BIOME
The BIOlogy Education MEssenger
(An ATBS eNewsletter)

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From The Editorial Team.....

The editorial team of Biome – the ATBS eNewsletter is back again with the fourth issue!! As the month of September approaches, we push ourselves hard to put together some useful material that you teachers might find interesting to read. Apart from the regular textbooks and reference books related to the curriculum that each of you teach, we hope these few pages of Biome would not only put a smile on your faces as you read it, but also that it gives you some anecdotes and material that you can share with your students as you teach them. We also hope that a few of the things you read here might set you thinking on certain issues that you can relate to.

This issue includes a concept based question that appeared in the theoretical exam of the Biology Olympiad Programme and the analysis of how students who took the exam answered it. Such an analysis could help teachers identify the parts of a certain concept that students misinterpret or do not understand clearly.

With this issue we also begin a series of articles on interesting incidences in the history of Biology. The first such article 'Shocking Extinction Stories: The Legend of Martha' written by Prof. B. B. Nath is included in this issue. We also have snippets of the performance of Team India at the International Biology Olympiad (IBO) held in Hanoi, Vietnam in July, 2016. A lab activity from the Biology Olympiad Cell at HBCSE is also included to help you organize some lab experience for your students.

Hope you enjoy reading all that we have put together in this issue! Glance through this issue of Biome while you celebrate being a teacher!!

With lots of good wishes for a happy teachers month!!

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Respiration in animals: Analysis of student responses to an Olympiad question

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The Indian National Biology Olympiad (INBO) is held every year in the last week of January. This is the second stage examination in the various stages of selection leading to the selection of the Indian team to represent India at the International Biology Olympiad (IBO). The IBO is held in July in a different host country every year.
The INBO 2016 was held on 31st January, 2016 in about 18 centres across India. About 300 students selected from the first stage exam, the National Standard Examination in Biology (NSEB) appeared for this exam.

INBO exam is a theoretical examination and includes questions of multiple choice, true or false, arrange in sequence, etc types. Thus the students are not required to write long answers in this exam. However, the attempt is to include questions which test students' conceptual understanding as well as their analytical skills instead of testing rote information.

One of the questions from the INBO 2016 paper is discussed here. Analysis of the responses of the students gives an insight to how students perceive this concept.

Question:

The slow rate of oxygen diffusion in water limits the efficiency of oxygen distribution from gas exchange surface to sites of cellular respiration. This is true for:

a. only aquatic animals.

b. only air-breathing animals.

c. only those animals in which no circulatory system has developed.

d. all animals.

The BIOLOGY OLYMPIAD PROGRAMME:

Stage 1: NSEB (National Standard Examination in Biology)

Stage 2: INBO (Indian National Biology Olympiad)

Stage 3: OCSC (Orientation cum Selection Camp)

Indian team of 4 students selected at the end of the OCSC!

Team undergoes training at HBCSE, Mumbai prior to departure!

Discussion about the question:

The process of respiration in animals involves gas exchange and cellular respiration. The gas exchange involves diffusion of gases across different types of surfaces such as skin, alveoli, gills or simply a plasma membrane. The process of diffusion, in turn, is driven mainly by concentration gradient and the solubility of a gas in water. As a result, oxygen and CO_2 must dissolve in the water molecules present on the moist surface of the cells for exchange to occur. It is important to note that the solubility of Oxygen in water is 26 times less as compared to CO_2 at 25^0C. Thus this is one of the most important factors that limits the rate of O_2 diffusion across respiratory surfaces.

The question deals with an important concept that the fundamental process of exchange of gases in all animals is the same, be it air breathers or water breathers, namely, dissolution of gases in water.

Absence of circulatory system can pose difficulty in transport of oxygen from the site of dissolution to the site where it would be required for cellular respiration. Truly, such life forms are much simpler in organisation owing to the latter problem.

Analysis of student responses:

Among the top 300 students selected for the INBO examination, the question was correctly answered by 21%of students only. 40% of the students considered that the limitation exists only for animals which possess no circulatory system. 11%of the students thought that it

only poses a problem for aquatic animals while 7% considered that it limits the rate of respiration in only air-breathers.

--------- Prof. Rekha Vartak

TEAM INDIA – IBO 2016

From Left to right: Dr. Dharmendra Shah, Prof. Rekha Vartak, Spandan Roy (student) , Lajjaben Patel (student), Vidushi Varshney (student), Tanish Modi (student), Ms. Anupama Ronad, Mr. Vikrant Ghanekar

INTERNATIONAL BIOLOGY OLYMPIAD (IBO) 2016 AT A GLANCE!

