Slide Program: A Case Study for 6th Grade Pupils in a Japanese Elementary School
Kitano, H. 88
The Concept and the Method of Environmental Education and the Way of Their Application: “The Kushiro Marsh Plan”
Ubakata, H. 91
A Historical Study on the Genetics Education in Japanese Secondary School Biology Subject Matters
Ikeda, H. 98
New Science Curricula for Non-Science Course in Upper Secondary Schools
Umeno, K. 103
Revolutionary Change in Biological Education at Nihon Daigaku College of Law by Adapting to New Curriculum
Sudzuki, M. 111

The Environment and Reproductive Rhythms in Mammals
Tang, P. L. and Chan, S. T. 120
A Study on the Effect of a Molecular Movement Based Instruction on Understanding of Diffusion and Osmosis and on Scientific Attitude
Cho, J.-I. 135

Poster Presentations
How Much Knowledge the University Students Have of the Trees on Campus
Takeuchi, K., Umeki, S. and Matsuka, M. 143
Comparison of Biology Curriculum for Upper Secondary Schools between Myanmar and Japan
Hiroki, M. and Ciin, N. K. 149
A Study on the Cognition of Natural Environment of High School Students in Japan and Korea
Fujishima, H. and Ka, H. 157
Cognitive Functions of Two Hemispheres and Biological Education
Kang, H.-K. and Rim, Y.-D. 162
The Analysis of Environment-relating Texts and the Personalization of Environment in the Environment Education
Chang, N.-K., Lee, J. E. and Park, M. 170
Time-lapse-video Display for the Intuitive Understanding of Plant Motility
Shihira-Ishikawa, I., Furukawa, T., Ohsu, T., Hosokawa, S., Makita, N. and Sugiyama, Y. 179
A Video Program Showing the Procedure for Collection and Observation of Diatoms Used for Evaluation of River Water Quality
Mayama, S., Ueyama, S., Mayama, N. and Kobayasi, H. 184
Semi-individualized Instruction for Students’ Activities
Fukuda, H., Shimizu, K., Sato, Y. and Murasugi, S. 190
A CAI (Computer Aided Instruction) Program and a Video Program, Terms of Biology and Its Use for Teaching Biology in High School
Kaga, T. 197
How to Obtain Protista Available for Biology and Environmental Education at School: Cultivation of Volvox and Paramecium
Mikami, K., Igarı, T. and Oka, K. 203
An Examination of a Freshwater Filamentous Green Alga Rhizoclonium riparium for the Development of Experimental Materials for Teaching Photosynthesis
Imai, M. and Katayama, N. 211
3-Dimentional Algal Specimen Is an Useful Teaching Material in Biology Education
Misonou, T. and Rinno, M. 216
Leaf Skeletonizing – A Practical Way to Livelihood Education through Science and Technology
   de la Torré, R. U. 222

Laboratory Exercises Using a Japanese Ladybird, Propylea japonica, for Teaching “Reproduction” and “Heredity” in Junior High School Science
   Kanaizuka, Y. and Katayama, N. 225

A Simple Volumetric Method for Measuring Photosynthesis and Respiration Rates even at Home
   Takaoki, T. 231

Improvement of Indigo Carmine Method at the Experiment of the Photosynthesis in Science Education
   Jinno, N. and Fujita, T. 239

On the Use of the Greenbelts at School Grounds
   Park, J.-K. 245

UST Mangrove Tree Planting: A Model for an Outdoor Class Activity in Environmental Biology
   Duque, S. M. and Madulid, R. 253

Education for the General Public on Marine Biology with the South-Izu Marine Ecology Society (S.M.E.S.): Observation Activities of Marine Organisms by Snorkel Diving
   Hirata, T., Aoki, M., Kurashima, A., Dasai, A. and Yokohama, Y. 258

Abstracts

Biology Teaching to Non-biology Majors through Out-of-school Education – The Role of Youth Environmental Non-governmental Organizations
   Hili, C. 264

Biology Course for Non-biology Majors at Chiang Mai University
   Sukchoitratana, M. 264

Biology Teaching to Undergraduate and Post-graduate Engineering Students - A Personal Experience -
   Tang, P. L. 265

An Educational Use of a Braconid Wasp, Cotesia (= Apanteles) glomerata (L.) in High School Biology
   Kawahara, H. 265

The Winning Works of the 10th Annual Nature Trail Contest in Japan
   Saitoh, M. 266

Workshop

A Student Activity for Nature Conservation in Tokyo Gakugei University
   Katayama, N. 267

List of Participants 268

Executive Committee Members 275

Acknowledgements 276

<XVI> Sixteenth Conference held in Chang Mai, Thailand (December, 1996)

