DEPARTMENT OF ZOOLOGY HONOURS

LIST OF STUDENTS: 35

1. ANUSHKA GHOSH
2. DIYA PATRA
3. JASMIN SULTANA
4. JAYESH GHOSH
5. MD USMAN GANI
6. MOUPRIYA GHOSH
7. MOUPRIYA MANNA
8. MRINMOYE PAL
9. PRIYA MONDAL
10. PRIYANKA MONDAL
11. SATASRI MONDAL
12. SK SAJID
13. SK WAYASEFUL ISLAM
14. SOUMYADIP SAHA
15. SOYFUDDIN SK
16. SURYA GARAI
17. SWAPRAVA BARI
18. TANISHA NASRIN
19. TANUSHREE CHANDRA
20. ANKIT MUKHERJEE
21. ARUNIMA GHOSH
22. AYAN BAIRAGYA
23. MUNMUN GHOSH
24. PRADIPTA KUMAR GARAI
25. RAJESH GHOSH
26. SNAHASIS MONDAL
27. SOUVIK NAYEK
28. SUBHOJYOTI GHOSH
29. TITHI BASU
30. BARUN HAZRA
31. SEHELI PARVEEN
32. MD RIYAJUDDIN
33. RAJRISHI MONDAL
34. SWAGATO MONDAL
35. CHANDRADEEP DAS

TITLE OF THE PROJECT:

1. Aquarium maintenance
2. Duck Breeding
3. Report on Poultry farm
4. Glimpses of India's Wildlife in context of World
5. Project report on Animal cell culture
6. "Whole Mounting & Identification of specific stages of Chick Embryos"
7. Larval Forms of Echinoderms
8. Project report on *Drosophila* culture

DURATION WITH DATE: **(1 – 3) 16.11.2022 to 17.11.2022**

4. 09.02.2023 (11 AM to 4 PM)

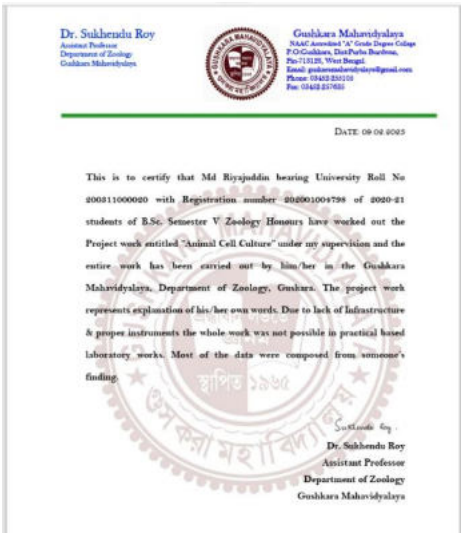
5. December 2022 to February 2023

6. February 2023 to June 2023

7. 25.06.2023

8. 28.06.2022

PROJECT WORK COMPLETION CERTIFICATE



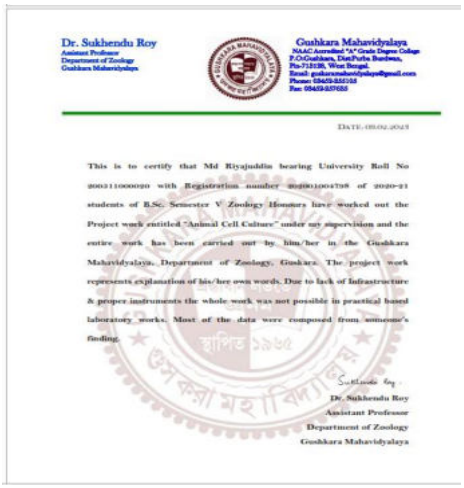
Dr. Sukhendu Roy
Assistant Professor
Department of Zoology
Gushkara Mahavidyalaya

Gushkara Mahavidyalaya

DATE: 09.02.2023

This is to certify that Md Riyajuddin bearing University Roll No 200311000020 with Registration number 202001004798 of 2020-21 students of B.Sc. Semester V Zoology Honours have worked out the Project work entitled "Animal Cell Culture" under my supervision and the entire work has been carried out by him/her in the Gushkara Mahavidyalaya, Department of Zoology, Guskara. The project work represents explanation of his/her own words. Due to lack of Infrastructure & proper instruments the whole work was not possible in practical based laboratory works. Most of the data were composed from someone's finding.

Dr. Sukhendu Roy
Assistant Professor
Department of Zoology
Gushkara Mahavidyalaya



Dr. Sukhendu Roy
Assistant Professor
Department of Zoology
Gushkara Mahavidyalaya

Gushkara Mahavidyalaya

DATE: 09.02.2023

This is to certify that Md Riyajuddin bearing University Roll No 200311000020 with Registration number 202001004798 of 2020-21 students of B.Sc. Semester V Zoology Honours have worked out the Project work entitled "Animal Cell Culture" under my supervision and the entire work has been carried out by him/her in the Gushkara Mahavidyalaya, Department of Zoology, Guskara. The project work represents explanation of his/her own words. Due to lack of Infrastructure & proper instruments the whole work was not possible in practical based laboratory works. Most of the data were composed from someone's finding.

Dr. Sukhendu Roy
Assistant Professor
Department of Zoology
Gushkara Mahavidyalaya

Dr. Saurabh Sarkar
Assistant Professor
Department of Zoology
Gushkara Mahavidyalaya

Gushkara Mahavidyalaya

DATE: 25.06.2023

Certificate

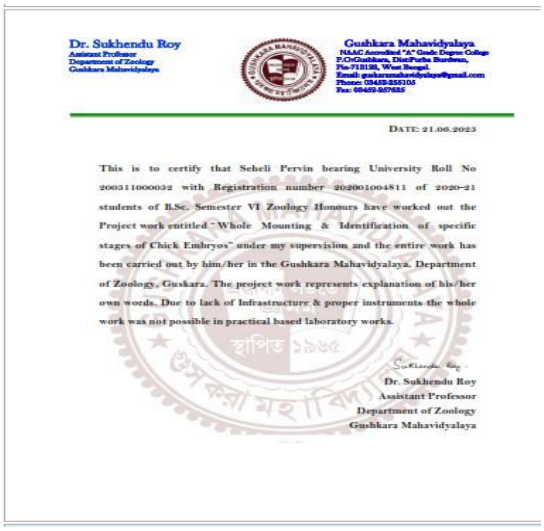
This is to certify that, the following students of B.Sc. Semester II Zoology Honours have worked out the Project work entitled "*Larval forms of Echinodermata*" under my supervision and the entire work has been carried out by him/her in the Gushkara Mahavidyalaya, Department of Zoology, Guskara. The project work represents an explanation of his/ her own words. Most of the data were composed from someone's findings with reference.

Name of the students:

1. ANUSHKA GHOSH
2. DIYA PATRA
3. JASMIN SULTANA
4. JAYESH GHOSH
5. MD USMAN GANI
6. MOUPRIYA GHOSH
7. MOUPRIYA MANNA
8. MRINMOYE PAL
9. PRIYA MONDAL
10. PRIYANKA MONDAL
11. SATASRI MONDAL
12. SK SAZID
13. SK WAYASEFUL ISLAM
14. SOUMYADIP SAHA
15. SOYFUDDIN SK
16. SURYA GARAI
17. SWAPRAVA BARI
18. TANISHA NASRIN
19. TANUSHREE CHANDRA

25.06.23

Dr. Saurabh Sarkar
Assistant Professor
Department of Zoology
Gushkara Mahavidyalaya



Dr. Sukhendu Roy
Assistant Professor
Department of Zoology
Gushkara Mahavidyalaya

Gushkara Mahavidyalaya

DATE: 21.06.2023

This is to certify that Seheli Pervin bearing University Roll No 200311000032 with Registration number 202001004811 of 2020-21 students of B.Sc. Semester VI Zoology Honours have worked out the Project work entitled "Whole Mounting & Identification of specific stages of Chick Embryos" under my supervision and the entire work has been carried out by him/her in the Gushkara Mahavidyalaya, Department of Zoology, Guskara. The project work represents explanation of his/her own words. Due to lack of Infrastructure & proper instruments the whole work was not possible in practical based laboratory works.

Dr. Sukhendu Roy
Assistant Professor
Department of Zoology
Gushkara Mahavidyalaya

LINK THE REPORT OF THE FIELD WORK: (PDF OF THE REPORT OF THE STUDENT)

1. **PDF OF MUNMUN GHOSH**
2. **PDF OF TANUSHREE CHANDRA**
3. **PDF OF SEHELI PARVEEN**
4. **PDF OF MD RIYAJUDDIN**
5. **GENERAL PDF FOR ALL STUDENTS**

SAMPLE PHOTOGRAPH OF THE FIELD WORK:

PERMISSION LETTER FOR FIELD WORK FROM COMPETENT AUTHORITY

GUSHKARA MAHAVIDYALAYA
P.O.: Gushkara, Dist.: Purba Bardhaman, Pin - 713128 (W.B.)
E-mail : guskaramahavidyalaya@gmail.com

Ref. No. E-5/418(b) Date 14.11.2022

To
The Assistant Project Officer
Golapbag Poultry Farm
Golapbag
Burdwan

Sub: Prayer for permission to visit the Golapbag Poultry Farm on 16.11.2022.

Madam/Sir,

I would like to draw your kind attention to that the Dept. of Zoology is going visit to Golapbag Poultry Farm, Burdwan with 5th Semester students of Zoology General Course on 16.11.2022 (Wednesday) in supervision of Dr. Saurabh Sarkar, Assistant Professor of Zoology and Mr. Uday Chand Mete, Technical Lab. Attendant. This is a part of their curriculum DSE-1 Practical (Applied Zoology; Item No. 5 & 6) and syllabus of Zoology General Course.

In this connection I hereby seek the necessary permission for the same.

Thanking you.

Yours faithfully,
Sabina Begum
Teacher-in-Charge
Gushkara Mahavidyalaya
Teacher-in-charge
Gushkara Mahavidyalaya

List of Students:

1.	Ankit Mukherjee -	5th Semester
2.	Ayan Bairagya -	Do
3.	Arunima Ghosh -	Do
4.	Munmun Ghosh -	Do
5.	Tithi Basu -	Do
6.	Souvik Nayek -	Do
7.	Snehasish Mondal -	Do
8.	Pradipta Garai -	Do
9.	Subhojit Ghosh -	Do
10.	Rajesh Ghosh -	Do

GUSHKARA MAHAVIDYALAYA
P.O.: Gushkara, Dist.: Purba Bardhaman, Pin - 713128 (W.B.)
E-mail : guskaramahavidyalaya@gmail.com

Ref. No. E-5/418(c) Date 14.11.2022

To
The District Science Officer
Science Centre (Burdwan)
Golapbag
Burdwan

Sub: Prayer for permission to visit the Science Centre on 16.11.2022.

Madam/Sir,

I would like to draw your kind attention to that the Dept. of Zoology is going visit to Science Centre, Burdwan with 5th Semester students of Zoology General Course on 16.11.2022 (Wednesday) in supervision of Dr. Saurabh Sarkar, Assistant Professor of Zoology and Mr. Uday Chand Mete, Technical Lab. Attendant. This is a part of their curriculum DSE-1 Practical (Applied Zoology; Item No. 5 & 6) and syllabus of Zoology General Course.

In this connection I hereby seek the necessary permission for the same.

Thanking you.

Yours faithfully,
Sabina Begum
Teacher-in-Charge
Gushkara Mahavidyalaya
Teacher-in-charge
Gushkara Mahavidyalaya

List of Students:

1.	Ankit Mukherjee -	5th Semester
2.	Ayan Bairagya -	Do
3.	Arunima Ghosh -	Do
4.	Munmun Ghosh -	Do
5.	Tithi Basu -	Do
6.	Souvik Nayek -	Do
7.	Snehasish Mondal -	Do
8.	Pradipta Garai -	Do
9.	Subhojit Ghosh -	Do
10.	Rajesh Ghosh -	Do

To
The TIC
Gushkara Mahavidyalaya
Gushara, Purba Bardhaman.

Sub: Permission regarding the completion of field works of Semester 1 Hons. Practical Syllabus

Respected Madam,

I would like to draw your kind attention to that the Department of Zoology is going visit to Ramsahagan Wildlife Sanctuary, Golapbag, Purba Bardhaman with 1st Semester Zoology Hons & General students on 08.09.2023 in supervision of Dr. Saurabh Sarkar, Assistant Professor of Zoology. This is a part of their curriculum CC1 & CC1A practical syllabus.

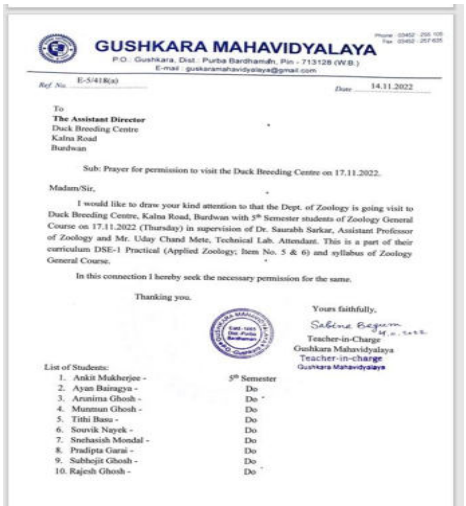
In this connection I hereby seek the necessary permission regarding the same.

Thanking you

Sincerely
Dr. Sukhendu Roy
Assistant Professor of Zoology
Gushkara Mahavidyalaya

1ST SEM

1. Anushka Ghosh
2. Dipa Patra
3. Jarnima Sultana
4. Md. Usman Gani
5. Moupriya Ghosh
6. Moupriya Manna
7. Mrinmoye Pal
8. Priya Mondal
9. Sk Waseeful Islam
10. Soumyadip Saha
11. Soyfuddin Saha
12. Tanusree Chandra
13. Priyanka Mondal
14. Sk. Sajid
15. Tanuka Nasrin
16. Surya Garai
17. Swapawa Bari
18. Ayu Ghosh
19. Sritoni Mondal
20. Subana Khatun
21. Anurag Chowdhuri
22. Raj Ghosh
23. Sk. Rajuddin



GUSHKARA MAHAVIDYALAYA
P.O.: Gushkara, Dist.: Purba Bardhaman, Pin - 713128 (W.B.)
E-mail : guskaramahavidyalaya@gmail.com

Ref. No. E-5/418(a) Date 14.11.2022

To
The Assistant Director
Duck Breeding Centre
Kalna Road
Burdwan

Sub: Prayer for permission to visit the Duck Breeding Centre on 17.11.2022.

Madam/Sir,

I would like to draw your kind attention to that the Dept. of Zoology is going visit to Duck Breeding Centre, Kalna Road, Burdwan with 5th Semester students of Zoology General Course on 17.11.2022 (Thursday) in supervision of Dr. Saurabh Sarkar, Assistant Professor of Zoology and Mr. Uday Chand Mete, Technical Lab. Attendant. This is a part of their curriculum DSE-1 Practical (Applied Zoology; Item No. 5 & 6) and syllabus of Zoology General Course.

In this connection I hereby seek the necessary permission for the same.

Thanking you.

Yours faithfully,
Sabina Begum
Teacher-in-Charge
Gushkara Mahavidyalaya
Teacher-in-charge
Gushkara Mahavidyalaya

List of Students:

1.	Ankit Mukherjee -	5th Semester
2.	Ayan Bairagya -	Do
3.	Arunima Ghosh -	Do
4.	Munmun Ghosh -	Do
5.	Tithi Basu -	Do
6.	Souvik Nayek -	Do
7.	Snehasish Mondal -	Do
8.	Pradipta Garai -	Do
9.	Subhojit Ghosh -	Do
10.	Rajesh Ghosh -	Do

PROJECT REPORT ON MAINTAINENCE OF FRESHWATER AQURIUM

Name : munmun Ghosh

University Roll : 20061101001 1 . Year : 3rd

Registration NO : 202001004851

session : 2022-23 (5th sem - Gen)

Page No.
Date.

Project Report on maintainence of Freshwater aquarium

সূচিপত্র

Teacher's Signature .....

ভূমিকা (Introduction) :

অ্যাকোয়ারিয়াম একটি বাস্তুতন্ত্রের একটি ক্ষুদ্র রূপ যা আমাদের বাড়ির সৌন্দর্য বাড়িয়ে তোলে।
এই প্রাকৃতিক 'জীবন্ত ছবি' আমাদের ঘরের পরিবেশকে আরও আকর্ষণীয় করে তোলে এবং পরিবারের সদস্য বা দর্শনার্থীদের মধ্যে আনন্দ উত্তেজনার সৃষ্টি করে।
অ্যাকোয়ারিয়াম রাখার প্রধান সুবিধাটি অন্যান্য পোষা প্রাণীর তুলনায় কম ~~খরচ~~ হয়। ব্যয় হয়।
মৎস্য লালনপালনকারীর জন্য, অমেরুদণ্ডী প্রাণী, উভচর, জলজ সরীসৃপ, কচ্ছপ, এবং জলজ উদ্ভিদ রাখার জন্য অ্যাকোয়ারিয়াম ব্যবহার করা হয়।
অ্যাকোয়ারিয়াম আধুনিক ইংরেজি অ্যাকুয়াটিক ভিভারিয়াম দুইটি নাম দ্বারা প্রচলিত।

সারসংক্ষেপ (Summary):

আমাদের পাঠ্যসূচীর একটি বিষয় maintance of of freshwater aquarium. এর জন্য আমাদের শ্রদ্ধেয় মহাবিদ্যালয়ের নির্দেশে আমাদের জন্য একটি field visit আয়োজন করা হয়।

16.11.২০২২ তারিখে নির্দিষ্ট দিনে আমরা শিক্ষকমহাশয়ের সহিত বর্ধমান science centre এ দুপুর 1 টা নাগাদ পৌঁছাই। সেখানে আমার Ticket কেটে প্রবেশের পর বিজ্ঞানের নানারকম যন্ত্রপাতি ও আরও অনেক ধরনের জিনিস দেখি। তারপর অ্যাকুরিয়াম গুলি দেখতে যাই। সেখা অনেকরকমের অ্যাকুরিয়াম ও তার ভেতর নানাধরনের মাছ ছিল।

তারপর ওখানকার আধিকারিকের সাথে আমাদের সাক্ষাৎ হয় তিনি এই অ্যাকোয়ারিয়াম গুলির পরিচর্যা করেন। উনি আমাদের অ্যাকোয়ারিয়াম পরিচর্যা সম্পর্কে নানারকম তথ্য দেন। কীভাবে অ্যাকোয়ারিয়াম গুলি পরিস্কার রাখা হয়, কীভাবে অ্যাকোয়ারিয়ামের জল পাল্টানো হয় ইত্যাদি।

তারপর বিকেল ৪টে নাগাদ আমাদের ৪টে নাগাদ আমাদের কাজ শেষ হয় এবং আমরা ওনাকে ধন্যবাদ জানিয়ে বের হই এবং শিক্ষক মহাশয়ের অনুমতিপূর্বক বাড়ির পথে রওনা হই।

Burdwan science centre

Report :- Maintainence of freshwater aquarium-

অ্যাকোয়ারিয়াম মাছ রাখার ইতিহাস হুবার্ট অনুসারে অ্যাকোয়ারিয়াম মাছ রাখার ধারণাটি পুরোনো, সুমেরীয়, আসিরিয়ান এবং মিশরীয়রা সবচেয়ে পুকুরে মাছ রেখেছিল। ~~ন~~ ~~[illegible]~~ ১৮৫৩ এর বসন্তকালে লন্ডনে রিজেন্টস পার্কে ফিশ হাউস খোলার বিষয়টি বিশ্বের প্রথম অ্যাকোয়ারিয়াম।

- সুন্দর ও নির্ভরযোগ্যতার কথা বিবেচনা করে নিম্নলিখিত বিষয়গুলি মনে রাখা উচিত।

(১) নান্দনিক সৌন্দর্য

(২) ট্যাঙ্কের আকার ও আকৃতি

(৩) জলের পরিমাণ।

Fabrication of the tank:

উৎপাদন অ্যাকোয়ারিয়াম সংযোজন একটি শিল্প যা আমরা দুই থেকে তিন দিনের ব্যবহারিক অভিজ্ঞতার মধ্যে শিখতে পারি। কাঁচের বণিকদের কাছ থেকে ভালো মানের কাঁচ, আঠা, গ্লাস কাটার এবং সিলিকন সিলান্ট কেনা যায়। গ্লাস কাটতে বা অর্ডার দেওয়ার জন্য পরিমাপ করার সময় কাচের প্রস্থ জেনে নেওয়া উচিত।

ট্যাঙ্কের উচ্চতা: অ্যাকুরিয়াম ট্যাঙ্কটি একটি লিভিং রুমে দাঁড়ানো এবং বসার অবস্থান থেকে দেখার জন্য সর্বদা ২০ সুর্ভ উচ্চতায় রাখা উচিত।

Teacher's Signature ....