- Venue: Hanoi, Vietnam
- Dates: 17th – 24th July, 2016
- No. of participating countries: 68
- No. of student participants: 253
- Medal tally of Team India:

  1 Gold & 3 silver medals!!

SHOCKING EXTINCTION STORIES: THE LEGEND OF MARTHA

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Prof. Bimalendu B. Nath is the former Head and a Professor at the Department of Zoology, Savitribai Phule Pune University. He is a passionate teacher and is actively involved in teaching as well as research for over three decades.

Believe it or not, our biosphere is currently in the midst of its sixth mass extinction of flora and fauna as estimated by many scientists. The situation can be more critical than the complete disappearance of Dinosaurs 65 million years ago. The present crisis is being mostly caused by humans. The International Union for Conservation of Nature (IUCN) has identified more than 15000 species which are categorized as 'threatened' with a risk of future extinction.

It is doubtful whether we can reverse the process of extinction due to natural causes, but we can certainly reverse the human activities which tend to reduce the loss of flora and fauna and we can certainly increase awareness

Examples of a few birds and animals that have reportedly become extinct:

- **Dodo (1662)**
- **Great Auk (1844)**
- **Tasmanian Tiger (1936)**
- **Steller's Sea Cow (1768)**
- **Pyrenean Ibex (2000)**
- **West African Black Rhinoceros (2011)**
- **Dutch Alcon Blue Butterfly (1979)**

amongst students whom we teach biological diversity in the classroom. I feel motivated to take this opportunity to share a few shocking stories of extinction of animals in recent times through a series of articles in BIOME. I would like to begin the series with one of the most shocking stories of the 20th century: the extinction of the passenger pigeon.

The passenger pigeon or the wild pigeon (*Ectopistes migratorius*; synonym of *Columbia migratoria* Linnaeus) was once an abundant bird endemic to North America which became extinct in less than 100 years. Although there was an estimated population of a few billion birds in the early 1800s, none were surviving by 1900. On September 1, 1914, the last survivor, named 'Martha' was found dead on the floor of the cage in the Cincinnati Zoo, U.S.A. Two years ago, Sept.1, 2014, was marked as the 100th anniversary of the passenger pigeon's extinction. The population of these birds reduced from billions to zero due to the senseless hunting and human apathy. Let me highlight a few salient features of this extinct wild pigeon which used to fly in flocks in millions but has now completely disappeared from this earth due to reckless massacre and indiscriminate slaughter. The extinction of the passenger pigeon reminds us of the story of Dodo, the flightless bird of Mauritius, which was also driven to extinction by reckless hunters.

The passenger pigeon belonged to the dove and pigeon family. The common name as well as the taxonomic name reflected the migratory habits of this species. Although it had adapted to the deciduous forests and to the woodlands of North America, these wild pigeons used to migrate elsewhere as well. They were habituated to migrate in huge flocks from one place to the other in search of food, shelter and breeding grounds, possibly to avoid intra-specific competitions whenever the population size increased beyond a threshold limit. Passenger pigeons did not have any fixed migratory patterns although it was usual for many other birds exhibiting annual migration to specific breeding place. The sky remained blackened for

Examples of a few birds and animals that have reportedly become extinct:

- **Round Island Burrowing Boa (1975)**
- **Javan Tiger (mid-1970s)**
- **Tecopa pupfish (1979)**
- **Golden Toad (1989)**

hours whenever the huge flock of passenger pigeons flew over a terrain. Archival records showed that in Southern Ontario, one flock of migratory passenger pigeons took 14 hours to pass a stretch of 500 kilometers and the birds covered a width of 1.5 kms. In many travelogues, explorers documented the migration of a vast number of pigeons darkening the sky. Indeed it sounds like an unbelievable fairy tale yet several monographs and diaries hold testimonies of such events by whoever witnessed the magnitude of migratory patterns of passenger pigeons in the bygone centuries.

The passenger pigeon was a part of religious rituals among a few North American tribes (e.g. the Huron tribes), who used to sacrifice these pigeons and eat the flesh during the 'feast of Dead' with a belief that the souls of ancestors had changed into passenger pigeons. The tribes of Ho-Chunk group respected the passenger pigeons as a special bird only worth for the king and these birds were served as special food only during the feast for the tribal chief. On the other hand, the Seneca tribal people never hunted these birds and used to express their respect to the wild pigeon by a special 'pigeon dance'. In many tribes of North America, killing of wild passenger pigeons was treated as a crime. However, the scenario changed dramatically with the arrival of Europeans on the American continent and these birds were killed by the early European settlers equipped with firearms whenever they spotted flocks of passenger pigeons. Over the years, pigeon food turned out to be a delicacy and the demands for passenger pigeon on the menu of hotels and restaurants kept increasing day by day. Consequently, the wild passenger pigeons which used to fly in millions got completely vanished from the face of the earth.