Excellence in Biology Teaching: Research, Practice and Experience

Welcome Address: Sukchoitratana, M. 1

Opening Address: Teetrakont, C. 2

30th Anniversary of AABE Speech:
   Koshida, Y. 4

Plenary Lectures

Experience in Practice and Research Lead to Excellence in Biology Teaching
   Hormchong, T. 6

Teaching Ecology through Environmental Issues:
   A Workshop Example in Thailand
   Tilling, S. M. 10

The Use of Lichens as Indicators of Environmental Change in Seasonal Tropical Forests of Northern Thailand: A Workshop Using Simple Techniques for Sampling Lichen Communities
   Wolseley, P. 31

Country Reports

Teaching and Learning Environmental Science in Schools of Thailand
   Boonklurb, N. 44

Biology Education in the Philippines: Prospect and Retrospect
   Angtuaco, S. P. 48

Current Status of Biology Education at the Primary and Secondary Levels in Japan
   Katayama, N. 53

Quality in Teaching and Learning – The Australian Universities’ Experience
   Wallis, R. L. and Boyd, B. 69

Contribution Papers

Enhancing the Development of Thinking Skills and Critical Thinking among Students of Natural Sciences
   Hafalla, J. R. 76

Biology Education by VTR and Field Works
   Tara, M. 81

Integrating HIV/AIDS Concepts in a Basic Biology Curriculum
   Gregorio, L. C. 87

Laboratory Exercises Using a Red Alga, Gigartina mamillosa, for Teaching Photosynthesis of Seaweeds in Junior High School Science
   Kanaizuka, Y. and Katayama, N. 92

Management of Dalbergia sisso Roxb, in Farm Conditions Using Different Pruning Intensities
   Kafle, S. K. and Dixit, P. M. 102

Australia’s Largest Owl. Diet and Conservation of Powerful Owls in the Yarra Valley
   Wallis, R. L. 112
Seventh Grade Students’ Informal Theories of Horn-Beetles
Hirata, A. 118

Frog Culture for Biology Study and Biological Research
Na Nagara, S. 124

Where and How to Collect the Small Benthic Marine Invertebrates
Chullasorn, S. 127

Inquiry into the Cell
Nakamichi, T. 134

Analysis or the Discriminatory Capacity of Questions in University Entrance Examinations in Japan
Koshida, Y., Maekawa, S. and Shimizu, T. 141

Repellency Effects of Neem and Synthetic Pesticides to Honeybees
Thapa, R., Wongwiri, S. and Prakobvitayakit, O. 147

Comparative Study on the Learning Achievement in Biology Course 045: Genetic Materials and Protein Synthesis of Mathayomsuksa 6 Students Focusing on the Use of Teaching Protein Synthesis* Magnetic Board during Teaching Process
Piriyakul, K. 153

Some Biological Investigation of Larval Trematodes from Chiang Mai Moat

Helminthological Survey of Rats from Urban Area of Chiang Mai
Namue, C. and Wongsawad, C. 172

Mt. Kwangdok as a Nature Trail
Park, I.-K. and Rim, Y.-D. 184

Pteridophyte Comparison in Different Forest Types at Doi Lohn, San Kampaeng District, Chiang Mai Province
Bañoc, L. M., Maxwell, J. F., Elliott, S. D. and Anusarnsunthorn, V. 190

Change of Nature Environment and Biological Education
Fujishima, H. 197

The Effect of Benzocaine in the Transpiration of Oreochromis niloticus Linn. and Chanos chanos Forskal
Sommamai, A., Kerdthongkai, S. and Srisangngam, S. 202

A Proposition to the International Solidatory of Environmental Education in Asia-Pacific Area
Satthoh, M. 208

Study of Marine Actinomycetes and Their Roles in Marine Microcosm
Srivibool, R. 216

List of Participants 232

Executive Committee Members 233
The Asian Association for Biology Education (AABE): Constitution and Rules 242

<B>Seventeenth Conference held in Manila, Philippines (December, 1998)<BR>Biology Education in the Third Millennium</B>

<i>Message: Editors</i> i
<i>Message: Kanapi, C. G.</i> ii

<i>Keynote Address</i>
Biology Education in the Third Millennium: Focus on Information Technology and Environmental Education
<i>Padolina, W. G.</i> 1

<i>Inspirational Talk</i>
Foundation and Development of the Asian Association for Biology Education
<i>Imahori, K.</i> 5

<i>Plenary Papers</i>
Biology Education in the Third Millennium: Focus on Information Technology and Environmental Education
<i>Madrazo, G.</i> 7

Biology Education at Risk
<i>Imahori, K.</i> 12

IT-Aided Adult Environmental Education
<i>Suselo, T.</i> 16

Biology of Dicyemid Mesozoans with Notes of Their Educational Use
<i>Koshida, Y., Furuya, H. and Tsuneki, K. A.</i> 25