Burdwan science centre

• অ্যাকোয়ারিয়াম স্থাপন: অ্যাকোয়ারিয়াম ট্যাঙ্কটি একটি সমতল পৃষ্ঠের উপর রাখা হয়, এক ইঞ্চি বেশি মার্সোফোমের শিটের উপর রাখুন। ~~একটি~~

অ্যাকোয়ারিয়াম স্থাপনের জন্য যতগুলি ধাপ আছে যা নিম্নরূপ —

(A) প্রথমে আমরা মাছ অনুযায়ী ট্যাঙ্ক নির্বাচন করব এবং একটি সঠিক অ্যাকোয়ারিয়াম জায়গায় ব্যবস্থা করব।

(B) তারপর একটি নির্দিষ্ট ট্যাঙ্কটির জন্য যে ধরনের ফিল্টার প্রয়োজন সেটি ট্যাঙ্কের ভিতর লাগাতে হবে।

(C) ট্যাঙ্কের তলায় অ্যাকোয়ারিয়ামের জন্য কেনা বালি, মাটি 2-3 cm বিছিয়ে দেব। এবং সাজানোর জন্য বিভিন্ন [illegible] মাছ শ্যাওলা ইত্যাদি ট্যাঙ্কের ভেতর লাগানো হল,

(D) তারপর ট্যাঙ্কের ভেতর জলদ্বারা পূর্ণ করা হল। তারপর ট্যাঙ্কের ~~ভেতর~~ ধারে ওয়াটার হিটার লাগানো হয়।

(E) জলে অনেকসময় ক্লোরিন থাকে যা মাছেদের মেরে ফেলে, তাই জলের ক্লোরিন এবং অন্যান্য ক্ষতিকারক পদার্থের পরীক্ষা করে ওয়াটার-ডি ক্লোরিনের যোগ করা হল।

(F) তারপর মাছ ছাড়ার আগে কিছু সপ্তাহ ধরে ট্যাঙ্ক কয়েকবার জল ভরে জল বের করার সাইকেল রাখা হয়। এবং এই দরুন কোনো সমস্যা দেখা না দিলে মাছ ছাড়া হয় অ্যাকোয়ারিয়ামে।

Teacher's Signature ....

• অ্যাকোয়ারিয়ামের নিম্নলিখিত রক্ষণাবেক্ষণ:

(1) মাছগুলি দিনে অন্তত দু-বার অ্যাড হিসেবে খাওয়া হয়।

(2) খাওয়ানোর সময় অত্যধিক খাদ্য এবং খাওয়ার সিলভার সুতোর বল্ল করে দেয়।

(3) অতিরিক্ত উত্তাপ এড়াতে হিটার, খাওয়ার সিলভার ব্যবহার করা হয়।

(4) শীতকালে বিকেলের হিটারের সমাধান পর্যবেক্ষণ করুন।

(5) অ্যাকের নিকট কীটনাশক স্প্রে করবেন না।
(6) সরাসরি আলো আলা করবে
(7) অ্যাকের অত্যধিক সংখ্যক পরীক্ষা করুন।

(8) সপ্তাহে সপ্তাহে অ্যাকের থেকে মৃত মাছগুলিকে অপসারণ।

(9) চৌম্বকীয় স্ক্রিনার ব্যবহার করুন।
(10) ক্লোরিনমুক্ত জল ব্যবহার করুন।

Ans: silver dollar

Red tail fish

Silver Dollar:

সাধারণত সমগ্র দক্ষিণ আমেরিকার গ্রীষ্মপ্রধান অঞ্চলে বিশেষ করে আমাজন নদীর তীরবর্তী অঞ্চলে সিলভার ডলার মাছদের দেখা যায়। এই মাছেরা হল সেরাসালমিডিয়া পরিবারের অন্তর্ভুক্ত। প্রায় এক ডজন প্রজাতির সিলভার ডলার দেখা যায়, তবে অ্যাকোয়ারিয়ামের জন্য সবচেয়ে জনপ্রিয় প্রজাতিটি হলো মেটাইনিস আর্জেন্টিয়াস। দেহের পাশের দৈর্ঘ্য বরাবর দাগ গুলি দেখে সহজেই সিলভার ডলার মাছদের চেনা যায়। এদের সমস্ত প্রজাতি দেখতে অনেকটা রূপোর মতো দেখতে ও চ্যাপ্টা ও গোলাকার যা রূপোর মুদ্রার সাথে তুলনা করে এদের নামকরণ করা হয়েছে। পূর্ণ বয়স্ক সিলভার ডলারের দৈর্ঘ্য ১৫ cm হয়।

Red Tail Shark:

লাললেজের এই হাঙররা হল সাইপ্রিনিডে পরিবারের সদস্য এবং উষ্ণ মিষ্টি জলের মাছ। এবং লোহিত ও কার্টুনের মাছ্যার টেইল, রেড টেইলড ব্ল্যাক শার্ক নামে পরিচিত। এই সাইপ্রিনিডে পরিবারের সদস্যদের থাইল্যান্ডের নদ নদী ও জলাশয়ে পাওয়া যায়। লাললেজের হাঙররা আলাদা থাকতে ভালোবাসে। খাওয়ার সময় এরা খুব হিংস্র প্রকৃতির হয়। এরা কখনও চুপচাপ থাকে না, খাদ্যের সন্ধানে সবসময় ছোটাছুটি করে। হাঙরের মতো দেখতে লাগে।

চিত্র : severum

চিত্র : Gold fish

Severum :-

ব্রাজিলের উত্তর আমাজন অঞ্চলে সেভেরাম মাছেদের দেখা যায়। এরা সিকলিডিয়া পরিবারের সদস্য। এরা ব্যান্ডেড সিকলিড, এবং স্ট্রেইপ সিকলিড বা মিডর সিকলিড নামেও পরিচিত।
এরা ঈষৎ উষ্ণ জলে এবং ২৪°c থেকে ২৯°c তাপমাত্রায় থাকতে ভালোবাসে। এরা বেড়ে ২০ cm এর মতো লম্বা হয় এবং দশ বছর অবধি বেঁচে থাকতে পারে।

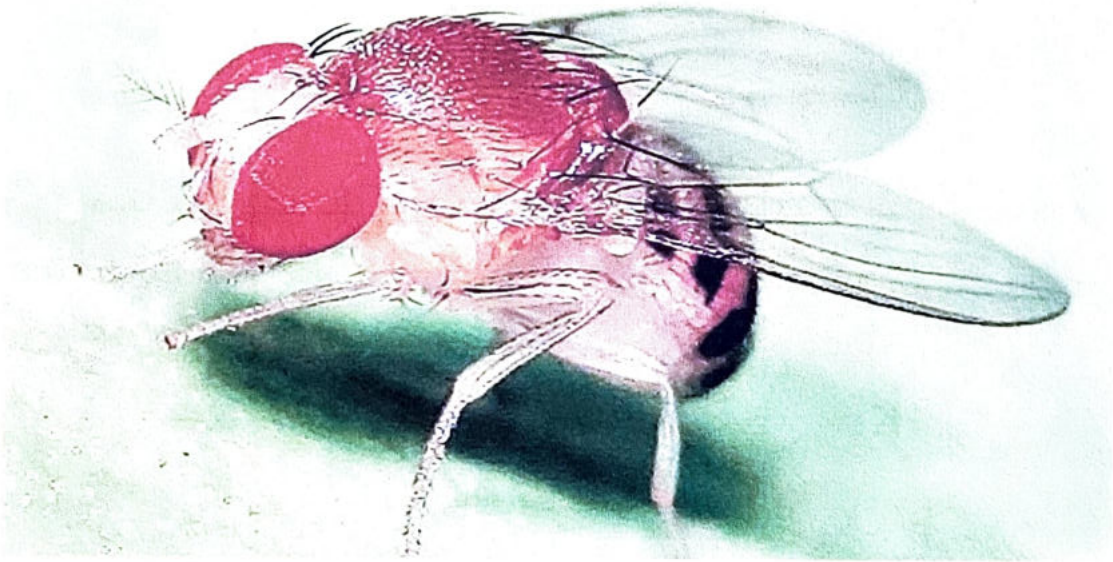
GOLD Fish :

আজ থেকে প্রায় 1000 বছর আগে চীন দেশীয় লোকেরা নিজেদের খাদ্যরূপে গোল্ড ফিসের আদি প্রজাতির চাষ শুরু করে। বর্তমানে যতরকমের গোল্ড ফিস আছে তা সবই ঐ প্রজাতি থেকেই এসেছে। যেমন কমেট, সিং টেল ফেল, ফ্যান টেল, লায়ন হেড, ভিয়েল, টেইল টেইল এবং ওরান্ডা ইত্যাদি।
কালো টেইল টেইল একটি বিশেষ প্রজাতির গোল্ড ফিস যার দৈর্ঘ্য প্রায় 9 cm. এদের চেহারা ফ্যান টেল এর মতনই মোটাসোটা। এদের লম্বা ল্যাজের পাখনাটি শরীরকে জড়িয়ে নীচের দিকে ঝুলে থাকে পর্দার মতো। এই গোল্ড ফিস সাধুটি অ্যাকোয়ারিয়ামে সাজানোর জন্য খুবই জনপ্রিয়।

PROJECT REPORT ON *Drosophila* CULTURE

PROJECT SUBMITTED IN PARTIAL FULFILMENT FOR

BACHELOR DEGREE OF SCIENCE IN ZOOLOGY



SEMESTER-VI HONOURS FINAL EXAMINATION (CBCS) – 2023

CC – 13: DEVELOPMENTAL BIOLOGY LAB (PRACTICAL)

THE UNIVERSITY OF BURDWAN

ROLL NO:- 20031100020

REGISTRATION NO:- 202001004798 OF 2020-21

Dr. Saurabh Sarkar
Assistant Professor
Department of Zoology
Gushkara Mahavidyalaya

Gushkara Mahavidyalaya
P.O: Gushkara, Dist: Purba Burdwan,
Pin-713128, West Bengal.
Email:guskaramahavidyalaya@gmail.com
Phone: 03452-255105 Fax: 03452-257635

CERTIFICATE

This is to certify that MD RIYAJUDDIN, bearing University Roll No:- 200311000020 with Registration number 202001004798 OF 2020-21 students of B.Sc. Semester VI Zoology Honours have worked out the Project work entitled "*Drosophila* Culture" under my direct supervision and the entire work has been carried out by him/her in the Animal House & Practical Laboratory, Department of Zoology, Gushkara Mahavidyalaya, Guskara. The project work represents explanation of his/her study. The pictures used in this project are captured during their own study.

Date: 24.06.23

Saurabh Sarkar

Dr. Saurabh Sarkar
Assistant Professor
Department of Zoology
Gushkara Mahavidyalaya

ACKNOWLEDGEMENT

*I express my deep sense of gratitude to **Dr. Saurabh Sarkar**, Assistant Professor, Department of Zoology, Gushkara Mahavidyalaya under whose direction this study was conducted.*

*I am ever grateful to to **Dr. Sukhendu Roy**, Assistant Professor, Head of the Department of Zoology, Gushkara Mahavidyalaya, for his continuous encouragement.*

*I am thankful to **Aparnita Nandi Roy**, SACT, Department of Zoology, Gushkara Mahavidyalaya, for her valuable suggestions.*

*I am also thankful to **Dr. Nabyendu Rakshit** & **Miss Poulomi Roy**, SACT, Department of Zoology, Gushkara Mahavidyalaya, for their continuous support.*

I am grateful to TIC, ***Dr. Sabina Begum**, Gushkara Mahavidyalaya, Gushkara, Purba Burdwan, West Bengal.*

*My appreciation goes to **Mr. Kalyan Kr. Mukherjee** and **Mr. Uday Chand Mete**, Laboratory Attendant for technical works, whose active involvement smoothen the entire tedious venture.*

I am thankful to my co-workers Barun, Rajrishi, Seheli, Swagata, Chandradeep for helping me to fulfilmy my project work.

I owe a lot to my parents, who encouraged and helped me at every stage of my personal and academic life.

Above all, I owe it all to Almighty God for granting me the wisdom, health and strength to undertake this project work and enabling me to its completion

Md Riyajuddin

Signature of the student

Name of the student

MD RIYAJUDDIN

ABBREVIATION

F_1	First filial generation
F_2	Second filial generation
Hsp70	Heat shock protein70
h/hr	Hours
mg	Milligram
min	Minute
mL	Millilitre
mM	Millimolar
nM	Nanomolar
P	Parental generation
ppm	parts per million
SDM	Standard Drosophila medium
µg	Microgram
µL	Microlitre
µM	Micromolar
gm	Gram
cm	Centimetre
mA	Milliamperes
kV	Kilovolt
M	Mole
EMS	Ethylmethanesulfonate
ENU	Ethylnitrosourea
DCV	Drosophila C Virus

CONTENTS

Introduction :

Drosophila derived from the Greek word drósos means dew loving. They belong to the Drosophilidae family; and are most frequently known as fruit flies or often called vinegar, wine or pomace flies. Their main distinguishing character is to stay on fruits, which are ripped or rotten. There is another related family Tephritidae, their members are also called as true fruit flies or fruit flies. Drosophilae are different from them. They feed primarily on unripe or ripe fruits. Many species of Drosophila are agricultural pests, especially the Mediterranean fruit flies. They oviposit through ovipositor and capable of colonizing in live fruits that are still in the process of ripening, causing massive agricultural damage. Currently, the genus Drosophila is considered as paraphyletic group. The entire genus, however, contains more than 1500 species , which are very diverse in their appearance, behavior, and breeding habitat . However, many members of the family Drosophilidae are categorized into two subgenera, in which around 1100 species belong to Drosophila sub-genera, moreover, about 330 species belong to Sophophora subgenera including *D. melanogaster*. Furthermore, another *Drosophila* species, i.e., Hawaiian spp. have more than 500 species in which only 380 species are described. Furthermore, they are occasionally documented as a separate subgenus or genus, i.e., *Idiomyia grimshawi*, but this is not widely accepted. About 250 species are part of the genus *Scaptomyza*, which arose from the Hawaiian *Drosophila* and later recolonized in continental areas. However, *Drosophila* spp. are distributed all over the earth; moreover, many species are found in the tropical regions. Furthermore, the alpine zones, cities, deserts, swamps, and tropical rainforest also confine them. Furthermore, hibernation takes place in many northern species.

Their breeding takes place in numerous types of rotten vegetation and mycological materials, comprising barks, flowers, fruits, mushrooms, and slime fluxes. However, the maggots of *D. suzukii* act as the pest and feed on fresh fruits. Moreover, some species of *Drosophila* have achieved the status of parasites and predators. Furthermore, several species attract to lure of mushrooms and fermented bananas, but others deny attracting to every type of bait. Furthermore, females and males are assembled for mating on appropriate propagating materials separate from breeding sites to form leeks. Also, many Drosophila spp., comprising *D. immigrans, D. melanogaster,* and *D. simulans*, are found neighboring and accompanying with humans and are called domestic species. Also, human activities, such as transporting of fruits and other fresh food items, are responsible for introducing many species throughout the world, including *D. immigrans, D. melanogaster, D. simulans, D. subobscura,* and *Zaprionus indianus* (**Farzana Khan Perveen 2018**).

Objective :

i) To introduce normal "wild type" and various mutant phenotypes.

ii) To conduct genetics experiment this span of generation.

iii) To introduce the use of the chi square statistic to test hypotheses concerning expected and observed ratios.

iv) To determine the ratio of monohybrid cross, dihybrid cross and sex linkage cross of Drosophila melanogaster.

v) To design genetic cross to illustrate segregation, independent assortment and sex linkage.

vi) To discuss the life cycle of Drosophila melanogaster.

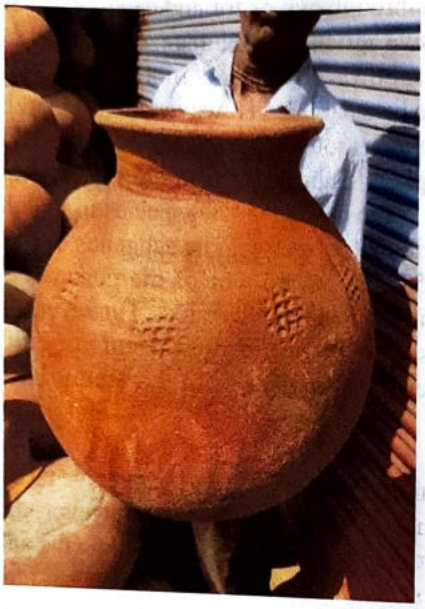
vii) To differentiate between male and female of Drosophila melanogaster.

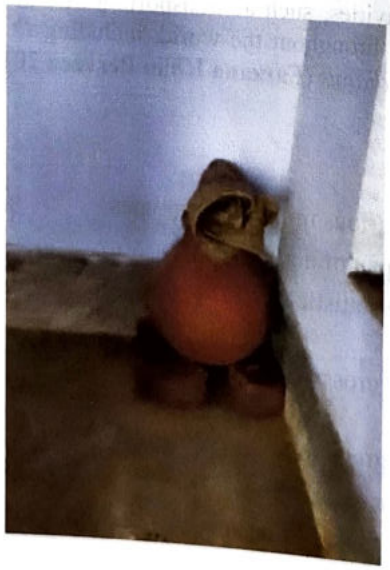
viii) To determine the progeny from the cross between wild type and vestigial (monohybrid cross), wild type and vestigial, Sepia eye (dihybrid cross) and wild type and white eyes (sex linkage cross).

ix) To familiarize you with an important research organism, the fruit fly, Drosophila melanogaster.

x) To help you understand the presence or absence of a genetic trait in an individual and the ratio of that trait in a population.

xi) To understand Mendelian genetics and inheritance of traits.





Systematic Position :

Domain: Eukarya

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Diptera

Family: Drosophilidae

Genus: *Drosophila* ("dew lover")

Species: *D. melanogaster* ("dark gut")

Characteristic Features

1. Physical Description

Drosophila matures through complete metamorphosis, as do all members of the order Diptera. Similar to all insects *Drosophila* is covered in a chitinous exoskeleton; has three main body segments; and has three pairs of segmented legs. Like other flies, *Drosophila melanogaster* has a single pair of wings that form from the middle segment of its thorax. Out of the last segment of its thorax (which in other insects contains a second pair of wings) develops set rudimentary wings that act as knobby balancing organs. These balancing organs are called halters. Other Physical Features: Ectothermic; heterothermic; bilateral symmetry. Sexual Dimorphism: Female larger; sexes colored or patterned differently.

2. Reproduction

Reproduction in *Drosophila* is rapid. A single pair of flies can produce hundreds of offspring within a couple of weeks, and the offspring become sexually mature within one week. As in all insect species *Drosophila melanogaster* lays eggs. The eggs are placed on fruit, and hatch into fly larvae (maggots), which instantly start consuming the fruit on which they were laid. Male flies have sex combs on their front legs. It has been theorized that these sex combs might be used for mating. However, when these combs are removed it seems to have little effect on mating success.

3. Lifespan

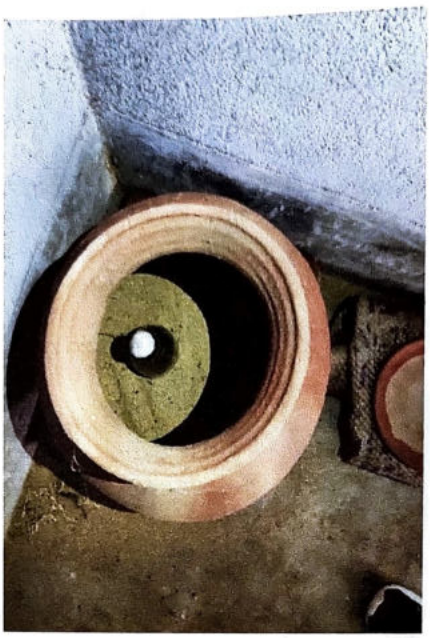
Average ligespan 0.3 years.

4. Behavior

The behavior of *Drosophila melanogater* is simplistic. They are easily drawn towards the smell of any food source, and will mate almost
indiscriminately with any individual of the opposite sex.

5. Food Habits

As the name implies, the fruit flies lives primarily on plant material. The adults thrive on rotting plants, and fruits; while eggs are usually laid on unripened/slightly ripened fruit, so by the time the larva develop the fruit will have just started to rot, and they can use the fruit that the egg was laid on as their primary source of nutrition. *Drosophila* are considered major pests in some area of the world for this reason.



6.Economic Importance for Humans

Positive impacts: This species is widely used in scientific research, Source of medicine and drug.

Negative impacts: *Drosophila melanogaster* has been known to over winter in storage facilites, where it can consume/ruin vast quatities of food. As stated above, the fruit fly also lays its eggs on unripened fruit, and is considered a pest in many areas.