A lot of research went into investigating the compelling reasons leading to the total extinction of passenger pigeons. Hunting was limited before Europeans started settling and many tribes considered disturbing migratory pigeons as a crime. Before European colonization,

Activities conducted by ATBS during 2015 - 2016!!

- **10th Annual Conference on Wetlands for our Future: Sustainable Livelihoods' held on 31st Jan., 1st and 2nd February, 2016 in collaboration with the Mangroves Society of India, B. N. Bandodkar College and SACON.**

the Native Americans would hunt and eat pigeons only on special occasions. In post-colonization days, there had been two major events that triggered the decline of passenger pigeons. Firstly, the European immigrants started clearing the vast forest areas to meet the increasing demands for agriculture and expansion of towns, lumber and fuel. Approximately, 180 millions of acres of forests were cleared between 1850 and 1910. This large-scale deforestation deprived the wild pigeons of their nesting and breeding sites. These birds were fond of acorns produced by white oak trees, which once dominated the forests of eastern North America. Mass-scale deforestation also resulted in a sharp decline of white oak trees which in turn led to the loss of foraging areas of wild pigeons. Secondly, passenger pigeons became a popular food for European settlers and the flavour of the flesh of juvenile pigeons tasted the best. The mass-scale decline of passenger pigeons began when professional hunters started netting and shooting the birds to sell them in the city markets.

After the civil war in America, there had been rapid expansion of rail and telegraphic communication links across the nation connecting towns and cities. Whenever migratory flocks of pigeons were sighted, the hunters were contacted by telegrams and the hunters used to reach nearby spots by rail and road. The pigeons were not only shot but were also trapped with nets. The hunters regularly burnt their roosters and suffocated the birds with burning sulphur. Pigeons were intoxicated by baiting them with whisky-soaked corn. The amateurs used to learn the use of guns through test killing of passenger pigeons. By 1850, thousands of personnel were hired and employed in the passenger pigeon industry. In 1855, around 18000 pigeons used to be processed per day in the city of New York. It has been estimated that in a single year, a billion passenger pigeons were harvested in Michigan alone. In many shooting competitions, hundreds of pigeons used to be kept as "living targets" for such sports activity. A bill

towards banning the netting of pigeons was passed in Michigan in the 1870s. Similar conservational efforts were brought in the form of legislations in other places like Ohio, Pennsylvania and so on. Unfortunately, it was too late to rescue the passenger pigeons from extinction. The greedy hunters and those in the hotel industry continued to kill these pigeons secretly.

In the 1880s, there were known to be around 1200 pigeon trappers across the United States. Market rates rose and fell depending on the availability and supply of pigeon flesh. Thousands of pigeons were trapped and kept in captive conditions and many birds died due to lack of food and water. By the end of the 1890s, the passenger pigeons had been a near-extinction species. The commercial organizations which used to thrive on selling passenger pigeons as one of the most demanding items on their menu started dwindling. The strong conservation efforts, legislations, imposition of penalty proved to be futile because it was too late to save passenger pigeons which had suffered a century-long massacre. This will remain as one of the black pages in the history of animal welfare. The last documented case of hunting was authenticated near a place called Pike County at Ohio when a female passenger pigeon was shot by a boy named Press Clay Southworth on March 22, 1900 by his air-gun. It was however later on recovered by a taxidermist who finally preserved the stuffed skinned model of passenger pigeon at Ohio historical society.

Amongst various organizations in the United States, the Cincinnati Zoo had initiated conserving passenger pigeons mostly around 1875. An initial population of around two dozen passenger pigeons was kept in cages with utmost care and attention. A female passenger pigeon lived in the Zoo for 25 years, later nick-named as 'Martha'. Martha's last cage-mate 'George' died on July 10, 1910. Some sources, made us believe that these last surviving couple of passenger pigeons were named as 'George' and 'Martha' honouring the then President of United States, George

Activities conducted by ATBS during 2015 - 2016!!

- **A Resource Generation Camp (RGC) for the Stage I exam of the Biology Olympiad was held on 28th , 29th and 30th April, 2016 at Homi Bhabha Centre for Science Education (HBCSE), Mumbai.**

Washington and his wife Martha. Finally, Martha died on September 1, 1914 probably due to old age and she was found motionless at around 1pm in her solitary cage where George lost his life four years earlier. Martha's body was preserved at the Smithsonian museum in Washington. A memorial statue of Martha can be found inside the Cincinnati Zoo in front of 'Passenger Pigeon Memorial Hut', where Martha and George spent their last phase of life together. A hundred years later, Martha is a constant reminder of a sad tale of human-induced extinction, simply caused by senseless massacre bringing one of the most abundant birds of this earth from 'billions to none'.

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Activities conducted by ATBS during 2015 - 2016!!