Toward a New Direction for Biology Educators: From Self Censorship to Mentoring
<i>Fortino, C. A.</i> 29

<i>Country Reports</i>
Biology Education and Environmental Education in the Third Millennium in Japanese Primary and Secondary Schools
<iKatayama, N.</i> 43

Environmental Education in the Philippines
<iRabago, K. M.</i> 50

Biotechnology Education in Tertiary Institutions in Hong Kong
<iTang, P. L.</i> 55

Biology in Australian Schools
<iWallis, R. L.</i> 56

<i>List of Seminar and Workshop Papers</i>
Biology Interactive: “Experience Life”
<iAcena, A. and XBI Team Xavier</i> 64

Clastogenicity of X-rays, Cobalt Chloride and Methyl Methane Sulfonate as Assayed in Allium cepa Seedlings
<iAlcaide, B., Ikeda, H. and Fujikawa, K.</i> 65

Interfacing Experiments with the Computer
<iGalvez, E. R., Catalan, M. H. C., Orbita, P. S.</i> 33
Archives: Proceedings of Past Biennial Conferences of the AABE

Integrating Sustainable Development into Tertiary Level Courses

David, M. A. B. and Esguerra, J. P. H.

Environmental Case Study: Ecological Succession in a Hay Infusion

Joaquin, C. C.

Laboratory Exercise Suitable for Teaching Relationship between Vertical Distribution of Seaweed and Their Photosynthetic Characteristics in Advanced Science Classes in Japanese Junior High Schools

Kanaizuka, Y. and Katayama, N.

Trials and Further Improvement of the “Simple Procedures for the Extraction and Separation of Photosynthetic Pigments”

Kanaizuka, N., Sato, H., Kanaizuka, Y. and Yokohama, Y.

Observation Material of Plant Chromosome for High School Students in Biology – with Special Reference to Morus nigra (Mulberry)

Kawashima, N. and Oshigane, K.

Pesticide Education in Upper Secondary School Biology Education in Japan: Based on a Survey of Biology Textbooks 1994-1995

Kitano, H.

The Use of Electronic Media, Both CDROM and Internet, in the Delivery of a Second Year University Course in Fish Biology

Laurenson, L. J. B. and Wallis, R. L.

The Use of the Internet System in Biology Education: Making Homepages about Experimental and Visual Information on Selected Topics in Biology

Ohshika, K., Treyes, R., Alcaide, B. and Ikeda, H.

Constructivism, Information Technology and Mediated Learning

Perez, T. R.

Microalgae: Potential Organism for Pollution Monitoring

Perez, T. R. and Tabbada, R. A.

A Rapid and Simple Experiment Utilizing Luminous Bacteria for the Classroom Demonstration of Biological Concepts of Cellular Toxicity, the Oxygen Effect on Bioluminescence and Catabolite Repression

Quinto, E.

My Experience in Biology Education – Observation of Plankton

Tara, M.

Differentiation of the Isolated Protoplasts from Gametophyte of the Tree Fern Cyathea contaminans (Hook) Copel. to Gibberelic Acid (GA_3, GA_4 and GA_7) Treatment

Treyes, R., Watanabe, S., Ohshika, K. and Ikeda, H.

Native Mammal Reintroductions to Predator-Controlled Habitat in Western Australia

Wallis, R. L.

List of Poster Presentation

Galls and Mine Growths on Philippine Plants

Alejandro, G. D., Madulid, R. and Schwettaman, K. D.

Enhancing the Learning Process of Biology Students via Community Study: A Centro Escolar University Experience

Austria, Z.

The Survival Plants of the Batak and Tagbanua Tribes in Palawan, Philippines

Bunquin, M. D. A.

Cyanobacterial Growth Response and Plasmid Copy Number in Salt-stressed Environment

Cao, E. P., Loveria, M. V., Rivero, G. C. and Roderos, R. R.

Phytoremediation of Heavy Metal Contamination in Soil and Water

Follosco, M. P. and Tel-Or, E.

Reproductive Biology of Some Ichthyofauna of the Agos River, Central Sierra Madre

Herrera, A. A.

Expression of Receptors to Biotinylated Probes in Transformed Breast Tissues

Herrera, A. A., Jacinto, S., Sioson, C., Gamboa, E., Amparado, E. and Casauay, A.

Effects of Acid Stress on Plasma Calcium Level and Histochemistry of Gonads of Oreochromis niloticus

Herrera, A. A. and Pador, G.

An Effective Method for Teaching Biology through the Internet in High School

Kaga, T. and Arai, M.

The Framework of Environmental Education from the Viewpoint of Biology

Kobayashi, T.