Characteristics of *Drosophila* that make it a good model organism:

1. Small, easy and cheap to maintain and manipulate
2. Short lifespan
3. Produce large numbers of offspring
4. Development is external
5. Availability of mutants
6. Lots of previous experiments and discoveries
7. Genome is sequenced
8. Homologues for at least 75% of human disease genes
9. Exhubit complex behaviours
10. Fewer ethical concern

Drosophila as a Model Organism :

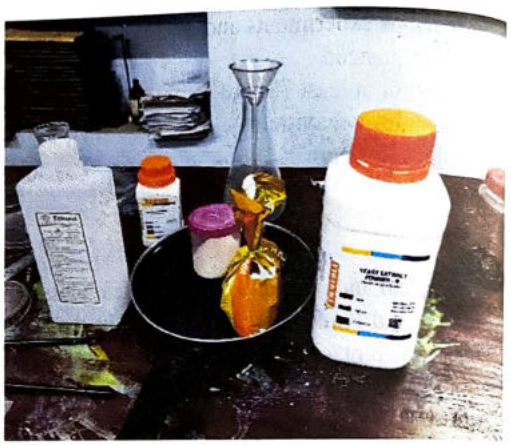
Both toxicological and pharmacological researchers use animals for scientific and research purposes **(Siddique et al. 2011, Ram and KarChowdhuri 2014)**. In recent years, for several emerging ethical issues, use of alternative model organisms replacing the established mammals are encouraged in research, study and testing purposes and for maintenance of ecological balance **(Mukhopadhyay et al. 2003)**. *Drosophila* is considered to be a well-established model organism for environmental toxicity monitoring studies as suggested by the European Centre for Validation of Alternative Methods **(Festing et al. 1998, Benford et al. 2000)**. Recently *Drosophila* has been used vastly as a model organism in clinical drug discovery processes also **(Bell et al. 2009, Giacomotto and Segalat 2010)**. Fully sequenced human and *Drosophila* genome analysis show that approximately 75% of known human disease genes have similar homologues in *Drosophila* **(Lu and Vogel, 2009)**. Hence *Drosophila* is considered as an established model insect for medical research purposes **(Reiter 2001)**.Rationality for selecting *Drosophila* as a model for this study is presented in the **following points:-**

- small size
- easy to handle
- short life cycle
- sexual dimorphism
- easy to anaesthetize
- low cost equipment for culture
- little space required for culture
- similarity with human genome

Life Cycle of *Drosophila sp* :

Drosophila completes entire life cycle within two weeks only at (25 ±1)°C **(Ashburner and Thomson 1978)**. As other holometabolous insects, life cycle of *Drosophila* consists of four stages - egg (embryo), larva, pupa and adult. A gravid female lays nearly 400 eggs (embryos) measuring 0.05 mm in length on sugar containing fruit or decaying materials. After nearly

12-15 h (at 25°C), 1st instar larvae which are coming out from eggs moult twice (2nd and 3rd instar larvae) at about 24 and 48 hours. Larvae feed on sugar in fruit and micro-organisms that decomposes the fruit. After that, larvae encapsulate itself in puparium for 4 days metamorphosis (at 25°C) and adult flies emerge from puparium **(Ashburner and Thompson 1978; Ashburner et al. 2005).**



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Identification of the Fly (male/ female; different species; different mutants) :

Male Fruit Fly:-

Male fruit flies have yellow-brown bodies with brick red distinguishable eyes. Males are smaller than females. Male fruit flies are easily identified by their color differences and certain characteristic features. However, certain features of males are less noticeable in newly emerged male flies. Sex combs are one of the best characteristic features found on the forelegs of male fruit flies that are used to cling onto female flies while trying to mate. In addition, males also have hairs called claspers around the reproduction parts that help to attach to a female during the copulation period.

Female Fruit Fly:-

The overall body colors of female and male fruit flies are similar. However, females are quite larger than male flies.
Female flies are ready to court with males after about 8-12 hours from their emergence. It takes around 15-20 minutes to complete the copulation. Females can lay about 400 eggs, and they lay eggs on rotting fruits and decaying mushrooms. The size of an egg is about 0.5 mm long . Once female fly lays eggs, it takes usually 12-15 hours to hatch them.

Different Fly Species:-Fruit flies, a kind of filth fly, are a nuisance in homes.
Many species in this category thrive in kitchens, bathrooms, and even sink drains. As a result, residents may refer to a variety of filth flies as house flies. All of these fly species reproduce quickly, and infestations can spread diseases to residents.
The first step to control the pests is to identify which of the different house fly species is causing problems. Divided into two groups by size, these types of house flies frequently invade homes.

Fruit Fly

Yellowish-white or tan with red eyes, these pests feed on sugary items. They develop inside drain lines or almost any source of moisture that accumulates inside poorly cleaned garbage containers. Fruit flies are kitchen invaders that drink and are attracted by vinegar, soda, or the juice of overripe produce. The insects crawl into liquids or sticky syrups, causing contamination. Fruit fly infestations typically are more frequent in summer and fall, but will occur anytime a suitable source of food and developmental sites are nearby.

Mutant Fruit Fly:-

The fruit flies in this exhibit show just a few of the mutations that occur in natural fruit fly populations.
The genetic instructions to build a fruit fly-or any other organism-are imprinted in its DNA, a long, threadlike molecule packaged in bundles
called chromosomes. Like a phone book made up of different names and addresses, each chromosome consists of many individual sections called genes.
Each gene carries some of the instructions for building one particular characteristic of an organism.
To build a complete organism, many genes must
work precisely together. A defect in a gene can cause a change in the building plan for one particular body part-or for the entire organism.

Mutations are neither good nor bad: some may be beneficial for an organism; others may be lethal. By creating new gene versions, mutations are a driving force for changes in evolution, sometimes leading to new species. Biologists learn about the proper function of any gene by studying mutations. If a defective gene causes short wings, for instance, scientists know that the healthy version of the gene is responsible for correct wing formation.

Rearing :

Basic instruments required for fly culture

I. Bottles (250 ml) and vials (40 ml ,100 ×25 – mm diameter)
II. Medium (fly food)
III. Microscope
IV. Autoclave
V. Anesthetization Equipments
VI. Environment test chamber
VII. Funnel
VIII. Insect pins
IX. Petridis
X. Brushes and forceps
XI. Cotton and cotton balls
XII. Bloating paper
XIII. Distil water
XIV. Alcohol
XV. Labeling tape

Environmental conditions required for fly culture

The easiest way to grow flies is at room temperature. However, the optimum rearing condition is a temperature of 25°C and 60% humidity. In these conditions generation time is shorter (9-10 days from egg to adult). Unless equipment is readily available this is unnecessary for successful rearing and crossing of flies. It is preferable to keep flies out of drafts and direct sunlight or heat sources. These will rapidly dry the media, necessitating frequent media changes and the potential to dehydrate the flies.

Collection of flies

Drosophila melanogaster stock was collected from one Drosophila Stock Centre in Toxicology Research Unit, Department of Zoology, The University of Burdwan. Stock flies are collected in vials and transferred to Animal House, Department of Zoology, Gushkara Mahavidyalaya, maintain the temperature.

Standard Drosophila Medium (SDM)

Drosophila melanogaster was selected for the present study as the model organism. Flies were cultured in Environmental test chamber at 22±1C. They were allowed to feed on Standard Drosophila Medium (SDM) containing corn powder, agar agar, sucrose and yeast extract powder. Nepagin and propionic acid were added as antifungal agents.

Components	Amount
Agar-agar (Qualigens, India)	**3 gm**
2. Corn meal (Victoria Foods PrivateLimited, Delhi, India)	**17 gm**
3. Yeast extract powder (Qualigens,India)	**6 gm**
4. Sucrose (Merck, India)	**15 gm**
5. Distilled water	**360 mL**
6. Propionic acid (SRL, India)	**1 mL**
7. Nepagin [n-methyl-p-hydroxy-benzoate] (Sigma Aldrich)	**1 gm**
8. 90% Alcohol	**1-2 mL**

Specified amount of Agar-agar, Corn meal, Sugar and Yeast were weighed and mixed in a 1000 mL glass beaker. Then required amount of distilled water (as specified in the above table) was added and boiled with continuous stirring to avoid unnecessary adhesion with container. Continuous boiling reduced the volume of the food to nearly one third (1/3) of its starting amount and a thick sticky consistency was achieved. Then propionic acid as well as nepagin powder (dissolved in 90% alcohol) were added as antifungal agents.
Food thus prepared was used as culture medium for the flies and was poured in bottles, phials and petri plates and was cooled subsequently,
followed by tight cotton plugging. Food containers were carefully monitored at regular intervals to avoid contamination as well as fungal and mite infections. Food medium was changed at an interval of every fifteen (15) days for normal fly culture. The flies used in this study have been maintained in the above mentioned laboratory conditions for nearly 300 generations. Hence the flies used in these experiments are expected to be homogenous in nature. Overcrowding of flies in culture was always avoided.

Fly maintenance

PROCEDURES

Keeping Stocks

Most large fly laboratories maintain stocks that are not in everyday use at 18°C on a 4-5-week generation cycle. Stocks should be kept as two to four independent cultures, and it may be convenient to keep these on alternating generations, 2 weeks apart. Stocks are normally maintained in vials.
Most stocks can be kept by dump-transfer of flies to fresh vials. However, it is important to avoid too overcrowded cultures and only 20 or so flies should be transferred. It is good practice to inspect the flies on transfer, to ensure that both sexes are present and that their phenotype is as expected. Fly laboratories may keep some stocks that require selection of each generation, and it is important that the stock keeper knows of any special requirements to keep any stock (these should be entered on the stock database, see below). The "sick tray" is an inevitable part of any stock room, a place where sickly stocks, or stocks going through a crisis, are kept under special attention. It

is very good practice to keep the old cultures for 2 weeks (at 18°C) after transfer, so that they can be used as a backup should the new stocks fail for any reason.

Collecting Virgins

Two general methods ensure that female *D. melanogaster* are virgin when used to set up a cross. These can be called the "biological" and "genetic" methods. Only the former is considered here; for genetic tricks useful for virgin collecting, see Chapter 12 of **Ashburner (1989).**

Although some variation between stocks exists, the general rule is that females will not accept a male mate until they are 10-12 hours old (i.e., after eclosion from the pupa). Thus, flies can be collected during this window (or, better, between 8 and 10 hr after eclosion), anesthetized, separated into males and females, and stored until needed in yeasted vials. The females will then usually be virgin when used. As a preliminary check, the vials that were used for storing the virgin females should be kept and inspected 3 or 4 days later for any signs of larvae. If larvae are present, it is clear that at least one female in that vial was not virgin. Of course it does not matter too much if a single female is incorrectly stored with the males (as long as she is discarded); but a single male in the tube of females will play havoc. The rule for sexing for virgin collecting, especially when tired or rushed is: If in doubt, it is a male. The following is a convenient schedule for virgin collection.

Day 0: Clear all flies from emerging cultures in the late afternoon or early evening (e.g., 5:00 p.m. to 6:00 p.m.). Discard these flies. Store emerging cultures at 18°C in the dark.

Day 1: Put cultures at 25°C in the light, first thing in the morning. Clear all flies from the cultures ~1 hour later, anesthetize, separate into males and females, and store these in separate vials at 18°C until required. The young females, i.e., those that are relatively unpigmented and/or have unexpanded wings, will almost certainly be virgin. Check that the emerging cultures have no adult flies. Return the emerging cultures to 25°C in the light and possibly collect virgins last thing in the evening. Keep the "female" vials after using the virgins and inspect 3-4 days later for larvae. If present, presume that any females from that vial were nonvirgin at the time of use. (Note that the presence of eggs in the female-holding vials is not evidence of nonvirginity, even virgin females will lay eggs, albeit at a low rate in comparison with mated females.)

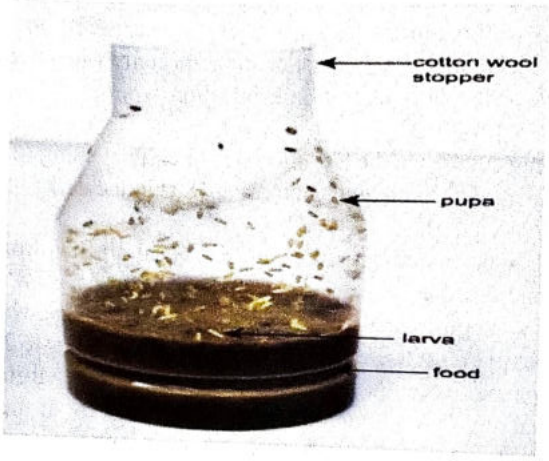
In practice, fly workers develop their own protocols for virgin collection that suit not only the flies, but also social and other activities. But please bear in mind, nonvirginity is *by far* the most common reason for an "unexpected" result from a cross. It is therefore very good practice to design crosses so that nonvirgin progeny will be evident by their phenotype, especially if unexpected nonvirgin progeny could confuse the analysis of an experiment (**Ashburner and Roote 2000**).

Controlling Plagues and Diseases:-

i. One reason for the success of *D. melanogaster* as a laboratory organism is that it is relatively resistant to plagues and diseases. The two most common problems are molds overgrowing the medium and mites. There have also been disturbing reports recently of viral infections in some laboratories. The culprit is apparently a picornavirus (*Drosophila* C virus, DCV) and the symptoms of infection are black, dying pupae. The extent to which fly laboratories suffer from molds and mites varies greatly, but there is some general advice that can be given. The most important advice is that prevention is better (*far* better) than cure; two main guidelines for preventing plagues and diseases are below.

ii. *Cleanliness.* Keep a clean fly room, fly kitchen, and culture environment. For cleaning surfaces in fly rooms, use alcohol or a spray disinfectant, e.g., "Astell D."

iii. *Isolation of new stocks.* Quarantine all incoming stocks, no matter from what source, even if the distributor swears that they are free of mold, mites, or viruses. A quarantine facility (e.g., a dedicated incubator) should be situated as distant from the normal fly and culture rooms as possible, and all materials, especially discarded vials, must be segregated from those in regular use. It is probably sufficient to quarantine for two generations, and only transfer the stocks to the regular facility when close inspection shows them to be free of infection or infestation. For flies brought to the laboratory straight from the wild, four generations of quarantine are recommended.


cotton wool stopper
pupa
larva
food

iv. If an infection or infestation occurs, the first rule is to isolate all affected cultures to a quarantine facility. What is done next depends very much on the nature and extent of the problem.

v. Bacteria

The most common bacterial problem is mucus-producing bacteria on the food, which often produce a reddish-brown pigment (e.g., *Acinebacter* sp.). The addition of antibiotics (streptomycin, tetracycline, or ampicillin) to the food at a concentration of 250 mg/liter is usually sufficient to cure the problem within one generation. If the problem is recurrent, then investigate the possible sources of the contamination (e.g., the yeast). The use of dextrose, rather than sucrose, in the fly medium should prevent most bacterial growth. The routine use of antibiotics in the food medium is not recommended, as this will inevitably lead to resistance.

vi. Molds

Molds, usually species of *Penicillium* or *Aspergillus*, are a common problem, as fly medium is an ideal substrate for their growth. With healthy cultures, the flies normally out-compete these fungi, but they can prove to be serious for weak stocks or for cultures at low density. It is now routine practice to include mold inhibitors in medium. Those most commonly used are Nipagin M or propionic acid. For both bacteria and molds, persistent infections that are refractory to treatment can best be overcome by washing eggs in 70% alcohol.

vii. Mites

Several species of mites can infect cultures of *Drosophila* (**Ashburner 1989**).

Broadly speaking, the mites may be interested either in the flies' food (food mites) or in the flies themselves. The fly mites are rarer than the food mites, but far more dangerous. Food mites often come in with the raw materials of fly medium (e.g., corn meal). For this reason, it is good practice to store bulk meal at - 20°C and to be scrupulous about cleaning up any spills. One of the commonest causes of a serious mite infestation is allowing old fly cultures to fester in culture rooms or the fly room. These rooms must be inspected regularly by someone with the authority to autoclave old cultures without question. This is not an issue where the liberal social attitude so characteristic of fly labs can be allowed to constrain effective management. If mites are found, then the affected cultures must be immediately quarantined (even better, autoclaved, but this is not always acceptable). If foam or muslin cotton-wool bungs are used, then replace these immediately with bungs of nonabsorbent, tightly balled cotton wool; these should prevent the mites spreading further.

Rapid (i.e., daily) transfer of stocks or cultures can rid them of mites, but this can be dangerous for weak stocks. An alternative is to collect eggs and wash them free of any mite eggs before transfer to clean vials, or to collect pupae on paper inserts and wash them free of mites and mite eggs in 70% ethanol, again before transfer to clean vials. Tedion has been found to be effective against some common species of food mite.

Tedion is available from the Sigma Aldrich Library of Rare Chemicals and also from local suppliers of agrochemicals. Dilute the commercial product (usually 8% active compound) to 5000 ppm in acetone and soak 7-cm filter papers in this solution. Allow filters to dry completely and introduce one into each culture. Serious endemic mite infestations should never be allowed to build up. If they do, then seek professional advice to combat them, since they will require complete fumigation of all fly-handling rooms and equipment (**Ashburner 1989).**

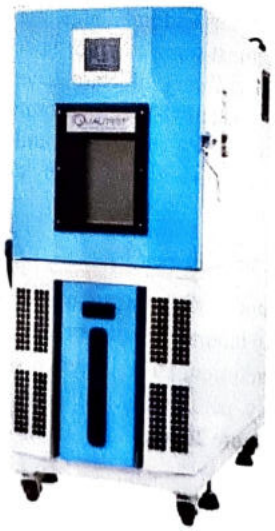
viii. Viruses

In contradiction to the statements in **Ashburner (1989)**, infection with the double- stranded RNA virus DCV has been found to be a serious problem in a few fly laboratories in recent years. Its symptoms are the presence of dying black pupae, particularly in old cultures. In addition, DCV infection seems to block the induction of transgenes under the Hsp70 heat shock promoter (T. Tully, pers. comm.). T. Tully (pers. comm.) has developed protocols for eradicating viral infection (**Ashburner and Roote 2000**).

Fly transfer

Flies should be transferred every 10 to 14 days. Students should maintain a backup culture of their flies and the instructor should maintain backup stock cultures of all fly strains. There are two basic ways to transfer flies when forming new cultures. One requires no anesthetizing but quick hands.

Place a funnel in the mouth of a fresh culture vial that already has media added. In the old vial (the one with flies in it), gently tap the flies down by softly tamping the vial on a soft surface, such as a mouse pad. The flies will fall



to the bottom and remain there for a few seconds (no more than that!), enough time to quickly take the plug off the vial, invert it into the funnel, and gently tamp, together, the two vials to force flies down into the new vial.
An alternative way is to put the flies in the freezer for about 8 minutes. This will cause the flies to fall into a state of stupor. After placing a funnel on the new vial, invert the vial with motionless flies into the funnel. This is not as much fun but you won't have any flies flying around the classroom.

Cleaning/ Sanitization

Keep a clean fly room, fly kitchen, and culture environment is very much important for Drosophila culture. For cleaning surfaces in fly rooms, use alcohol or a spray disinfectant, e.g., "Astell D." (**Ashburner and Roote 2000**).

Importance of *Drosophila* fly culture

1. The relationship between fruit fly and human genes is so close that often the sequences of newly discovered human genes, including disease genes, can be matched with equivalent genes in the fly.

2. 75 per cent of the genes that cause disease in humans are also found in the fruit fly.

3. Drosophila have a short, simple reproduction cycle. It is normally about 8-14 days, depending on the environmental temperature. This means that several generations can be observed in a matter of months.

4. Fruit fly are small (3 mm long) but not so small that they can't be seen without a microscope. This allows scientists to keep millions of them in the laboratory at a time.

5. They are inexpensive to maintain in the laboratory.

6. They require a simple diet consisting of simple sources of carbohydrates (cornmeal) and proteins (yeast extract).

7. The only care they need is having their food changed regularly (every 10-14 days at 25°C or 5-6 weeks at 18°C).

8. Drosophila have 'polytene' chromosomes, which means that they are oversized and have barcode-like banding patterns of light and dark. During early Drosophila research scientists could therefore easily identify chromosomal rearrangements and deletions under the microscope.

9. It is relatively straightforward to mutate (disrupt or alter) fruit fly genes.

10. The fruit fly provides a simple means of creating transgenic animals that express certain proteins, such as the green fluorescent protein of jellyfish.

11. The long and distinguished history of research devoted to the study of the fruit fly means that a remarkable amount is now known about its biology.

Conclusion

The fruit fly *Drosophila melanogaster* is a versatile model organism that has been used in biomedical research for over a century to study a broad range of phenomena. There are many technical advantages of using *Drosophila* over vertebrate models; they are easy and inexpensive to culture in laboratory conditions, have a much shorter life cycle, they produce large numbers of externally laid embryos and they can be genetically modified in numerous ways. Research using *Drosophila* has made key advances in our understanding of regenerative biology and will no doubt contribute to the future of regenerative medicine in many different ways. (**Jennings 2011**).