- **A State level essay competition for students of Class VIII – XII is being held in September-October, 2016.**

In the Bio Lab……..

Extraction of nucleic acids – genomic DNA, extrachromosomal DNA and RNA has become an integral part of any lab using molecular biology techniques. Here, we present a simple protocol for the extraction and visulaisation of DNA from cells of the spinach leaf. This protocol can easily be followed in any junior/undergraduate college lab. Although this protocol makes use of a plant source, simple modifications to the given protocol can be made depending on the source being used.

Materials Used:

1. Spinach leaves
2. Salt
3. Liquid detergent
4. Guanidine isothiocyanate
5. Ethanol
6. Tris
7. Glacial acetic acid
8. EDTA
9. Agarose
10. Ethidium bromide
11. Gel loading dye
10. Ice

EXTRACTION AND VISUALISATION OF NUCLEIC ACIDS FROM A PLANT SOURCE

Extraction of nucleic acids from spinach leaves and visualization of the extracted nucleic acids is described here. Before proceeding to the actual extraction protocol one requires to prepare TAE buffer.

Preparation of TAE Buffer

TAE (Tris-acetate-EDTA) buffer, pH 8.0 is the buffer that is used during agarose gel electrophoresis of nucleic acids. Prepare the buffer as follows:

Step 1: Preparation of 0.5M EDTA solution
Take required quantity of EDTA to get 0.5M in 100 ml solution. Initially add 50ml distilled water to this. Adjust the pH of this solution to 8.0 very carefully using 10N NaOH. After the pH is adjusted, make up the volume to 100ml.

Step 2: Preparation of TAE buffer
4.84 gm Tris base + 1.14 ml glacial acetic acid + 2ml of 0.5M EDTA (pH8.0)

Apparatus required:

- Mortar and Pestle
- Test tubes
- Pipettes
- Filter paper
- Beakers
- Funnel
- Droppers
- Eppendorf vials
- Gloves

Add Tris base to 900 ml distilled water. Add glacial acetic acid and EDTA to the solution and mix. Make the total volume to 1 litre. Check the pH.

PART 1: Extraction of Nucleic acids

1. Dissolve 0.6 gm NaCl in 18 ml distilled water. To this add 2 ml 50% solution of detergent.
2. Take 2 leaves of spinach. Wash well and cut to pieces in a mortar and pestle. Add 10 ml of above solution and macerate well without much frothing.
3. Transfer this extract into a test tube and heat at 60^0C in a water bath for exactly 15 mins.
4. Cool the test tube immediately in ice for 5 min.
5. Filter the solution through filter paper.
6. To the filtrate, add 3 drops of Guanidine isothiocyanate solution (GI) -instead of proteinase using a plastic dropper. Keep the tube at room temperature for 5 mins.
7. Add 3 ml chilled ethanol gently from the sides of the tube. Keep the tube immersed in ice for 5 min.
8. Nucleic acid strands will be visible at the interface.
9. With the help of a micropipette and cut tips, transfer the white strands into a vial. Centrifuge the vial at 2000 rpm for 5 mins.
10. Discard the supernatant and add 70% ethanol to the pellet to wash the pellet. Centrifuge the vial at 2000 rpm for 5 mins.
11. Repeat the ethanol wash twice.
12. Allow the pellet to dry for a few minutes and add 50µl TAE buffer to dissolve the pellet.
13. Refrigerate at 4^0C till further use.

Instruments required:

- Hot water bath
- Hot plate
- Centrifuge
- Agarose gel electrophoresis apparatus
- Power supply
- UV Transilluminator

PART 2: Visualisation of Nucleic acids

Agarose gel electrophoresis is a technique in which the molecules separate on the basis of charge as well as molecular weights. The following procedure is used to prepare agarose gel and to run the nucleic acid sample in it.

1. Prepare the sample for loading in the agarose gel: 15 µl of refrigerated nucleic acid sample + 5 µl of loading dye

   The loading dye contains glycerol which helps in increasing the density of the sample so that the sample settles at the bottom of the in the well. It also contains bromophenol blue and xylene cyanol which help track the movement of the DNA while the separation is in progress.

2. Load the sample in 0.8% agarose gel. Prepare the gel by dissolving agarose in the TAE buffer and set it in a UV transparent tray. Add ethidium bromide to the slightly cooled agarose solution to get a final concentration of 1 microgram/ml.

   Note: Ethidium bromide is a carcinogenic chemical. Hence, use hand gloves while adding the chemical as well as during handling the gel in all further steps.

3. Allow the separation to occur for about half an hour at 70mV.
4. Remove the tray and view the gel under UV light using a UV transilluminator.

---- By the Biology Olympiad Cell, HBCSE, TIFR

Recreation Corner .....

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