Environmental Studies in Senior High School

Nakamichi, T.

The Isolation and Characterization of Bacteria Isolated from the Seawater Samples Collected along the Breakwater Area beside the Folk Arts Theater of Manila Bay

Quinto, E.

Biosensor for Water Toxicity Based on a luminous Bacterium: Photobacterium leiognathi USTCMS2116

Quinto, E. and Sevilla, F.

Scanning Electron Microscopic Studies of the Phytoplankton Flora in Talin Bay, Lian, Batangas
Relon, M. L. 180
Bioremediation Potential of Two Philippine Microalgal Isolates Bat-09 (Chroococcus) and CAV-25 (Desmococcus) Exposed to Copper and Cadmium
Rivero, G. C., Lintongan, P. B., Cao E. P., and Roderos, R. R. 181
Isolation and Cultivation of Microalgae from Philippine Waters
Roderos, R. R., Calugay, R. J., Cao, E. P., and Rivero, G. C. 197
Distribution of Mosquitoes and Incidence of Mosquito-borne Diseases in Metro Cebu
Rivero, J. 201
An Invitation to the Annual Nature Trail Contest in Japan
Saitoh, M. 207

AUTHOR INDEX

A
Acena, A. XVII-64
Adler, J. H. IV-376, VIII-229
Alcaide, B. XVII-65, XVII-92
Alejandro, G. D. XVII-113
Alfonso, P. J. II-47
Al-Jalili, A. R. I-192
Allen, D. IV-291
Allon, Y. IV-593
Amanus, W. B. XIV-86
Amos de Shalit Science teaching Center, Hebrew University IV-187
Amparado, E. XVII-133
Andaya, A. A. XI-3
Angtuaco, S. P. XVI-48
Antonio, B. XIII-304
Anusarnsunthorn, V. XVI-190
Aoki, M. XV-258
Arai, M. XVII-158
Aranez, A. T. XIII-304, XIV-228
Archbold, N. W. XIV-155
Arcilla, J. G. XIII-286
Asai, N IX-106, IX-119
Asis, C. V. III-64
Atchia, M. VIII-47
Attacho, C., et al XIV-57
Avadhanii, P. N. V-83
Austria, Z. XVII-114

B
Bañoc, L. M. XVI-190
Bar-on, E. IV-323, IV-326
Baryam, M. IV-326
Baskaran, K. XV-12
Basnayake, V. I-48, III-167, VIII-41
Beal, J. B. IV-263
Ben-Chanan, M. IV-483

Bennett L. M. V-268
Ben-Shaul IV-455
Bingman, K. J. IV-155
Bingman, R. M. IV-155
Blum, A. IV-215, IV-234, IV-492
Boonklurb, N. XVI-44
Boyd, B. XVI-69
Brüunner, H. 166
Bunquing, M. D. A. XVII-116