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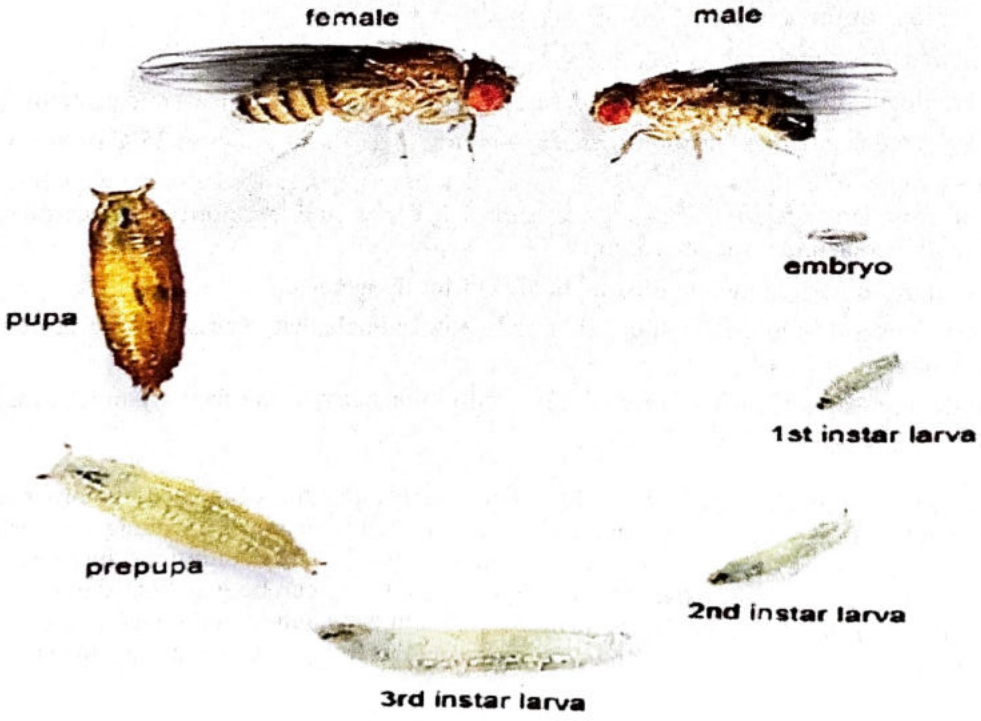
The life cycle of *Drosophila melanogaster*



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Examined
28/06/2023

VISIT TO A DUCK BREEDING CENTRE

Name :- Munmun Ghosh

University Roll :- 20061101011, year : 3rd

Registration No :- 202001004851

Session :- 2022-23

Bsc-gen-5th sem

Page No.
Date.

Visit to a Poultry farm and Duck breeding centre

सूचीपत्र

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Teacher's Signature ....

ভূমিকা (Introduction) :
সকল প্রাণীই খাদ্য অ্যানিম্যালিয়ার অন্তর্গত। বাস্তুবায়ন বলতে সেইসব প্রাণীদের বোঝায় যাদের সুসমন্বিত প্রাণীকূলে প্রতিপালিত করা হয়, যেমন - গবাদি পশু ও পোল্ট্রি পাখি। এসমস্ত সুসমন্বিত প্রাণী থেকে আমরা খাদ্য (দুধ, ডিম, মাংস), বস্ত্র ও অন্যান্য সামগ্রী পেয়ে থাকি এবং এদের বিভিন্ন প্রজাতির কাজে ব্যবহার করা হয় তাদের প্রাণীজ সম্পদ বলে।

■ পোল্ট্রি (POULTRY)
যে শিল্প খাদ্য উৎপাদনকারী অর্থনৈতিক গুরুত্বসম্পন্ন পাখিদের পালন করা হয় তাকে পোল্ট্রি বলে।
পোল্ট্রি পাখি প্রধানত দুটি কলের হয়, যথা - মুরগী ও হাঁস

মুরগী	হাঁস
গর্ব : কর্ডাটা	গর্ব : কর্ডাটা
উপগর্ব : ভার্টিব্রাটা	উপগর্ব : ভার্টিব্রাটা
শ্রেণী : অ্যাভিস	শ্রেণী : অ্যাভিস
উপশ্রেণী : নিওর্নিথিস	উপশ্রেণী : নিওর্নিথিস
বর্গ : গ্যালিফর্মিস	বর্গ : অ্যানসারিফর্মিস
গণ : গ্যালাস	গণ : অ্যানাস
প্রজাতি : ডোমেস্টিকাস	প্রজাতি : প্লাটিরিংকোস

● কয়েকটি মুরগীর প্রজাতি : হোয়াইট লেগহর্ন, রোড আইল্যান্ড রেড, অরপিংটন, সাসেক্স ইত্যাদি।
● কয়েকটি হাঁসের প্রজাতি : ইন্ডিয়ান রানার, বাফ, কাকি ক্যাম্পবেল, হোয়াইট পিকিন ইত্যাদি।

Teacher's Signature .....

সারসংক্ষেপ (Summary):

গত 16/11/2022 আমরা ডোমকল মহাবিদ্যালয়ের ছাত্রছাত্রী ও শিক্ষকরা মিলে রাজ্য মুরগী খামার দেখতে গিয়েছিলাম। প্রথমে আমরা বাসে করে বর্ধমান নবাবহাট বাসস্ট্যান্ড-এ আসি তারপর টোটোতে করে গোলাপবাগ এ পৌঁছালাম। 10.30 নাগাদ স্যার আসার পর আমরা রাজ্য মুরগী খামারে গেলাম। প্রথমেই আমাদের চুনজলের ওপর দিয়ে হেঁটে আসতে হলো।

সেখানে উপস্থিত ছিলেন গোলাপবাগ রাজ্য মুরগী খামারের Assistant প্রফেসর Dr. Prabir Sengupta. তিনি আমাদের মুরগী পালন সম্পর্কিত নানা তথ্য আমাদের সামনে উপস্থাপন করেছিলেন যা আমরা খাতায় লিপিবদ্ধ করেছিলাম।

প্রথমে মুরগীর ডিমকে জীবাণুমুক্ত করা হয় $KMnO_4$ + ফরমালডিহাইডের ধূপন দিয়ে। তারপর ইনকিউবেশন করা হয় এবং 18 দিন পর ইনকিউবেশন পিরিয়ড শেষ হয়। তারপর হ্যাচারে রেখে 2-3 দিন পর ডিম ফুটে বাচ্চা হয়। আমরা সমস্ত ~~মেশিন~~ ইনকিউবেটরের মেশিন ভালোভাবে দেখলাম। তারপর ব্রুড হাউসে গিয়ে বিভিন্ন পদ্ধতিতে তৈরি মুরগীর ঘর দেখলাম। এছাড়া, মুরগীর পার্থক্য নিরূপণ করতে পারছিলাম সবাই।

তারপর Dr. Prabir Sengupta Sir আমাদের পোলট্রি সম্পর্কিত বিভিন্ন তথ্য, ডিমের গুণাগুণ, পুষ্টিমূল্য ইত্যাদি সম্পর্কে আলোচনা করেছিলেন।

তারপর আমরা পুরো মুরগী খামার ঘুরে দেখার পর পরবর্তী গন্তব্যস্থলের জন্য রওনা দিলাম।

Page No. 03

Date.

গত 17/11/2022 আমরা সকাল 7:30 নাগাদ বর্ধমান ষ্টেশনে পৌঁছালাম। আজ আমাদের গন্তব্যস্থল ছিল হাঁস প্রজনন কেন্দ্র। এরপর স্যার আসার পর আমরা টোটো করে সবাই duck breeding centre এ পৌঁছে গেলাম। সেখানে উপস্থিত ছিলেন Duck breeding centre এর Ast. Proffesor Dr. R.S Banerjee.

তিনি আমাদের হাঁস পালন সম্পর্কিত নানা তথ্য আমাদের বলছিলেন যা আমরা খাতায় লিপিবদ্ধ করছিলাম.

এরপর আমরা ব্রুড হাউসে গেলাম এবং হাঁসগুলিকে ভালোভাবে পর্যবেক্ষন করলাম।

মূলত খাকি ক্যাম্পবেল হাঁস পালন করা হচ্ছে প্রজনন কেন্দ্রে। এরপর আমরা ইনকিউবেশন মেশিন বাইরে থেকে দেখলাম। ইনকিউবেশন পিরিয়ড থাকে 28 দিন। সাধারনত 5 মাস পর থেকেই হাঁস ডিম দেয়। খাবার হিসাবে অ্যাজোলা, কাচা হলুদ, Aloverra, শাক ইত্যাদি। কাচা হলুদের জন্য Liver strong হয়।

আমরা খুব ভালোভাবে সবকিছু পর্যবেক্ষন করলাম।

এরপর আমরা ওখান থেকে বেরিয়ে বাড়ির জন্য রওনা দিলাম।

पंजिरान duck breeding centre

Report:

হাঁস প্রজনন কেন্দ্র:

বর্তমানে গ্রামীন অর্থ সামাজিক উন্নয়নে হাঁস পালনের ভূমিকা স্বীকৃতি লাভ করেছে। গ্রামের গরিব খেটে খাওয়া মানুষের বাড়িতেও ২-৪ টি হাঁস পোষার রেওয়াজ আছে।

ডিম উৎপাদনের উদ্দেশ্যে পালিত বিভিন্ন জাতের হাঁসের মধ্যে খাঁকি ক্যাম্পবেল খুবই জনপ্রিয়।

- খাঁকি ক্যাম্পবেল হাঁসের বৈশিষ্ট্য:
- ওজন - পুরুষ হাঁস - ২.২-২.৭ কেজি, স্ত্রী হাঁস - ২.০-২.২ কেজি
- সাড়ে ৭ মাস বয়স থেকেই ডিম পাড়তে শুরু করে এবং বছরে ৩০০টি পর্যন্ত এই হারে ডিম পাড়ে। ২.৫ বছর পর্যন্ত এই হাঁস পালন করা লাভজনক।
- এই হাঁসের ডিম, সুস্বাদু, পুষ্টিকর এবং আকৃতিতে বড়ো হওয়ায় বাজারে বেশী দামে বিক্রি হয়।
- খাবার ও মাথা পর্যন্ত ডোবানোর জন্য প্রয়োজনীয় জল পেলেই এরা স্বাচ্ছন্দে বেঁচে থাকতে পারে, তাই বড় জলাশয় বা পুকুর ছাড়াও এই হাঁস পালন করা হয়।
- হাঁসের ঘর: খোলা জায়গায় পুকুরসহ বাগানে হাঁস পালন করলে শুধু রাত্রিবাসের জন্য হাঁসের ঘরের প্রয়োজন হয়। অন্যত্রে হাঁস প্রতি ২.৫ বর্গফুট জায়গা দিলেই হয়। হাঁসের ঘর বেশী উঁচু করার দরকার নেই। ঘরের চাল টিন বা টালি, খড় দিয়ে তৈরি করা যেতে পারে। ঘরের মেঝে এক দিকে ঢাল করতে হবে যাতে কোনমতে জল না দাঁড়াতে পারে। ঘরের দেওয়াল তারের জাল দিয়ে তৈরি করা হয়।

Teacher's Signature ....

Date.

• হাঁসের জন্য একটি সুষম খাদ্যের উপাদান:

প্রতি 100 kg সুষম খাদ্য তৈরি করার জন্য সাধারণত গম বা ভুট্টা জাতের দানাশস্য 35 kg, দানাশস্যের উপজাত দ্রব্য (খুদকুঁড়া, ভুসি, খুদ) 30 kg, প্রোটিন জাতীয় উপাদান 10 kg, শামুকের গুঁড়ো 20 kg, ভিটামিনযুক্ত খনিজ লবণের মিশ্রণ 5 kg মিশাতে হবে। হাঁসকে পরিমাণমতো শামুক খাওয়াতে পারলে মিনারেল না খাওয়ালেও চলে। হাঁসের খাবারে যেন ছত্রাক না থাকে সেদিকে নজর দিতে হবে।

• হাঁসের প্রজনন ও ডিম ফোটানো:

পুরুষ হাঁসের উপস্থিতি ছাড়াও খাকি ক্যাম্পবেল স্ত্রী হাঁস ডিম পাড়ে। তবে এ ধরনের ডিম থেকে বাচ্চা হয় না - এদের খাওয়া ডিম বলে। উর্বর ডিম পেতে গেলে স্ত্রী হাঁসের সঙ্গে পুরুষ হাঁস রাখা জরুরী হয়। হাঁস খামারে 10 টি স্ত্রী হাঁসের সঙ্গে দুটি পুরুষ হাঁস রাখা হয়।

খাকি ক্যাম্পবেল হাঁস সাধারণত ডিমে তা দিতে চায় না। সেজন্য দেশী হাঁস বা মুরগীর সাহায্যে এই ডিম ফোটানো যেতে পারে। এছাড়া ইনকিউবেটরের সাহায্যে মুরগীর মতোই হাঁসের ডিমও ফোটানো হয়। হাঁসের ডিম ফুটে বাচ্চা হতে 28 দিন সময় লাগে।

কৃত্রিম উপায়ে ডিম ফোটানোর জন্য ইনকিউবেটরের তাপমাত্রা 37 - 37.5° C এবং আপেক্ষিক আর্দ্রতা 70-75% বজায় রাখা দরকার।

Teacher's Signature ....

– Duck Breeding Center

হাঁসের যত্ন ও রোগ প্রতিরোধ:

- খাকি ক্যাম্পবেল হাঁসের ৭ দিন বয়স পর্যন্ত সব থেকে বেশী যত্নের প্রয়োজন। এই সময় এদের তারের জালের ঘেরাটোপে অথবা মাটিতে কাঠের গুঁড়ো বিছিয়ে রাখা হয়।

- ৭ দিন পর এদের সাধারণ ঘেরাটোপে ও মুরগির মতো ছাড়া হয়।

- ৪২ দিন পর্যন্ত বাচ্চা হাঁসদের তাপ দিতে হবে। একে ব্রুডিং বলে। ইলেকট্রিক বাল্ব বা কেরোসিন ল্যাম্পের সাহায্যে তাপ দেওয়া যেতে পারে।

- বাচ্চা অবস্থায় জলের পাত্রের গভীরতা যেন ৫-৭.৫ সেমির বেশী না হয়। বড়ন্ত ও ডিম পাড়া হাঁসের জলের পাত্রের গভীরতা বাড়াতে হবে।
- ঠিকমতো ডিম পাড়ার জন্য হাঁসের ঘরে ১৪-১৬ ঘণ্টার আলো থাকা দরকার। অর্থাৎ ১২ ঘণ্টার দিনের আলো ছাড়াও ২-৪ ঘণ্টা কৃত্রিম আলোর প্রয়োজন।

- পরিষ্কার ডিম উৎপাদনের জন্য হাঁসের ঘরে ডিম পাড়ার বাক্স রাখলে ভালো হয়।
- হাঁসের বিশেষ দুটি রোগের বিরুদ্ধে প্রতিষেধক টীকা দেওয়া জরুরী। এই রোগ দুটি হলো প্লেগ এবং ডাক কলেরা।

Teacher's Signature ....

ঁহাঁস প্রজনন কেন্দ্র

হাঁস পালন

কৃতজ্ঞতা স্বীকার:

আমাদের পাঠ্যসূচীর অন্তর্গত Field Visit থেকে আমরা অনেক জ্ঞান ও ধারণা লাভ করেছি। এর জন্য আমরা অকপটভাবে আমাদের মহাবিদ্যালয় ও আমাদের Department এর কাছে বিশেষভাবে কৃতজ্ঞ। অন্যান্য ও আমাদের বিশেষভাবে সাহায্য করেছেন সেই শিক্ষকমহাশয় গৌরব সরকারের কাছে এবং রাজ্যসুরাজী মাধ্যমের প্রোজেক্ট অফিসার এবং ডাবগ্রামডি সেন্টার এর প্রোজেক্ট অফিসার Dr. Prabir Sengupta এবং Dr. R.S. Banerjee মহাশয় এর কাছে বিশেষভাবে কৃতজ্ঞ।

16/12/2022

PROJECT REPORT CUM POWERPOINT PRESENTATION

ON

LARVAL FORMS OF ECHINODERMS

Submitted by

Tanushree Chandra
University Roll No. 220311000045
Registration No. 202201004519 of 2022-23

Dr. Saurabh Sarkar
Assistant Professor
Department of Zoology
Gushkara Mahavidyalaya

Gushkara Mahavidyalaya
NAAC Accredited Degree College
P.O: Gushkara, Dist: Purba Burdwan,
Pin-713128, West Bengal.
Email: guskaramahavidyalaya@gmail.com

DATE: 25.06.2023

Certificate

This is to certify that, the following students of B.Sc. Semester II Zoology Honours have worked out the Project work entitled "***Larval forms of Echinodermata***" under my supervision and the entire work has been carried out by him/her in the Gushkara Mahavidyalaya, Department of Zoology, Guskara. The project work represents an explanation of his/ her own words. Most of the data were composed from someone's findings with reference.

Name of the students:

1. ANUSHKA GHOSH
2. DIYA PATRA
3. JASMIN SULTANA
4. JAYESH GHOSH
5. MD USMAN GANI
6. MOUPRIYA GHOSH
7. MOUPRIYA MANNA
8. MRINMOYE PAL
9. PRIYA MONDAL
10. PRIYANKA MONDAL
11. SATASRI MONDAL
12. SK SAJID
13. SK WAYASEFUL ISLAM
14. SOUMYADIP SAHA
15. SOYFUDDIN SK
16. SURYA GARAI
17. SWAPRAVA BARI
18. TANISHA NASRIN
19. TANUSHREE CHANDRA

Saurabh Sarkar

25.06.23

Dr. Saurabh Sarkar
Assistant Professor
Department of Zoology
Gushkara Mahavidyalaya

INTRODUCTION

- Echinoderms are unisexual animal with no sexual dimorphism.
- Fertilization external
- Echinoderms are deuterostomes and hence cleavage is radial, holoblastic and indeterminate.
- Development is mostly indirect having larval stage in between.

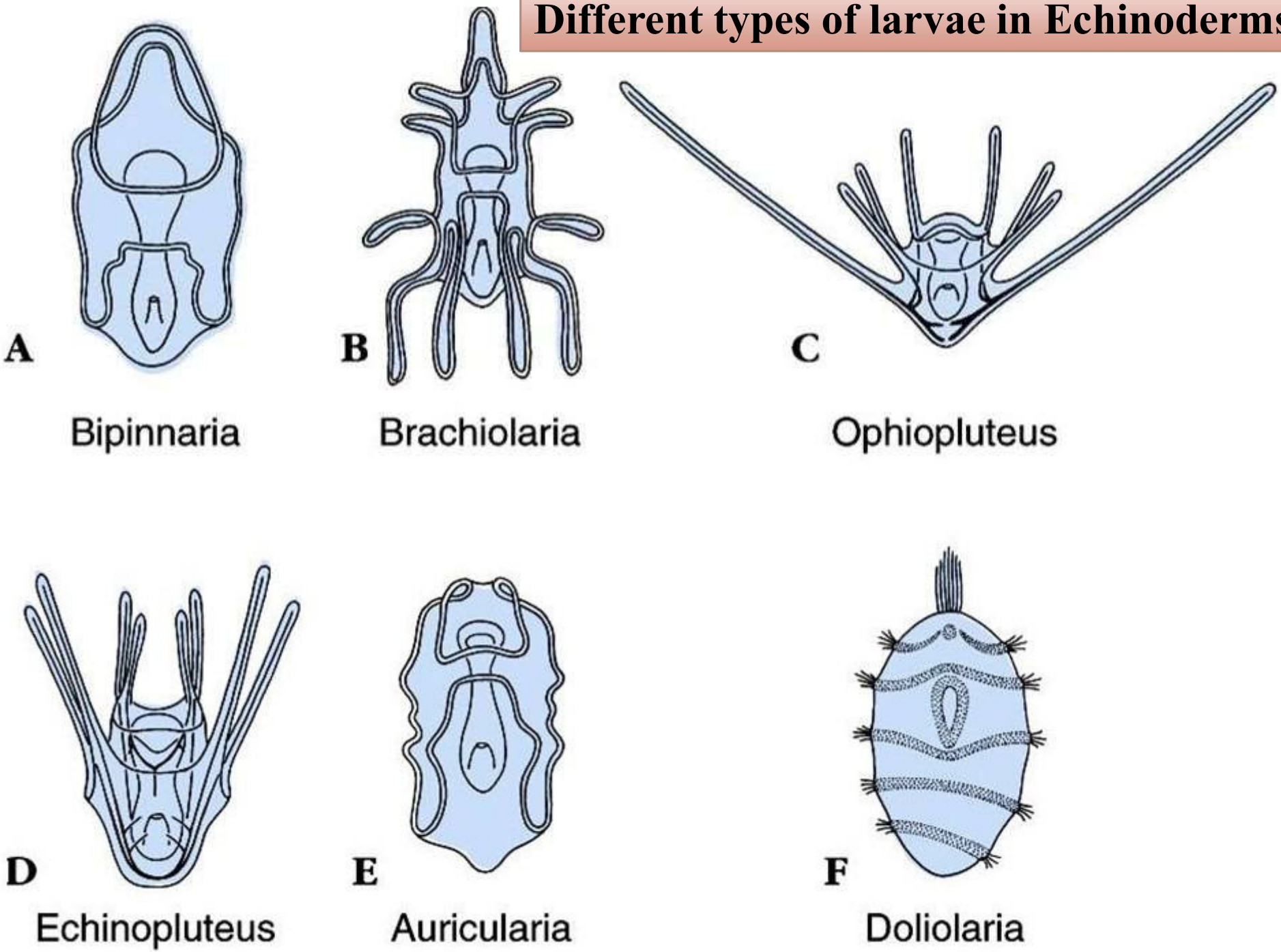
LARVA

- The larvae hatch in water, feed and grow through successive larval stages to become adults.
- Larvae of Echinoderms are bilaterally symmetrical but lose symmetry during metamorphosis.
- Different classes of Echinoderms show structurally different larval stages.
- Comparison of the larval stages of different classes can reveal their evolutionary ancestry.