Calugay, R. J. XVII-197
Cao, E. P. XVII-117, XVII-181, XVII-197
Care, R. A. IV-465, IV-606
Carter J. VI-113
Casauay, A. XVII-133
Catalan, M. H. C. XVII-69
Chambers, P. J. XIV-253
Chan, J. Y. IV-155
Chan, S. T. XV-120
Chang, N.-K. XV-35, XV-170
Chanthesarakul, V. VIII-105, X-47
Charles, S. VII-97
Cheah, C. K. V-13
Chelliah, T. VII-233, IX-34
Cherdshewasart, W. XV-149
Chiowanich, X-3, X-233, XI-207
Chou, L. M. VII-153, VIII-113
Chouhdry, A. S. VIII-155
Chullasorn, S. IX-14, XIV-197, XVI-127
Chung, W. H. XIII-361
Chung, Y. J. XI-73, XII-20, XIII-72
Chye, Y. O. V-1
Cini, N. K. XV-149
Cocude, M. VII-157
<table>
<thead>
<tr>
<th>Name</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crusz, H.</td>
<td>I-48, I-76, IV-509</td>
</tr>
<tr>
<td>Cruz, J. M.</td>
<td>IX-215</td>
</tr>
<tr>
<td>D</td>
<td></td>
</tr>
<tr>
<td>Daniel, C. J. S.</td>
<td>III-32</td>
</tr>
<tr>
<td>Dasai, A.</td>
<td>XV-258</td>
</tr>
<tr>
<td>David, M. A. B.</td>
<td>XVII-70</td>
</tr>
<tr>
<td>Dearing, S. J.</td>
<td>XI-131</td>
</tr>
<tr>
<td>de la Torrè, R. U.</td>
<td>XIII-259, XIV-251, XV-222</td>
</tr>
<tr>
<td>Diong, C. H.</td>
<td>XIV-183</td>
</tr>
<tr>
<td>Dissanayake, P.</td>
<td>III-37</td>
</tr>
<tr>
<td>Dixit, P. M.</td>
<td>XVI-102</td>
</tr>
<tr>
<td>Dixon, J.</td>
<td>XIV-253</td>
</tr>
<tr>
<td>Doraiswami, S.</td>
<td>IV-573</td>
</tr>
<tr>
<td>Dowdeswell, W. H.</td>
<td>I-65, II-64, IV-434</td>
</tr>
<tr>
<td>Dreyfus, A.</td>
<td>XI-138</td>
</tr>
<tr>
<td>Duong, D. N.</td>
<td>I-293</td>
</tr>
<tr>
<td>Duque, S. M.</td>
<td>XV-253</td>
</tr>
<tr>
<td>Dwidjoseputro, D.</td>
<td>V-146, VIII-285, IX-69</td>
</tr>
<tr>
<td>E</td>
<td></td>
</tr>
<tr>
<td>Edling, J. V.</td>
<td>IV-434</td>
</tr>
<tr>
<td>Eggleston, J. F.</td>
<td>IV-122</td>
</tr>
<tr>
<td>Elkana, Y.</td>
<td>IV-595</td>
</tr>
<tr>
<td>Elliot, A. B.</td>
<td>V-43</td>
</tr>
<tr>
<td>Elliott, S. D.</td>
<td>XVI-190</td>
</tr>
<tr>
<td>Elton, L. R.</td>
<td>IV-285, IV-430</td>
</tr>
<tr>
<td>Erinyagama, I.</td>
<td>III-9, III-13, IV-380</td>
</tr>
<tr>
<td>Escarlos, J. A.</td>
<td>XIII-310, XIV-237</td>
</tr>
<tr>
<td>Esguerra J. P. H.</td>
<td>XVII-70</td>
</tr>
<tr>
<td>Etrog, A.</td>
<td>IV-326</td>
</tr>
<tr>
<td>F</td>
<td></td>
</tr>
<tr>
<td>Fakoor, S. R.</td>
<td>I-145, II-111</td>
</tr>
<tr>
<td>Follosco, M. P.</td>
<td>XVII-123</td>
</tr>
<tr>
<td>Fortino, C. A.</td>
<td>XVII-29</td>
</tr>
<tr>
<td>Fuji, K.</td>
<td>VIII-425</td>
</tr>
<tr>
<td>Fujikawa K.</td>
<td>XVII-65</td>
</tr>
<tr>
<td>Fujishima, H.</td>
<td>XV-157, XVI-197</td>
</tr>
<tr>
<td>Fujita, T.</td>
<td>XV-239</td>
</tr>
<tr>
<td>Fukuda, H.</td>
<td>XV-190</td>
</tr>
<tr>
<td>Fukuda, Y.</td>
<td>VIII-337, IX-106, IX-119</td>
</tr>
<tr>
<td>Furtado, J. I.</td>
<td>VII-243</td>
</tr>
<tr>
<td>Furuhata, T.</td>
<td>XI-62</td>
</tr>
<tr>
<td>Furukawa, T.</td>
<td>XV-179</td>
</tr>
<tr>
<td>Furuya, H.</td>
<td>XVII-25</td>
</tr>
<tr>
<td>Furuya, K.</td>
<td>VIII-159, X-187</td>
</tr>
<tr>
<td>G</td>
<td></td>
</tr>
<tr>
<td>Galakhov, V. J.</td>
<td>IV-465</td>
</tr>
<tr>
<td>Galton, G.</td>
<td>IV-122</td>
</tr>
<tr>
<td>Galvez, E. R.</td>
<td>XVII-69</td>
</tr>
<tr>
<td>Gamboa, E.</td>
<td>XVII-133</td>
</tr>
<tr>
<td>Garcia, F. C.</td>
<td>IV-506, V-153, VI-164</td>
</tr>
<tr>
<td>Gargett, C.</td>
<td>XIV-263</td>
</tr>
<tr>
<td>Ghani, Z.</td>
<td>VI-78, VII-87</td>
</tr>
<tr>
<td>Glass, B.</td>
<td>II-56, II-335</td>
</tr>
<tr>
<td>Glassman, B.-G.</td>
<td>IV-178</td>
</tr>
<tr>
<td>Goldwin, A.</td>
<td>III-122</td>
</tr>
<tr>
<td>Goodall, M. H.</td>
<td>XIV-253</td>
</tr>
<tr>
<td>Gottlieb, S.</td>
<td>IV-552</td>
</tr>
<tr>
<td>Goyal, K. C.</td>
<td>III-111</td>
</tr>
<tr>
<td>Gregorio, L. C.</td>
<td>X-58, XVI-87</td>
</tr>
<tr>
<td>Grime, H. L.</td>
<td>XI-15</td>
</tr>
<tr>
<td>Grobman, A. B.</td>
<td>I-136</td>
</tr>
<tr>
<td>Grobman, H.</td>
<td>I-24, IV-1</td>
</tr>
<tr>
<td>Gnanamuthu, E.</td>
<td>VII-255</td>
</tr>
<tr>
<td>Gunaratne, M. M.</td>
<td>I-155</td>
</tr>
<tr>
<td>Guru, G.</td>
<td>IV-573</td>
</tr>
<tr>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Hafalla, J. R.</td>
<td>XIV-47, XVI-76</td>
</tr>
<tr>
<td>Hamiddon, F.</td>
<td>VII-xi</td>
</tr>
<tr>
<td>Han, S. B.</td>
<td>XIII-226</td>
</tr>
<tr>
<td>Harlen, W.</td>
<td>IV-73, IV-107, IV-147</td>
</tr>
<tr>
<td>Harman, A.</td>
<td>IV-592</td>
</tr>
<tr>
<td>Harris, H.</td>
<td>I-199, II-121</td>
</tr>
<tr>
<td>Hasumi, O.</td>
<td>XV-4</td>
</tr>
<tr>
<td>Hatakeyama, T.</td>
<td>IX-118</td>
</tr>
<tr>
<td>Haynes, L. J.</td>
<td>IV-399, IV-409</td>
</tr>
<tr>
<td>Heang, K. B.</td>
<td>VII-313</td>
</tr>
<tr>
<td>Hernandez, D. F.</td>
<td>I-117, III-64, IV-563, VI-38, VII-5, IX-49, XVII-147</td>
</tr>
<tr>
<td>Herrera, A. A.</td>
<td>XVII-124, XVII-133,</td>
</tr>
<tr>
<td>Herr, A.</td>
<td>XII-37, XIII-209, XVI-118</td>
</tr>
<tr>
<td>Hirata, T.</td>
<td>XV-258</td>
</tr>
<tr>
<td>Hili, C.</td>
<td>XV-264</td>
</tr>
<tr>
<td>Hiroki, M.</td>
<td>V-1173, XV-149</td>
</tr>
<tr>
<td>Hisatake</td>
<td>II-333</td>
</tr>
<tr>
<td>Homma, S.</td>
<td>VIII-397</td>
</tr>
<tr>
<td>Hong, L. C.</td>
<td>V-51</td>
</tr>
<tr>
<td>Hoole, G. J.</td>
<td>III-25</td>
</tr>
<tr>
<td>Horiuichi, S.</td>
<td>X-24</td>
</tr>
<tr>
<td>Hormchong, T.</td>
<td>V-237, VI-211, VIII-53, XV-5, XVI-6</td>
</tr>
<tr>
<td>Hosokawa, S.</td>
<td>XV-179</td>
</tr>
<tr>
<td>Hunwald, A.</td>
<td>II-69</td>
</tr>
<tr>
<td>I</td>
<td></td>
</tr>
<tr>
<td>Igari, T.</td>
<td>XV-203</td>
</tr>
<tr>
<td>Ikeda, H.</td>
<td>XV-98, XVII-65, XVII-92, XVII-107</td>
</tr>
<tr>
<td>Imahori, K.</td>
<td>VI-113, II-27, VIII-433, IX-77, XV-2, XVII-12</td>
</tr>
<tr>
<td>Imai, M.</td>
<td>XV-211</td>
</tr>
<tr>
<td>Inbar, S.</td>
<td>IV-326</td>
</tr>
<tr>
<td>Isarankura, K.</td>
<td>X-207</td>
</tr>
<tr>
<td>Itoh, R.</td>
<td>II-337</td>
</tr>
<tr>
<td>Iwase, T.</td>
<td>V-64</td>
</tr>
<tr>
<td>J</td>
<td></td>
</tr>
<tr>
<td>Jacinto, S.</td>
<td>XVII-133</td>
</tr>
<tr>
<td>Jafaruddin, T. H. S.</td>
<td>VII-xiii</td>
</tr>
<tr>
<td>Jain, S. C.</td>
<td>VI-124, XI-219</td>
</tr>
<tr>
<td>James, S. L.</td>
<td>IV-388</td>
</tr>
<tr>
<td>Jinno, N.</td>
<td>XV-239</td>
</tr>
<tr>
<td>Joaquin, C. C.</td>
<td>XV-44, XVII-71</td>
</tr>
<tr>
<td>Joaquin, J. C.</td>
<td>XIV-54</td>
</tr>
<tr>
<td>Johnson, A.</td>
<td>V-31</td>
</tr>
<tr>
<td>Johri, B. M.</td>
<td>I-164, II-1, IV-470, V-207</td>
</tr>
<tr>
<td>Jones, M.</td>
<td>IV-122</td>
</tr>
</tbody>
</table>
Archives: Proceedings of Past Biennial Conferences of the AABE