LARVAL FORMS OF DIFFERENT CLASSES

CLASS	LARVAL FORMS
ASTEROIDEA	Bipinnaria
	Branchiolaria
OPHIUROIDEA	Ophiopluteus
ECHINOIDEA	Echinopluteus
HOLOTHUROIDEA	Auricularia
	Doliolaria
CRINOIDEA	Doliolaria
	Pentacrinoid larva of antedon/ Cystidean

Different types of larvae in Echinoderms

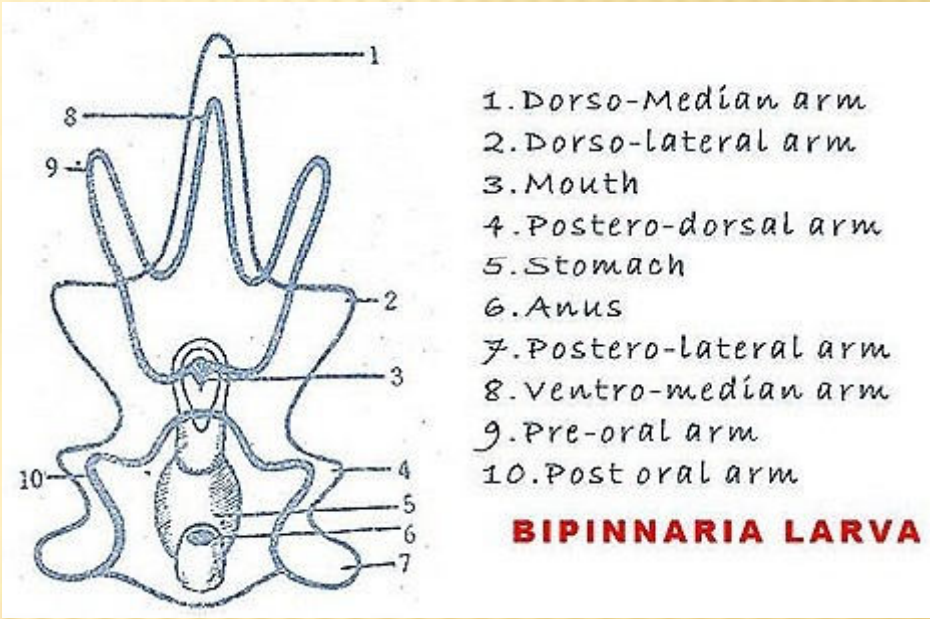


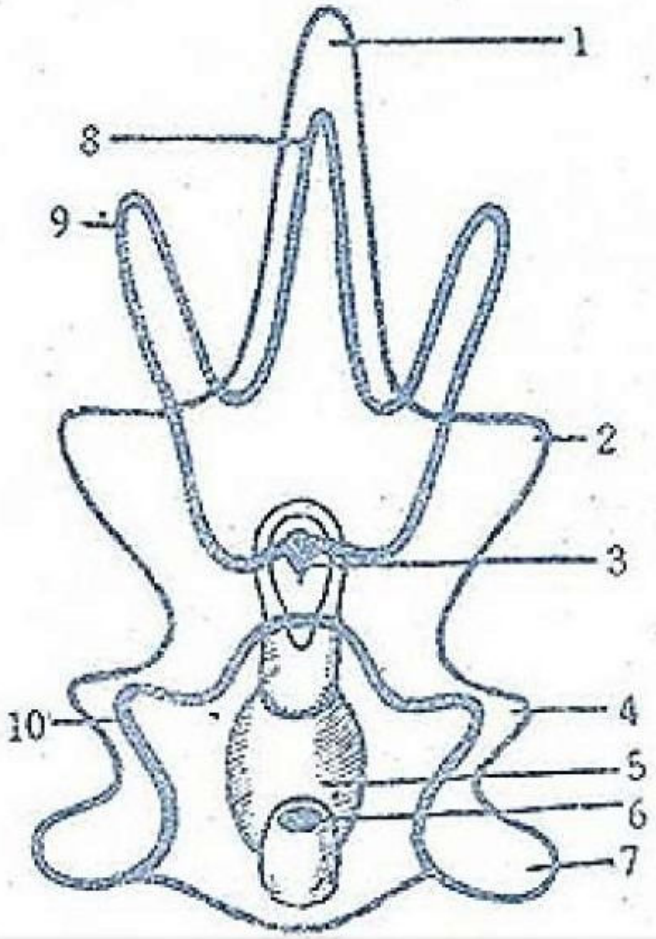
BIPINNARIA LARVA

- It is the first larval form of Asteroidea.
- It is a bilaterally symmertrical, free swimming, pelagic larva.
- The pre oral region is elongated, postoral region is broad.
- It possesses two ciliated bands, the pre oral and post oral bands
- The anterior end of the archenteron develop as mouth whereas the blastopore becomes the anus.
- The pre oral and post oral ciliated bands are continued over a series of prolongation called arms.
- The bipinnaria larva is free swimming and free feeding form.
- After a short period of time, it transforms into branchiolaria larva.

The following are the names and the number of arms developing from pre oral and post oral ciliated bands :

- Postero lateral arm - two
- Post oral arm - two
- Postero dorsal arm - two
- Antero dorsal arm - two
- Pre oral arm - two
- Ventero median arm - one
- Dorso median arm - one



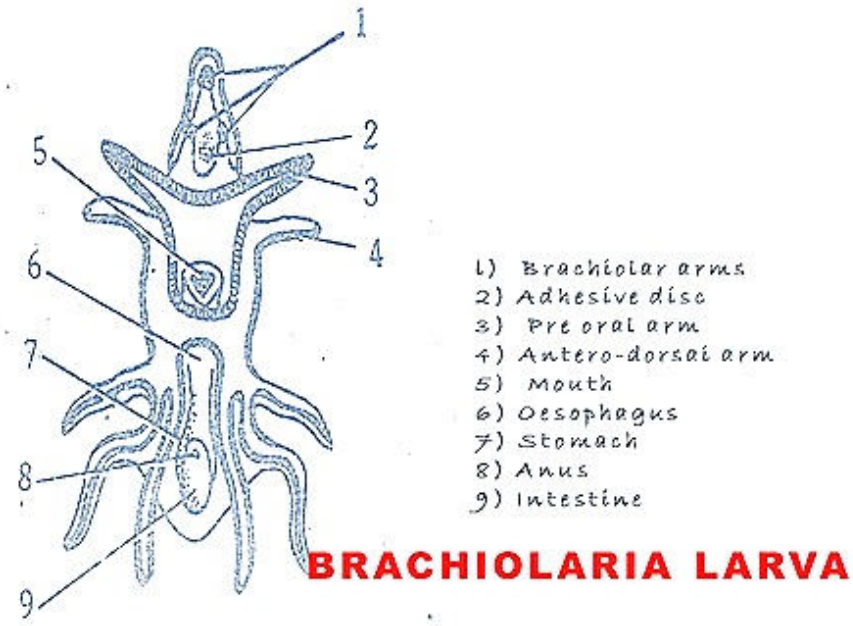


1. Dorso-Median arm
2. Dorso-lateral arm
3. Mouth
4. Postero-dorsal arm
5. Stomach
6. Anus
7. Postero-lateral arm
8. Ventro-median arm
9. Pre-oral arm
10. Post oral arm

BIPINNARIA LARVA

BRANCHOLARIA LARVA

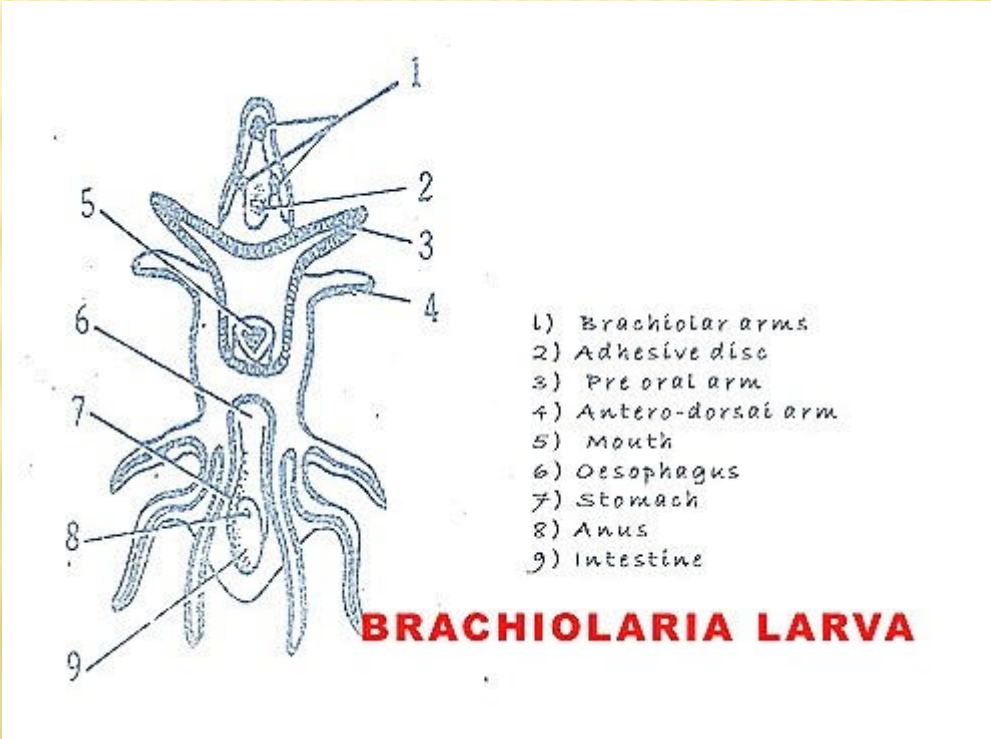
- Three additional arms are present on this larval form known as branchiolarian arms.
- These help the larva to adhere with the substratum.
- These arms are neither ciliated nor have calcareous rods and the coelomic cavity extends into these arms
- The three short arms are at pre oral lobe, one median and two lateral arms.
- They contain adhesive cells at their tips which act as a sucker.
- The rest arms degenerate and become long, narrow and slender.

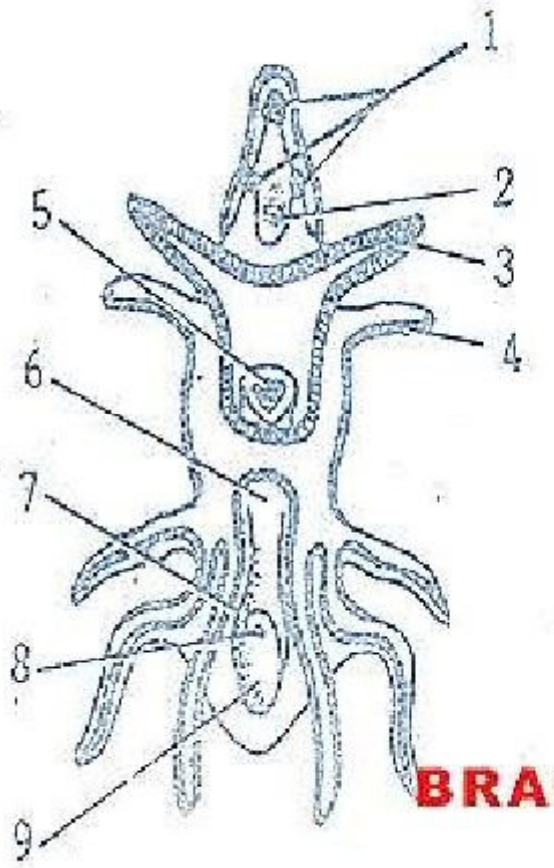


BRACHIOLARIA LARVA

METAMORPHOSIS OF BRANCHIOLARIA

- With the help of adhesive structures, it attaches to some object.
- Anterior portion acts as stalk for some time while posterior part having gut and coelomic chambers convert into a young starfish.
- This detaches itself and starts leading a free life.



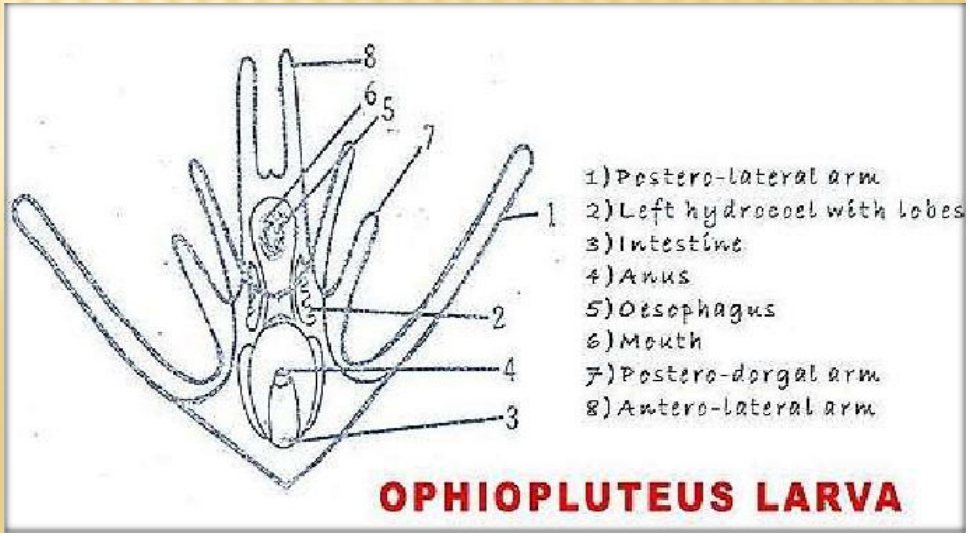


1) Brachiolar arms
2) Adhesive disc
3) Pre oral arm
4) Antero-dorsal arm
5) Mouth
6) Oesophagus
7) Stomach
8) Anus
9) Intestine

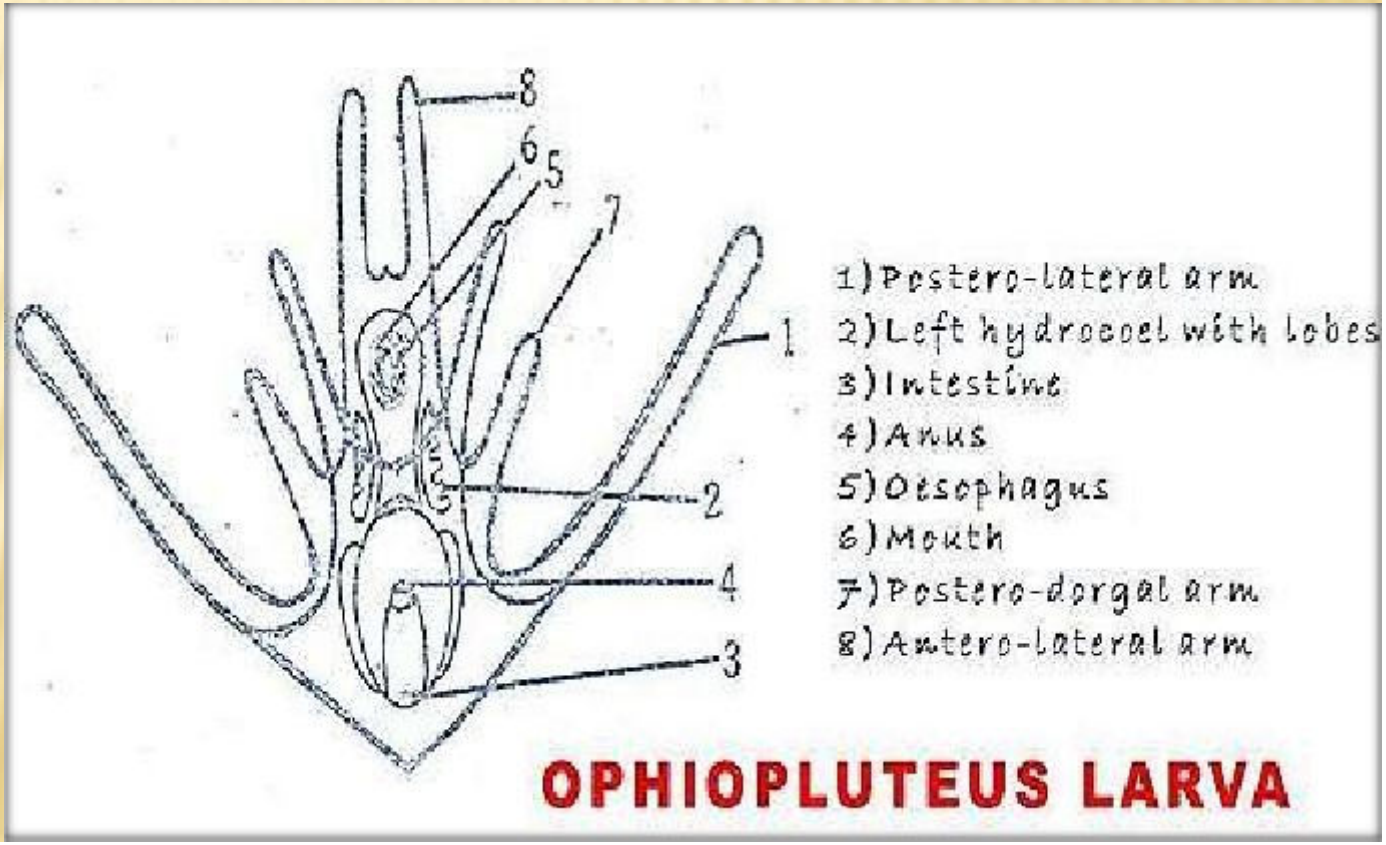
BRACHIOLARIA LARVA

OPHIOPLUTEUS LARVA

- This is the larval form of class Ophiuroidea
- This is free swimming, bilateral symmetrical form having a single ciliated band.
- It possesses long arms with ciliated bands at the margin.
- It has two anterio lateral, two post oral, two posterio dorsal and two posterio lateral arms.
- Out of these, posterio lateral arms are the longest and directed forward



- It has comparitively smaller, pre oral lobe.
- The post anal part of the body is quite well developed.
- Larva consists of coelomic chambers and archenteron.
- There being no attachment stage.
- Free swimming larva, metamorphose into tiny serpent star, which sinks to the bottom to begin its adult existence.



ECHINOPULTEUS LARVA

- It is a microscopic, free swimming larva of Echinoidea.
- It resembles the Ophiopluteus larva where the only difference is that it has more arms.
- This larva shows ciliated bands which are developed into arms.
- Fully developed larva consists of six arms supported by calcareous rods and its tips are pigmented.
- Postero lateral arms are very short and directed outwards or backwards.
- Locomotion is by ciliated bands, which in some cases become thickened and called Epaulettes
- There is no attachment stage.
- Metamorphosis is extremely rapid taking place in about an hour.