K

Ka, H. XV-157
Kaffe, S. K. XVI-102
Kaga, T. XV-197, XVII-158
Kahn, S. M. H. III-59
Kai, Y. C. II-186
Kaji, A. XIV-135
Kanagasabai, S. VII-33, VIII-95
Kanaizuka, Y. XIV-243, XV-225, XVI-92, XVII-72, XVII-73
Kanapi, C. G. XIV-86, XVII-ii
Kang, H.-K. XV-35, XV-162
Kapili, P. H. V-133
Kattmann, U. VI-47, VI-60, VII-277
Kawahara, H. IX-169, XV-265
Kawasaki, T. XVII-79
Kawashima, N. XVII-79
Kelly, P. J. I-65, IV-35, VI-18, VIII-1, VIII-435
Kennedy, M. H. VIII-215
Kerdriengkai, S. XVI-202
Kille, R. A. VIII-239
Kim, C. M. I-202, III-53
Kim, Y. S. XIII-398, XIII-450
Kimura, I. IX-24, X-219, XI-111
Kobayashi, K. VIII-337, IX-106, IX-119
Kobayashi, T. XVII-63
Kobayasi, H. VIII-345, XIV-63, XV-184
Koh, T. P. I-106, I-288
Koutnik, P. G. IV-155
Kramer, L. IV-314
Krishnamra, T. VI-207
Kurashima, A. XV-258
Kuthebutheen, A. J. VI-171
Kwan, L. P. V-195, V-288
Lee, C. H. V-13
Lee, J. E. XV-170
Lee Liu, H. C. III-42
Leong, T. Y. V-74, VIII-323
Lev, C. IV-376
Lev, H. III-122
Levey, A. IV-326
Levin, R. IV-326
Lewis, M. IV-295
Ley, A. IV-51
Libaee, Y. IV-329
Lindsay, A. XIV-91
Lintongan, P. B. XVII-181
M

Madan, D. R. I-58, I-121
Madrazo, G. XVII-7
Madulid, R. XV-253, XVII-113
Maekawa, S. XVI-141
Makita, N. XV-179
Manuel, J. L. III-218
Marandawala, P. III-1
Marinopoulos, J. XIV-91
Marsh, A. R. VII-57
Mayer, M. IV-240
Mayer, W. V. II-15, II-75, IV-118, IV-415
Medina, G. F. III-220
Meyer, R. G. VIII-199
Mino, S. XIII-310
Mishra, A. K. VI-72
Misonou, T. XV-216
Mitchell, J. L. S. J. II-137, II-149
Miyake, A. II-335
Mohta, R. K. XI-168, XIII-353
Moraes, A. T. II-335
Morikawa, H. II-87, II-99
Morimasa, S. VIII-55
Morita, T. VIII-425
Moss, G. D. IV-412
Murasugi, S. XV-190
N

Nah, C. K. V-195
Nakajima, Y. II-132, III-165, III-207, IV-531, V-104, VI-154, VIII-289
Nakamichi, T. XVI-134, XVII-168
Nakayama, K. I-206, II-340, VIII-373, VIII-441
Nalliah C. V-161
Namue, C. XVI-172