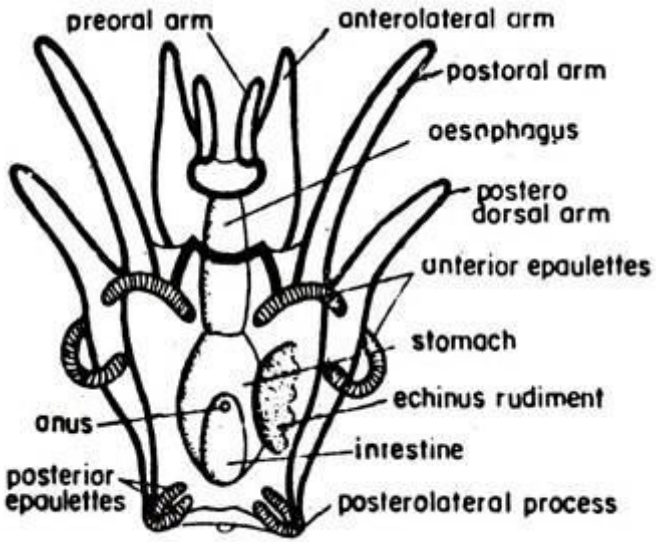


Fig. 27.11. Echinopluteus larva

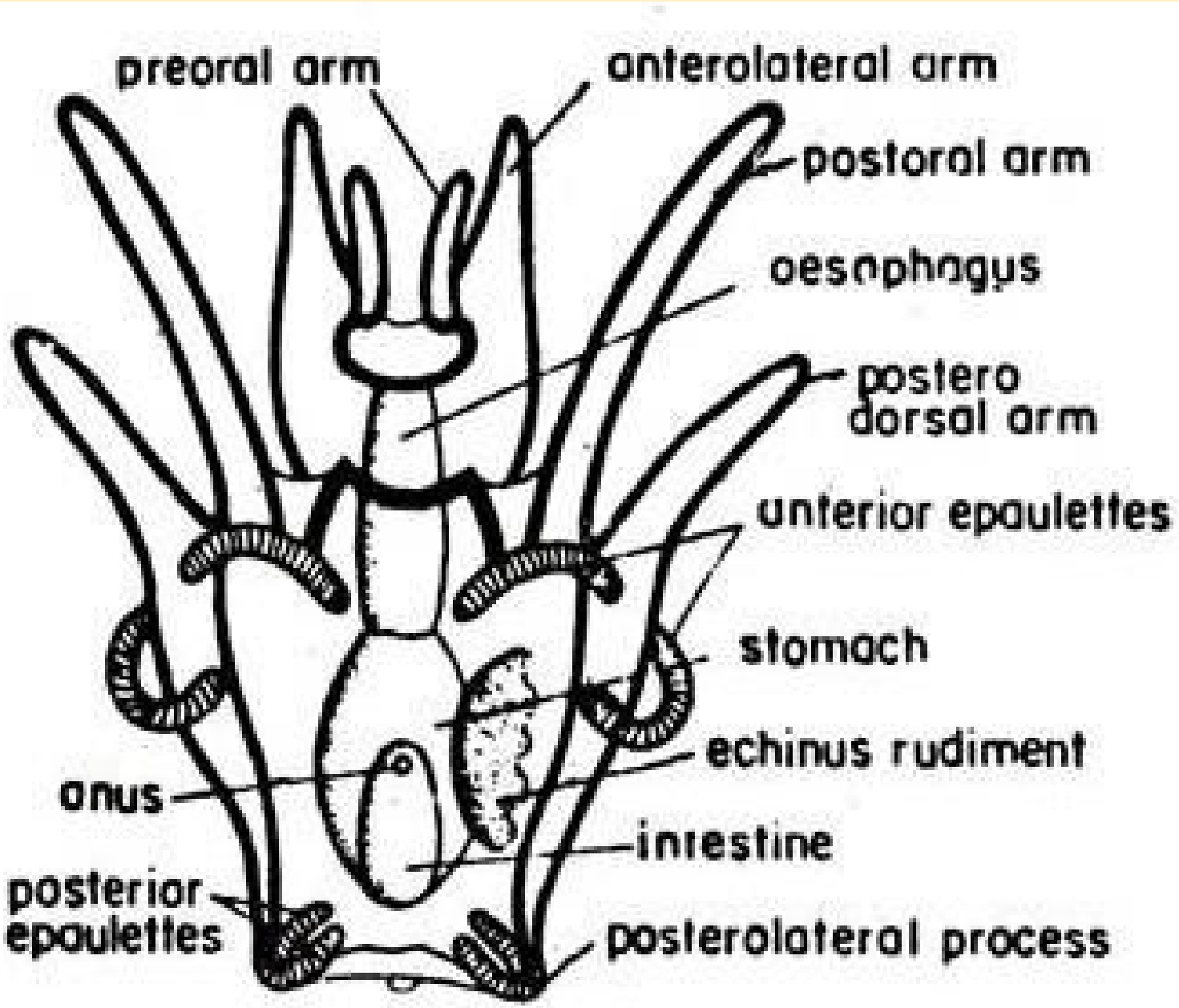


Fig. 27.11. Echinopluteus larva

AURICULARIA LARVA

- It is the first larval form of Holothuroidea.
- It is transparent, free swimming, pelagic larva of about 0.5-1 mm in length.
- Arms are absent. Ciliated bands are well developed.
- It swims about by a ciliated band which forms pre oral loop and an anal loop.
- Alimentary canal is developed which opens with mouth and ends with anus.
- Internally the larva has a curved intestine with sacciform stomach

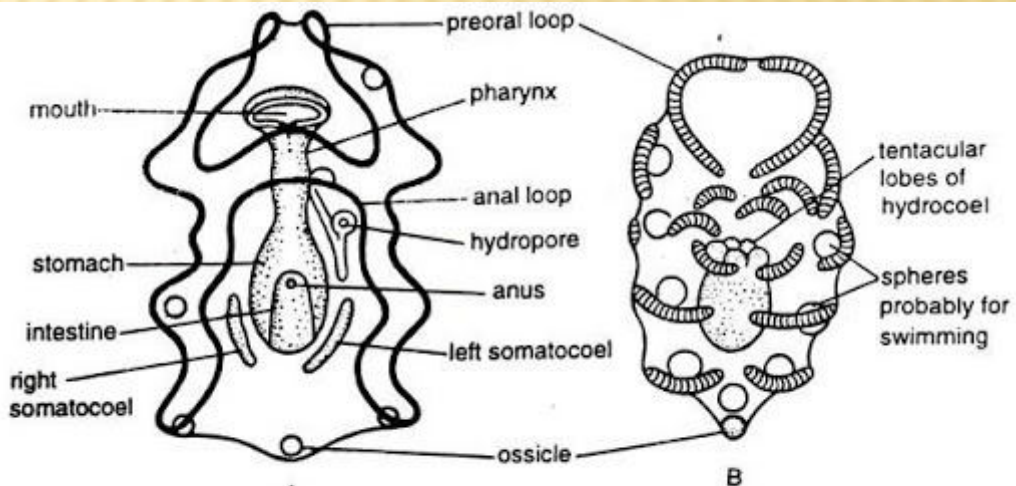


Fig. 27.12. A. Auricularia larva B. Transitional stage from Auricularia to Doliolaria larva

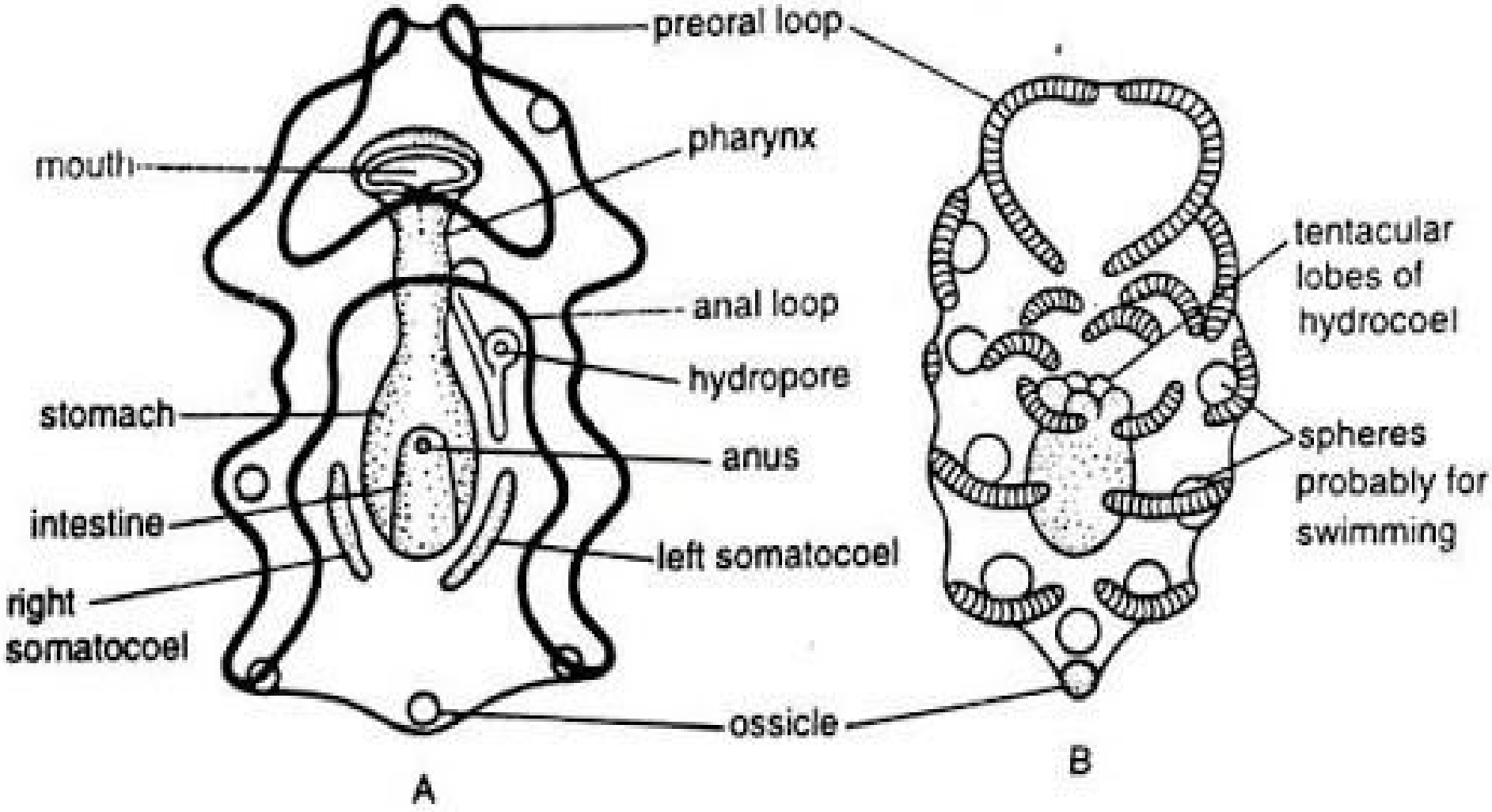


Fig. 27.12. A. Auricularia larva B. Transitional stage from Auricularia to Doliolaria larva

DOLIOLARIA LARVA

- It is the second larval form of Holothuroidea.
- It is a transitional stage from Auricularia larva.
- It is barrel shaped with continuous ciliated band which breaks into three to five flagellated rings.
- Mouth is shifted to anterior and anus to posterior pole.
- Metamorphosis is gradual, during which it acquires five tentacles and one to two functional podia.
- After appearance of more tentacles and podia, sea cucumber settles to the sea bottom and leads an adult mode of life.
- As such it is sometimes called Pentacula.
- In some cases, there is no Auricularia stage, the embryo directly develops into Doliolaria larva.

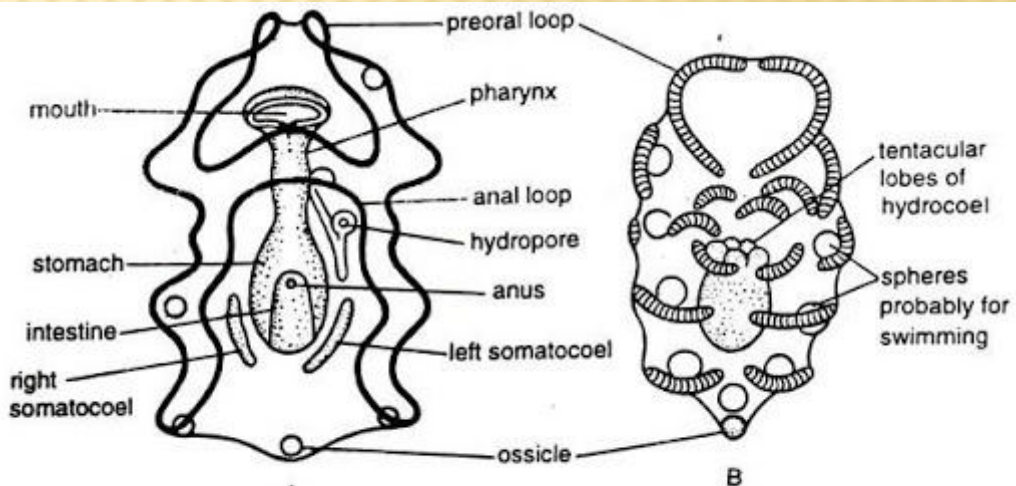


Fig. 27.12. A. Auricularia larva B. Transitional stage from Auricularia to Doliolaria larva

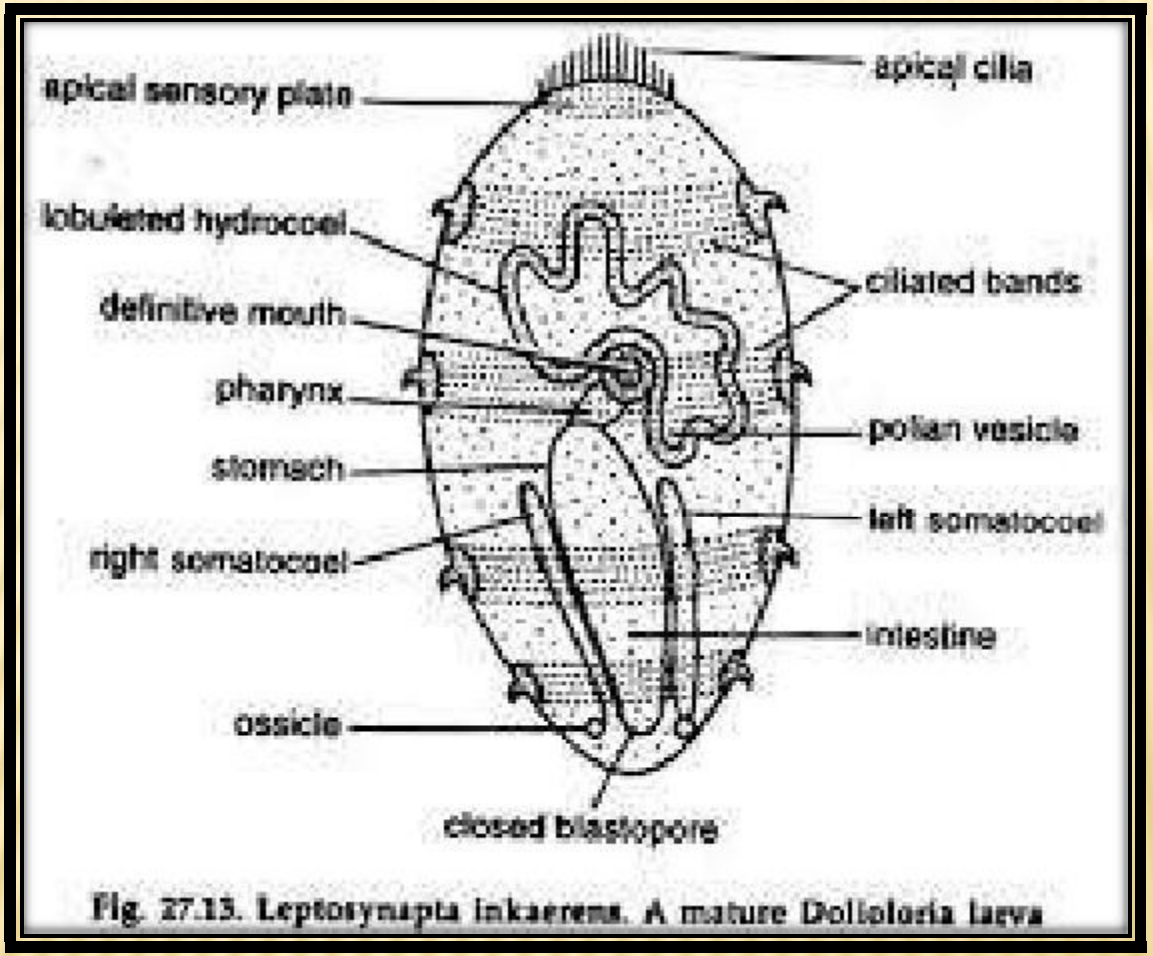
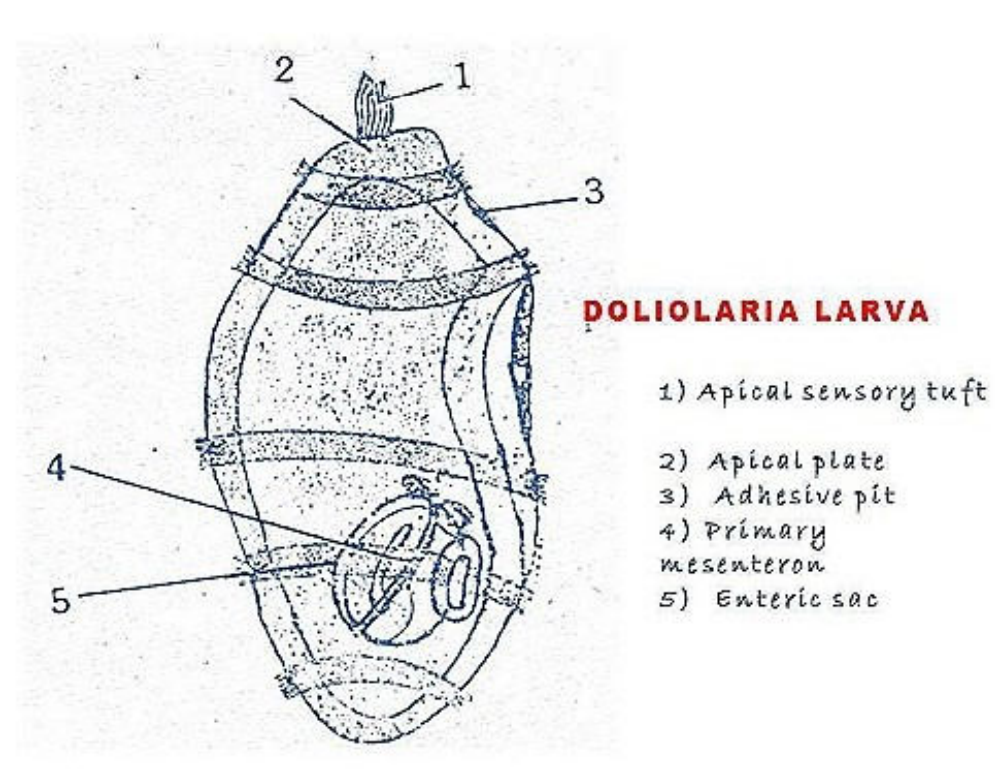


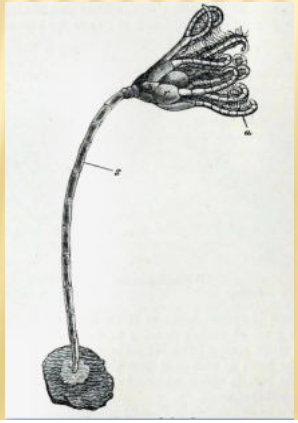
Fig. 27.13. Leptosynapta inkaerens. A mature Dolioloria larva

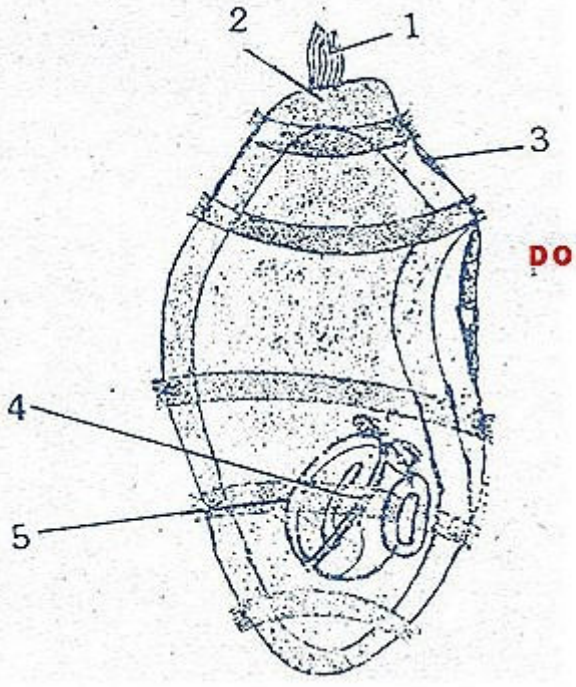
DOLIOLARIA LARVA

- It is the larval form of Crinoidea.
- It is a free swimming larva having four to five ciliated bands.
- It contains an apical tuft of cilia which will be sensory.
- On the mid ventral line, near apical plate, adhesive pit will be present over the first ciliated band.
- Between second and third ciliated band lies stomodeum or vestibule



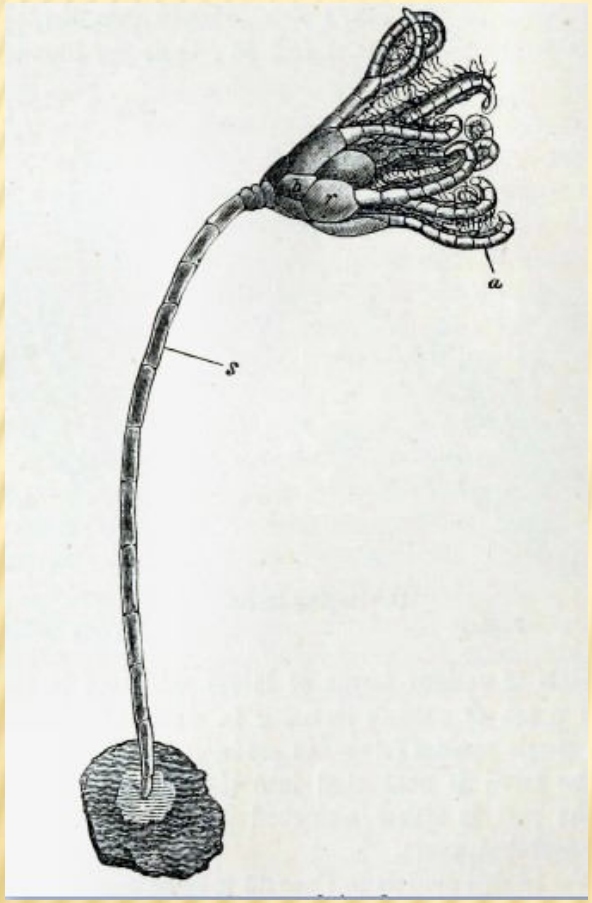
- Skeleton also develops at this larval stage.
- After swimming for some time, it will develop a stalk.
- It is called Pentacrinoid larva.
- Larva now attaches itself and internal organs rotate to 90 degree from ventral to posterior position.
- Larva forms a stalk and is now called as Cystidean or Pentacrinoid larva.
- This after somtime metamorphoses into an adult.