Asian Journal of Biology Education Vol. 10 37
Sood, J. K. VII-71, VIII-277
Soriano, L. B. I-v, I-333, II-337, III-224, IV-589
Soydhurum, P. VI-196, X-11, X-47
Srisangngam, S. XVI-202
Srividool, R. XVI-216
Stannard, F. IV-401
Strauss, G. III-122
Starobinetz C. IV-326
Stokes, D. M. VII-79, XIV-91
Subbarini, M. S. VIII-255
Sudzuki, M. XI-251, XV-111
Sugiyama, Y. XV-179
Sukchotratana, M. XI-243, XIII-13, XIII-100, XV-264, XVI-1, XVI-157
Surh, K. H. XIII-433
Suselo, T. XVII-16
Suvattananacupt, S. XVI-157
Swami, P. III-111
T
Tabbada, R. A. XVII-97
Tagliano, T. XIII-304
Takahashi, K. XV-11
Takaoki, T. XII-85, XIII-345, XV-231
Takasugi, S. II-24
Takeuchi, K. XV-143
Tamanoi, I. VIII-337, IX-106, IX-119, X-34
Tan, J. V-57
Tanaka, R. VIII-155
Tandon, S. L. II-1
Taniguchi, H. VIII-179
Tara, M. VIII-295, XV-80, Xviii-81, XVII-102
Tarmir, P. IV-178, IV-205, IV-240, IV-335, V-119, VI-131, VIII-81, VIII-229
Tate, T. VIII-251, IX-164, X-161, XI-178, XIII-244, XIV-189
Teetranont, C. XVI-2
Tel-Or, E. XVII-123
Thapa, R. XVI-147
Theodor, E. IV-332
Thitasut, P. X-7
Tieng, M. T. N. III-107
Tilling, S. M. XVI-10
Tokunaga, Y. X-187
Treloar, A. XIV-91
Treyes, R. XVII-92, XVII-107
Tribe, A. A. V-90
Tsuneki, K. A. XVII-25
U
Ubukata, H. XV-91
Ueda, H. VIII-125
Ueyama, S. XIV-63, XV-184
Umaly, R. C. XIII-135, XIV-4
Umeki, S. XV-143
Umeno, K. XI-185, XIII-56, XV-103
Ungson, L. B. XVII-213
V
Vangsayanya, C. VI-209
Vergara, B. S. III-64, III-82
Villavicencio, R. R. VI-89, VIII-26
Vohra, F. C. II-155, VI-1, VII-xviii, VIII-63, VIII-405, XI-192
W
Wallis, A. M. XIV-263
Watanabe, S. XVII-107, XVII-214
Wayan Seregeg, G. VII-137
Weerasinghe, A. III-20, IV-509, V-217, V-285
Weiss, M. IV-459
Wescott, G. XIV-91
Wilasdachanont, W. X-207
Wolseley, P. XVI-31
Wong, R. H. K. V-210
Wongsawad, C. XVI-157, XVI-172
Wongsawad, P. XVI-157
Wongsiri, S. XVI-147
X
XBI Team Xavier XVII-64
Y
Yamada, T. VIII-135
Yamagiwa, T. VIII-75, VIII-135
Yamazaki, S. X-90
Yang, J. H. I-285, II-117
Yokohama, Y. VIII-159, X-187, XII-52
Yonezawa, Y. VIII-155, X-117
Yoon, I. B. XIII-22
Yoong, C. S. I-254, V-164, VI-26, VII-xxiii, VIII-189
Yoshida, O. VIII-337, IX-106, IX-119
Yoshida, T. II-50
Yoshiki, M. VIII-437
Young, Y. M. XIX-72
Yu, W. I. XIII-375
Z
Zamora, P. M. XIII-263
Zamora, R. I. II-181, IV-522, VI-84
Zuzovsky, R. IV-253
Publications

*Biology Education for Social and Sustainable Development* (ISBN: 978-94-6091-925-1) was published in 2012 by Sense Publishers, Rotterdam, Netherlands (http://www.sensepublishers.com/). Some papers presented at the 23rd Biennial Conference of the AABE which was held in Singapore in October 2010 were compiled in this book by Dr. Mijung Kim and Dr. C. H. Diong. You can refer to the abstracts of these papers in the sixth volume of the *Asian Journal of Biology Education* (2012).

*Biology Education and Research in a Changing Planet (2015)* (ISBN 978-981-287-523-5) was published by Springer (http://www.springer.com/in/book/9789812875235). Some papers presented at the 25th Biennial Conference of the AABE which was held in Malaysia in October 2014 were compiled in this book by Dr. Esther Gnanamalar Sarojini A Daniel. The abstracts of these papers were included in the eighth volume of the *Asian Journal of Biology Education* (2015).

From the Editor-in-Chief

The tenth volume of *Asian Journal of Biology Education* (AJBE) contains one practical report and one country report. It also contains the contents of past conference proceedings (Vol. 1 – 17). Although this volume is a little thinner than the previous ones of AJBE, I have decided to publish it because one article included was accepted for publication more than a year ago, in March last year.

The next issue will be published possibly by the end of April, next year. It will include a report on the 27th Biennial Conference of AABE which will be held at Emerald Hotel Bangkok, Thailand, from the 30th of November to the 2nd of December this year, and the abstracts of papers presented at the conference, as well as some contributed articles.

One of the roles of AJBE is to report the latest biennial conference of AABE. However, the core object of AJBE is to publish reports on biology education research and practices. So, the publication of AJBE is mainly dependent on the number of contributed articles. Therefore, I would like to ask the readers of this journal, AABE members or non-members, to submit their articles (research papers, practical reports, reports on biological resources, etc.) to AJBE.

The articles contributed to AJBE during the last two years have been reviewed by Professor Kim Kyoungho (Gongju National University of Education, South Korea), Professor Morakot Sukchotiratana (Chiang Mai University, Thailand), and Dr. Robert Wallis (Federation University, Australia), as well as the Editorial Board members. I am very thankful to them for their efforts to review the articles.

Dr. Nobuyasu Katayama