DOLIOLARIA LARVA

1) Apical sensory tuft
2) Apical plate
3) Adhesive pit
4) Primary mesenteron
5) Enteric sac



Pentacrinoid stage of *Antedon bifida*; a) arms: b) basals; r) radials; s) stalk

Development of a Crinoid - A, morula; B, free larva, with bands of cilia; C, young crinoid.

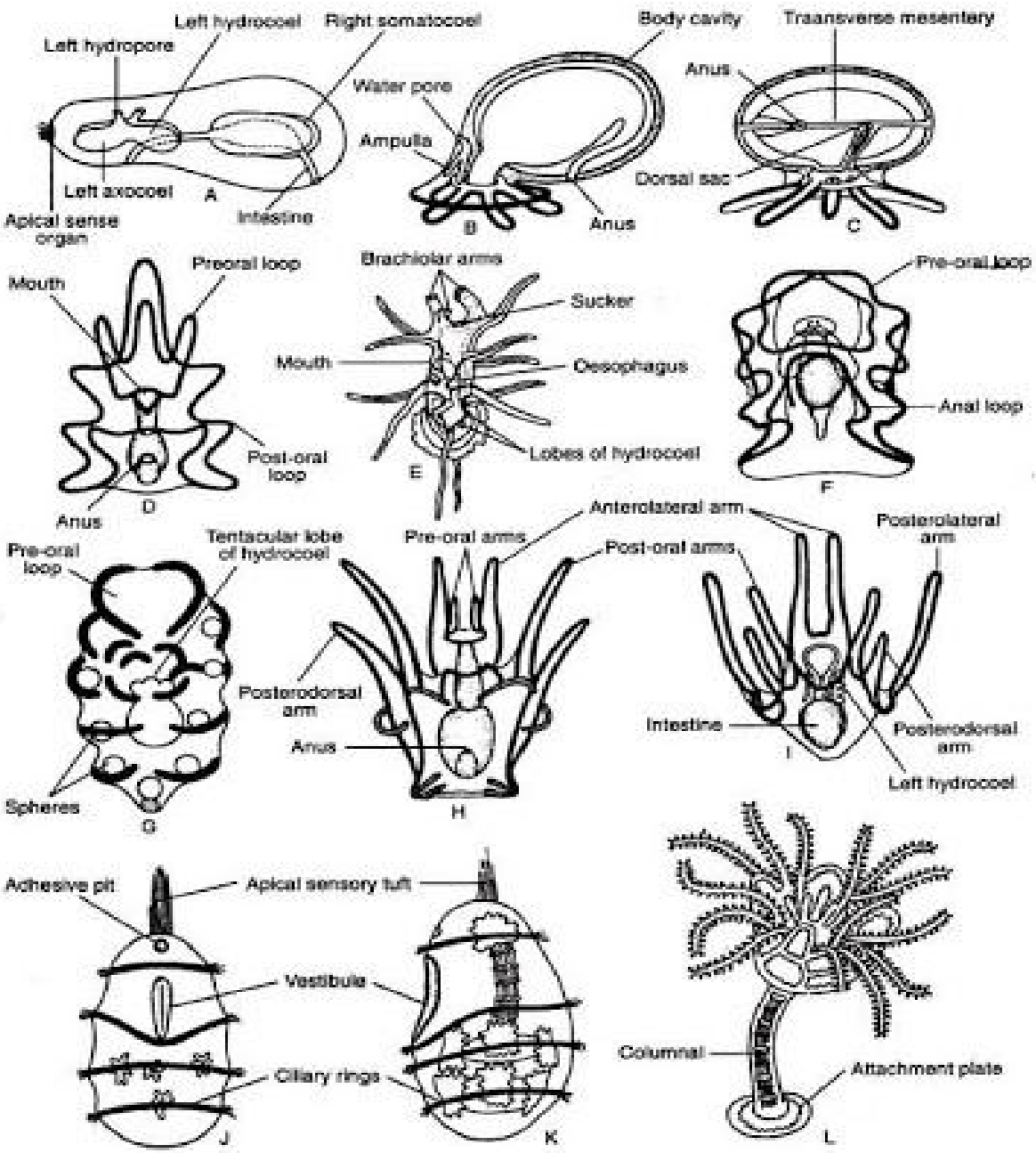


Fig. 21.39: Different larval forms in Echinodermata. A. Hypothetical *Dipleurula* larva. B. Bilateral stage of *Pentactula* larva. C. *Pentactula* larva after torsion of radial position. D. *Bipinnaria* larva. E. *Brachiolaria* larva. F. *Auricularia* larva. G. Transitional stage from *Auricularia* to *Doliolaria* larva. H. *Echinopluteus*. I. *Ophiopluteus*. J. *Doliolaria* or *Vitellaria* larva of *Antedon*. K. Late *Doliolaria* larva of *Antedon*. L. *Pentacrinoid* stage of *Antedon*.

HOMOLOGY AND PHYLOGENY OF ECHINODERM LARVAE

Except for the Crinoids, a sedentary group, the larvae of Asteroidea, Holothuroidea, Echinoidea and Ophiuroidea exhibit some fundamental resemblances:

- Having Pre-oral and Post-oral loops.
- Having V-shaped ciliated bands.
- Presence of gut with its divisions and openings.
- Coelom enterocoelic.

These are some common features indicating that they had a common ancestor.

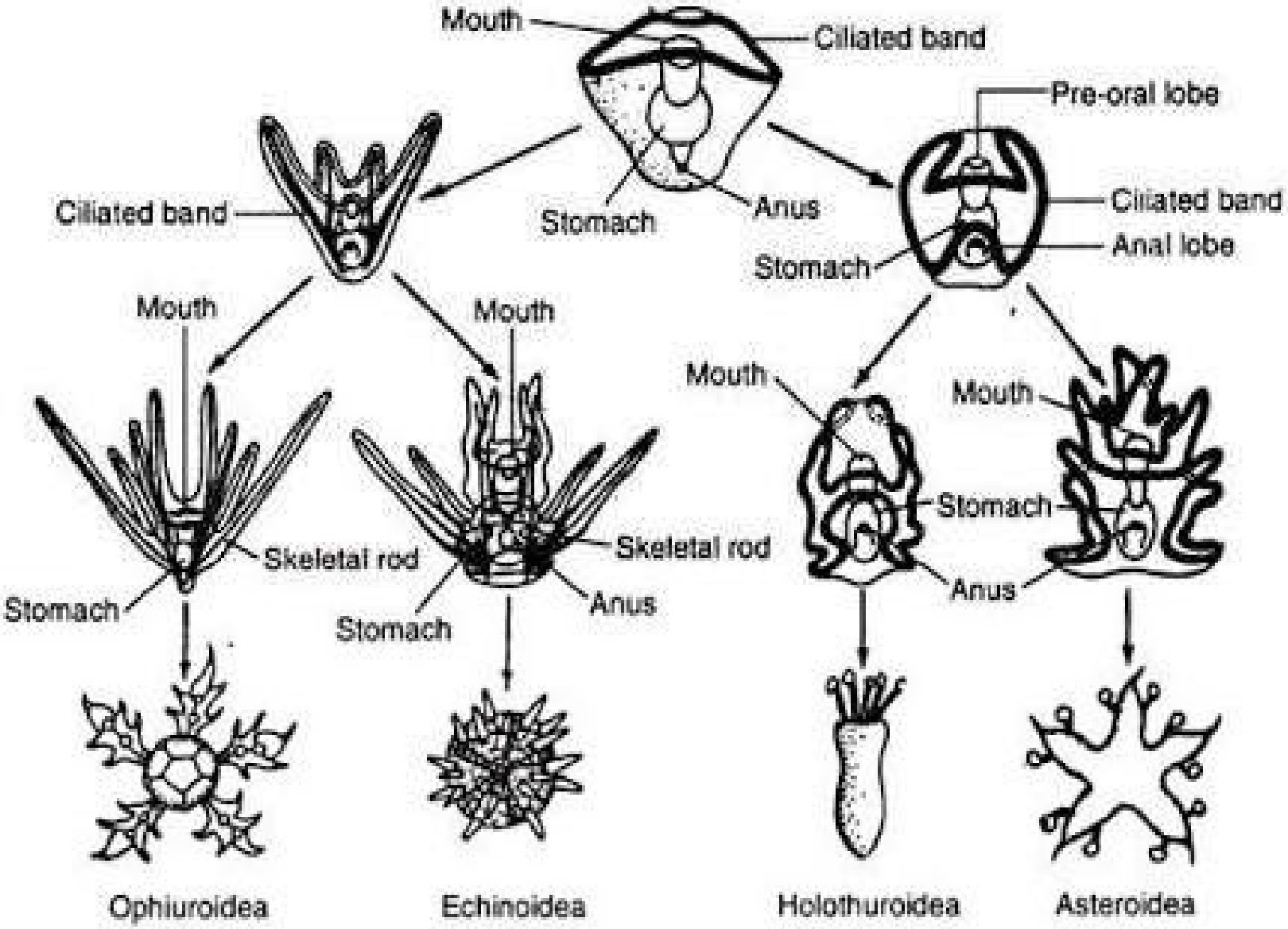


Fig. 21.33: Showing the development of radially symmetrical adult echinoderms from the bilaterally symmetrical larva (after Parker and Haswell).
Note that (i) the Ophiuroidea and Echinoidea are closely related and (ii) Holothuroidea and Asteroidea are closely related.

Hypothetical basic larval ancestral forms are:

1. **Dipleurula** type ancestral larva

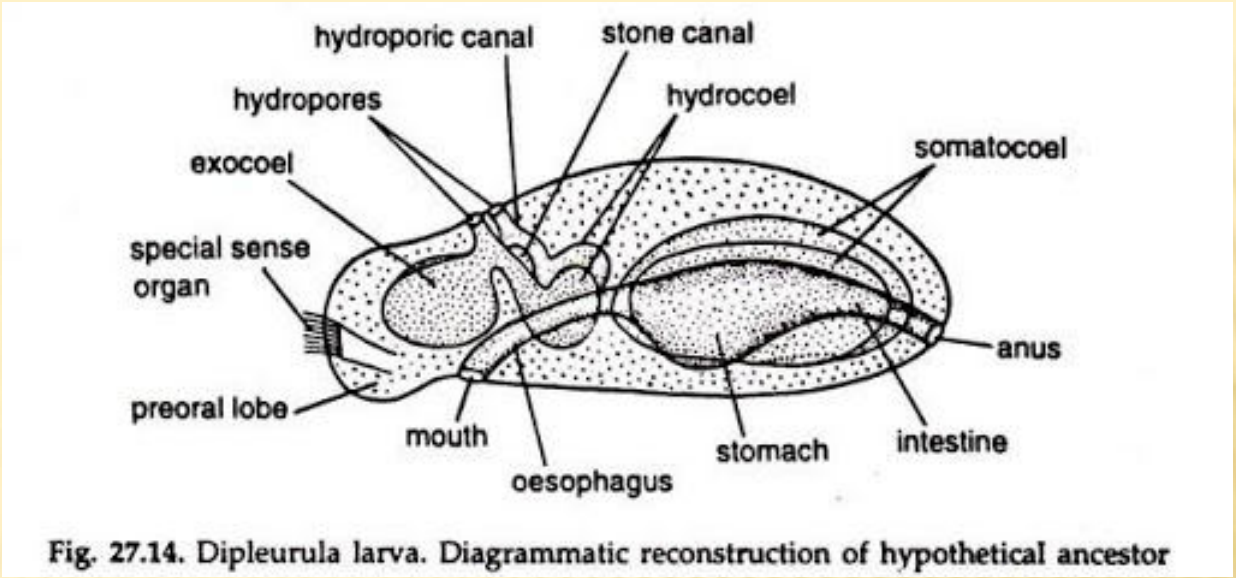


Fig. 27.14. Dipleurula larva. Diagrammatic reconstruction of hypothetical ancestor

2. **Pentactula** type ancestral larva

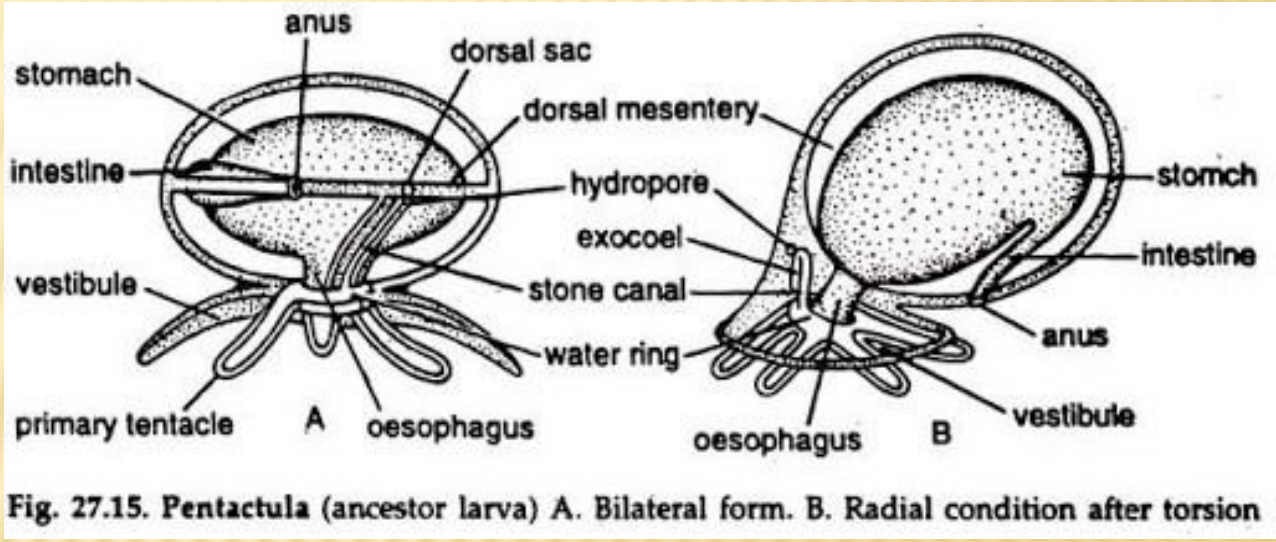


Fig. 27.15. **Pentactula** (ancestor larva) A. Bilateral form. B. Radial condition after torsion

SIGNIFICANCE OF ECHINODERM LARVAE:

1. **Bilateral larvae** are transformed in **radically symmetrical** adult.
2. **Deuterostomes**

THANK YOU!

VISIT TO A POULTRY FARM

Name :- Munmun Ghosh

University Roll :- 20061101011, Year :- 3rd

Registration No :- 202001004851

session :- 2022-23

BSc - gen - 5th sem

ভূমিকা (Introduction):

সকল প্রাণীই রাজ্য অ্যানিম্যালিয়ার অন্তর্গত। পশুপালন বলতে সেইসব প্রাণীদের বোঝায় যাদের গৃহপালিত প্রাণীরূপে প্রতিপালিত করা হয়। যেমন - গবাদি পশু ও পোলট্রি পাখি। সেসমস্ত গৃহপালিত প্রাণী থেকে আমরা খাদ্য (মাংস, ডিম, দুধ), বস্ত্র ও অন্যান্য সামগ্রী পেয়ে থাকি এবং যাদের বিভিন্ন অর্থনৈতিক কাজে ব্যবহার করা হয় তাদের প্রাণীজ সম্পদ বলে।

■ পোলট্রি (Poultry)

যে সকল খাদ্য উৎপাদনকারী অর্থনৈতিক গুরুত্বপূর্ণ পাখিদের পালন করা হয় তাকে পোলট্রি বলে।

পোলট্রি পাখি প্রধানত ২ রকমের হয়, যথা - মুরগী ও হাঁস

■ মুরগী	হাঁস
পর্ব : কর্ডাটা	পর্ব : কর্ডাটা
উপপর্ব : ভার্টিব্রাটা	উপপর্ব : ভার্টিব্রাটা
শ্রেণী : অ্যাভিস	শ্রেণী : অ্যাভিস
উপশ্রেণী : নিওর্নিথিস	উপশ্রেণী : নিওর্নিথিস
বর্গ : গ্যালিফর্মিস	বর্গ : অ্যানসেরিফর্মিস
গণ : গ্যালাস	গণ : অ্যানাস
প্রজাতি : ডোমেস্টিকাস	প্রজাতি : প্ল্যাটিরিনকোস

- কয়েকটি মুরগীর প্রজাতি : হোয়াইট লেগহর্ন, রোড আইল্যান্ড রেড, অরপিংটন, আসেল ইত্যাদি।
- কয়েকটি হাঁসের প্রজাতি : ইন্ডিয়ান রানার, খাকি, খাকি ক্যাম্পবেল, হোয়াইট পিকিন ইত্যাদি।

সারসংক্ষেপ (Summary):

গত 16/11/2022 আমরা তুফানগঞ্জ মহাবিদ্যালয়ের ছাত্রছাত্রী ও শিক্ষকগণ মিলে রাজ্য মুরগী খামার দেখতে গিয়েছিলাম। প্রথমে আমরা বাসে করে বর্তমান নবনির্মিত বাসস্ট্যান্ড -এ আসি তারপর টোটোতে করে গোলাপাড়া এ পৌঁছালাম। 10:30 নাগাদ স্যার আসার পর আমরা রাজ্য মুরগী খামারে গেলাম। প্রথমেই আমাদের চুনজলের উপর দিয়ে হেঁটে আসতে হলো।

সেখানে উপস্থিত ছিলেন গোলাকপাড়া রাজ্য মুরগী খামারের Assistant প্রফেসর Dr. Prabir Sengupta. তিনি আমাদের মুরগী পালন সম্পর্কিত নানা তথ্য আমাদের সামনে উপস্থাপন করেছিলেন যা আমরা খাতায় লিপিবদ্ধ করেছিলাম।

প্রথমে মুরগীর ডিমকে জীবাণুমুক্ত করা হয় $KMnO_4$ + ফরমালডিহাইডের ধোঁয়া দিয়ে। তারপর ইনকিউবেশন করা হয় এবং 18 দিন পর ইনকিউবেশন পিরিয়ড শেষ হয়। তারপর বাচ্চাদের খেতে 2-3 দিন পর তিন মুহূর্তে বাচ্চা হয়। আমরা ~~সমস্ত~~ ~~মেশিন~~ ইনকিউবেটরের মেশিন ভালোভাবে দেখলাম। তারপর ব্রুড হাউসে গিয়ে বিভিন্ন পদ্ধতিতে তৈরি মুরগীর ঘর দেখলাম। এছাড়া, মুরগীর লিঙ্গ নির্ধারণ করতে পারছিলাম সবাই।

তারপর Dr. Prabir Sengupta Sir আমাদের পোলট্রি সম্পর্কিত বিভিন্ন তথ্য, ডিমের মূল্যায়ন, পুষ্টিমূল্য ইত্যাদি সম্পর্কে আলোচনা করেছিলেন।

তারপর আমরা পুরো মুরগী খামার ঘুরে দেখার পর পরবর্তী গন্তব্যস্থলের জন্য রওনা দিলাম।

আজ 17/11/2022 আমরা সবাই 7.30 নাগাদ বর্ধমান স্টেশনে পৌঁছালাম। আজ আমাদের গন্তব্যস্থল ছিল হাঁস প্রজনন কেন্দ্র। তারপর স্যার আসার পর আমরা টোটো করে সবাই duck breeding centre এ পৌঁছে গেলাম। সেখানে উপস্থিত ছিলেন Duck breeding centre এর Ast. Proffesor Dr. R.S Banerjee.

তিনি আমাদের হাঁস পালন সম্পর্কিত নানা তথ্য আমাদের বলছিলেন যা আমরা খাতায় লিপিবদ্ধ করছিলাম। তারপর আমরা খুব ঘুরতে গেলাম এবং হাঁসগুলিকে ভালোভাবে পর্যবেক্ষণ করলাম।

মূলত ক্যাম্বেল হাঁস পালন করা হয়েছে প্রজনন কেন্দ্রে। তারপর আমরা ইনকিউবেশন মেশিন বাইরে থেকে দেখলাম। ইনকিউবেশন পিরিয়ড থাকে 28 দিন। সাধারণত 5 মাস পর থেকেই হাঁস ডিম দেয়। খাবার হিসাবে অ্যাজোলা, কাঁচা হলুদ, Alovera, শাক ইত্যাদি। কাঁচা হলুদের জন্য Liver strong হয়।

আমরা খুব ভালোভাবে সবকিছু পর্যবেক্ষণ করলাম। তারপর আমরা ওখান থেকে বেরিয়ে বাড়ির জন্য রওনা দিলাম।

পশ্চিমবঙ্গ রাজ্য সুরক্ষী খামার

Report

(i) [illegible] রাজ্য মুরগী খামার:

মুরগী পালন আজ একটি অর্থকরী শিল্প। মুরগী পালন করে অনেক বেকার যুবক যুবতী নিজেদের সংসার চালাচ্ছে। কিন্তু বেশীর ভাগ খামারি এই ব্যবসা সম্বন্ধে প্রাথমিক বৈজ্ঞানিক জ্ঞানের অভাবে লোকসান করে বন্ধ করে দিতে বাধ্য হচ্ছে।

উন্নত মুরগী: ডিমের জন্য পোষা মুরগীকে লেয়ার পাখী বলে। ৮ সপ্তাহ পর্যন্ত এদের চিক বলা হয়, ৯-১৮ সপ্তাহ পর্যন্ত গ্রোয়ার বলে এবং ১৯ সপ্তাহের পর ডিম পাড়া আরম্ভ করলে তাকে লেয়ার বলে। ~~[illegible]~~

পালন পদ্ধতি: কেজ ও ডিপলিটার পদ্ধতি।

■ কেজ: কম জায়গায় বেশী মুরগী পালন করা যায়। কিন্তু এই পদ্ধতিতে প্রাথমিক খরচ অনেক বেশী। কেজে দুটি লেয়ার পাখীর জন্য 16" x 10" এবং উচ্চতা 17" জায়গার প্রয়োজন হয়।

■ ডিপলিটার পদ্ধতি:- আলো বাতাসযুক্ত সাধারণ ঘরে বা কাঠের প্ল্যাটফর্মের ওপর খাঁচার মতো ঘর তৈরি করে নেওয়া হয়। ঘরের মেঝেতে ভালোভাবে চুন, ব্লিচিং পাউডার ছড়িয়ে ঘর পরিষ্কার ও শুকনো করা হয়। তারপর ঘরের মেঝেতে লিটার বিছানো হয়। লিটার তৈরির জন্য প্রয়োজনীয় উপাদানগুলি হলো কাঠের গুঁড়ো, [illegible], তুলোবীজ, খড়, তুষ, ছোবড়া ও ধানের তুষ, ভুট্টা ও আখের খোসা ও ছোটো ছোটো করে কাটা খড়। প্রথমে কাঠের গুঁড়ো ছড়িয়ে তার ওপর কাটা খড় বিছানো হয়। ~~[illegible]~~ তারপর 10-15 cm পর্যন্ত পুরু লিটার তৈরি করা হয়। এই লিটার মুরগীর বিষ্ঠা দ্বারা ভালোভাবে মিশে গেলে তখন এই লিটারের [illegible] হতে ডিপ লিটার প্রস্তুত হয়।

Teacher's Signature ....

cardeeing

■ খাবার জল: পানীয় জল জীবাণুমুক্ত হওয়া উচিত।

■ ঘরের আলো: আলোর প্রয়োজন হয় মুরগীর দেহের সার্বিক বৃদ্ধি ও ডিম উৎপন্ন করার জন্য। প্রথম ৩ সপ্তাহ ২৪ ঘন্টার কৃত্রিম আলোর ব্যবস্থা করতে হবে। চতুর্থ সপ্তাহ থেকে দিনের ও কৃত্রিম আলো মিলিয়ে ১৪ ঘন্টা দিতে হবে। ব্রয়লার মুরগীর ক্ষেত্রে ২৩ ঘন্টার আলোর প্রয়োজন হয়।

■ ঘর শোধন: ঘরের ফাটলের অংশ আগে জলে ভিজিয়ে ব্লো ল্যাম্প দিয়ে পুড়িয়ে ৫% কপার সালফেট জল দিয়ে স্প্রে করে তারপর ১০% ফিনাইল জল দিয়ে ঘর ধুতে হবে।
জলের পাত্র ও খাবারের পাত্র নিয়মিত পরিষ্কার করতে হবে।

■ বাচ্চা আনার পদ্ধতি:
হাইব্রিড লেয়ার মুরগীর বাচ্চা পালন করার উপযুক্ত সময় হল ফেব্রুয়ারীর ১৫ মার্চ থেকে মার্চ মাসের ১৫ তারিখ। এই সময় বাচ্চা কেনে যায় ও পাখিরা অক্টোবর-সেপ্টেম্বর মাস থেকে ডিম পাড়তে শুরু করে।

■ ব্রুডিং: একদিনের বাচ্চাকে কৃত্রিম উপায়ে লালন পালন করার ব্যবস্থাকে ব্রুডিং বলে। এই সময়ে তাপমাত্রার সাহায্যে তাপ দিয়ে বাচ্চার হজম শক্তি বাড়ানো হয় এবং তাড়াতাড়ি পালক উঠতে সাহায্য করা হয়। সাধারণত ২১ দিন এই তাপ দেওয়া হয়। শীতকালে ৬ সপ্তাহ বয়স পর্যন্ত তাপ দেওয়ার প্রয়োজন হয় লেয়ার পাখির ক্ষেত্রে।

■ ব্রুডার ঘর: ১০০ বাচ্চার জন্য ৭ সপ্তাহ বয়স পর্যন্ত ৩৫-৫০ বর্গফুট জায়গা লাগে। প্রথম সপ্তাহে ২০ বর্গফুট, ২য় সপ্তাহে ৩০ বর্গফুট ও ৩য় সপ্তাহে ৭০ বর্গফুট জায়গা দিতে হবে।

খাদ্য গুদাম

হ্যাচারি

• আলোসমূহ: ইলেকট্রিকে ৬০ ওয়াটের ১টি বাল্বে 100 বাচ্চাকে আলো দেওয়া হয়। 100 ওয়াটের কার্বাইড বাল্বে 100 বাচ্চাকে আলো দেওয়া হয়, 1টি হ্যাজাকে 100টি বাচ্চাকে এবং 1টি ~~হ্যারিকেনে~~ হ্যারিকেনে 20টি বাচ্চাকে আলো দেওয়া হয়।

• চিকগার্ড: ব্রুডারের চারপাশে ঘেরা জায়গাকে ~~চিক~~ চিকগার্ড বলে। চিকগার্ডের উচ্চতা হবে 1.5 ফুট এবং ব্রুডার থেকে 2.5 ফুটের দূরত্বে থাকবে। চতুর্থ দিনের পর চিকগার্ড সরিয়ে জায়গা বাড়াতে হবে।

• খাবারের ব্যবস্থা:- প্রথম ২-৩দিন ডিম রাখার ট্রে তে খাদ্য দেওয়া হয়। চতুর্থ দিন থেকে খাবার পাত্রে করে দেওয়া হয়।

• মুরগীর খাদ্য: লেয়ার মুরগীর জন্য চিক, গ্রোয়ার এবং লেয়ার তিনরকম ম্যাশ খেতে দিতে হবে।

• ডিবীকিং: ডিবীকিং মানে ঠোঁট কাটা। মুরগীদের ঠোঁট কেটে দিলে খাবার কম নষ্ট হয় ও ঠোকরা ঠুকরি করার প্রবণতা কমে যায়।

• কালিং: কালিং মানে ছাঁটাই। ব্যবসার পক্ষে অনুপযুক্ত মুরগীদের বেছে নিয়ে তাদেরকে বিক্রয় করে দেওয়া উচিত।

* পশ্চিমবঙ্গে প্রয়োজনের তুলনায় ডিমের উৎপাদন খুব কম তার কারণ — (1) আমাদের রাজ্যের দেশী মুরগী খুবই কম এবং ওরা বছরে মাত্র 60- 80 টি ডিম পাড়ে।

(২) বড়ো আকারের ডিমপাড়া মুরগীর খামার অলাভজনক হওয়ার কারণে একরকম নেই বললেই চলে।

(৩) একমাত্র বড় আকারের খামার তৈরি করতে হবে এবং অবশ্যই বাজার থেকে তৈরি খাদ্য না কিনে কাঁচামাল নিজেকেই ~~উৎ~~ তৈরি করতে হবে।

[redacted]

কৃতজ্ঞতা স্বীকার:

আমাদের পাঠ্যসূচীর অনুযায়ী Field visit করতে আমরা অনেক জ্ঞান ও ধারণা লাভ করেছি। এর জন্য আমরা প্রথমেই আমাদের মহাবিদ্যালয় ও আমাদের Department এর কাছে বিশেষভাবে কৃতজ্ঞ। অনুপ্রেরণা ও আমাদের বিশেষভাবে সাহায্য করেছেন সেই শিক্ষকমন্ডলীর সৌজন্যে আমাদের কাছে অত্যন্ত গুরুত্বপূর্ণ আমাদের প্রোজেক্ট এবং ডাকস্টিচিং সম্পর্কে এর প্রোজেক্ট অফিসার Dr. Prabin Sengupta এবং Dr. R.S. Banerjee মহাশয় এর কাছে বিশেষভাবে কৃতজ্ঞ।

16/12/20

Project Title: Glimpses of India's Wildlife in context of world

- The natural wealth of the Indian subcontinent has remained unique, mysterious and fascinating for nature lovers.
- India's wildlife is both rich and varied. More than 4% of India's land is under forest cover. Variety of India's wildlife can be seen in the 90 National Parks, 482 wildlife sanctuaries and 23 tiger reserves established by the Government of India in an attempt to conserve this vital resource.
- According to one study, India along with 17 mega diverse countries is home to about 60-70% of the world's biodiversity.
- India, lying within the Indomalaya eco-zone, is home to about 7.6% of all mammalian, 12.6% of avian, 6.2% ofIndia, lying within the Indomalaya ecozone, is home to about 7.6% of all mammalian, 12.6% of avian & 6.2% of reptilian.
- For Wildlife Enthusiasts, India is the perfect place to see wild animals in their natural habitat.

Endangered Species

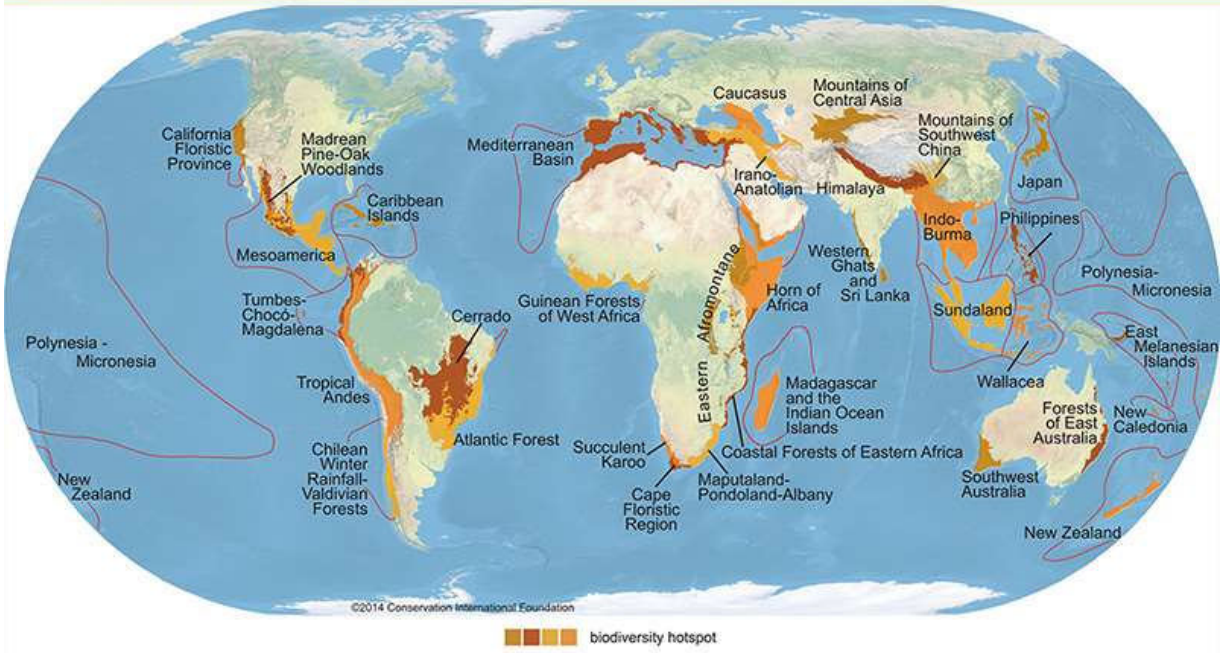
- **Asiatic Black Bear**
- **Asiatic Lion**
- **Asiatic Wild Dog/ Dhole**
- **Banteng**
- **Blue Whale**
- **Capped Leaf Monkey**
- **Chiru /Tibetan Antelope**
- **Wild Cat**
- **Fin Whale**
- **Ganges River Dolphin**
- **Golden Leaf Monkey**
- **Great Indian Rhinoceros**
- **Hispid Hare**
- **Indian Elephant or Asian Elephant**

- **Indus River Dolphin**
- **Andaman Shrew**
- **Asian Arowana**
- **Kashmir Stag / Hangul**

Conservation of the Species:

- In recent decades, human encroachment has posed a threat to India's wildlife.
- Since India is home to a number of rare and threatened animal species, wildlife management in the country is essential to preserve these species.
- Article 48 of the Constitution of India specifies that, "The state shall endeavour to protect and improve the environment and to safeguard the forests and wildlife of the country" and Article 51-A states that "it shall be the duty of every citizen of India to protect and improve the natural environment including forests, lakes, rivers, and wildlife and to have compassion for living creatures."
- The system of National Parks and protected areas, first established in 1935, was substantially expanded.
- In 1972, India enacted the Wildlife Protection Act
- In 1972, Project Tiger & Project Elephant started in 1992
- Further federal protections were promulgated in the 1980s.
- Forest Rights Act was established in 2008
- Along with over 500 wildlife sanctuaries, India now hosts 15 biosphere reserves & 25 wetlands.
- India contains 172, or 2.9%, of International Union for the Conservation of Nature and Natural Resources (IUCN)-designated threatened species. These include the Asiatic lion, the Bengal tiger, and the Indian white-rumped vulture.
- The most endangered Indian top predator of 2010, the dhole is on edge of extinction. Less than 2500 members of the species remain in the world.
- There are 39 Project Tiger wildlife reserves in India covering an area more than of 37,761 km^2.

There are a total of 34 Biodiversity Hotspots in the world that are listed as:



Conservation International (conservation.org) defines 35 biodiversity hotspots — extraordinary places that harbor vast numbers of plant and animal species found nowhere else. All are heavily threatened by habitat loss and degradation, making their conservation crucial to protecting nature for the benefit of all life on Earth.

Biodiversity Hotspots

North and Central America

1. California Floristic Province
2. Caribbean Islands
3. Madrean pine-oak woodlands
4. Mesoamerica

South America

5. Atlantic Forest
6. Cerrado
7. Chilean Winter Rainfall-Valdivian Forests
8. Tumbes-Chocó-Magdalena
9. Tropical Andes

Europe and Central Asia

10. Caucasus
11. Irano-Anatolian
12. Mediterranean Basin
13. Mountains of Central Asia

Africa

14. Cape Floristic Region
15. Coastal Forests of Eastern Africa
16. Eastern Afromontane
17. Guinean Forests of West Africa
18. Horn of Africa
19. Madagascar and the Indian Ocean Islands
20. Maputaland-Pondoland-Albany
21. Succulent Karoo

South Asia

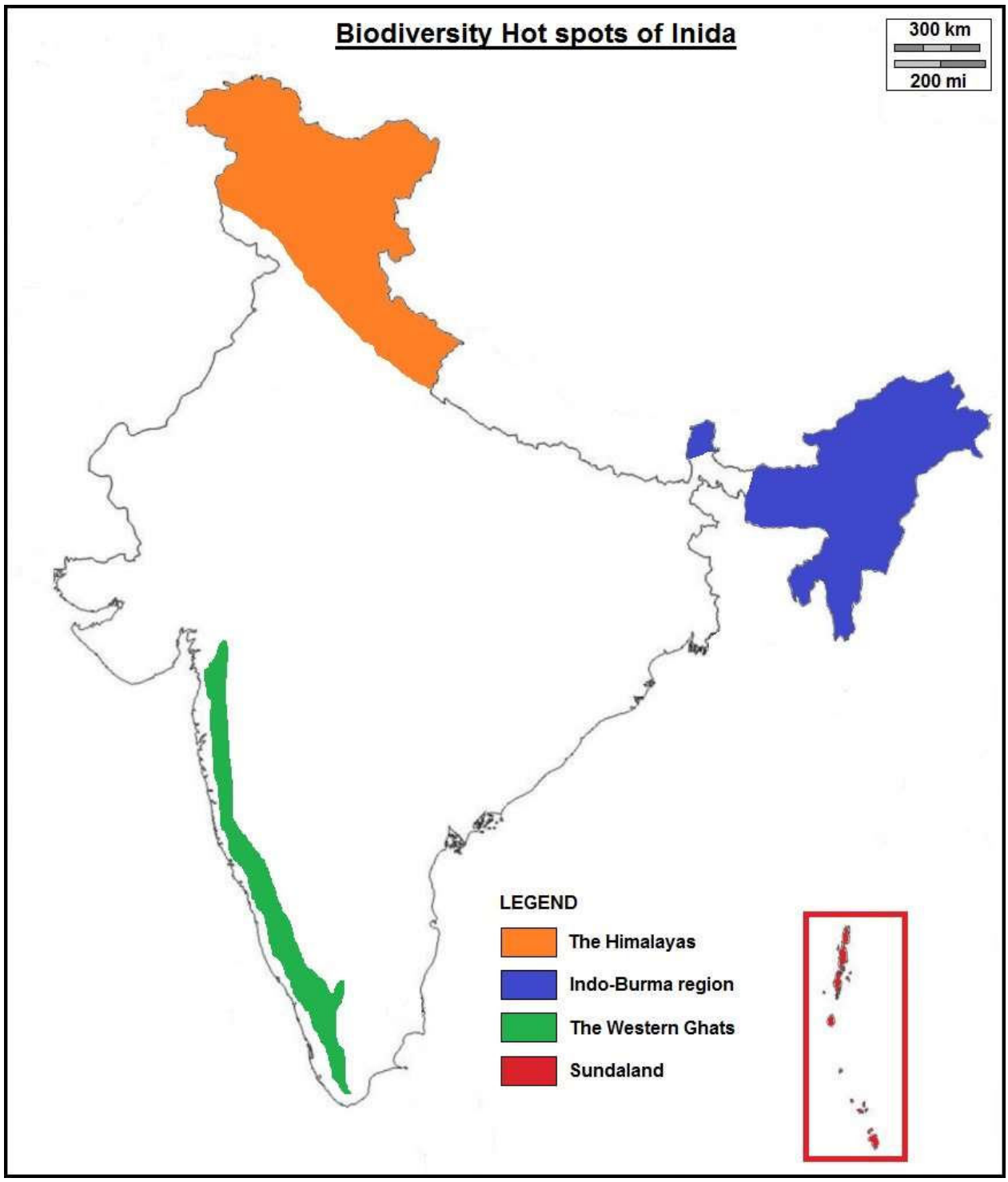
22. Eastern Himalaya, India
23. Indo-Burma, India and Myanmar
24. Western Ghats & Sri Lanka

South East Asia and Asia-Pacific

25. East Melanesian Islands
26. New Caledonia
27. New Zealand
28. Philippines
29. Polynesia-Micronesia
30. Southwest Australia
31. Sundaland
32. Wallacea

East Asia

33. Japan
34. Mountains of Southwest China


Biodiversity Hot spots of Inida
300 km
200 mi
LEGEND
The Himalayas
Indo-Burma region
The Western Ghats
Sundaland

1. Himalaya

http://bsienvis.nic.in/files/Biodiversity%20Hotspots%20in%20India.pdf

Species diversity and endimism

Taxonomic Group	Species	Endemic Species	Endemism (%)
Plants	10,000	3,160	31.6
Mammals	300	12	4.0
Birds	977	15	1.5
Reptiles	176	48	27.3
Amphibians	105	42	40.0
Freshwater Fishes	269	33	12.3

Rhododendron

1. Lammergeier 2. Black Eagle 3. Northern Goshawk
4. Tibetan Partridge

1. Back-Striped Weasel 2. Namdapha Flying Squirrel
3. Red Panda 4. Takin

2. Indo-Burma

Species diversity and endemism

Taxonomic Group	Species	Endemic Species	Endemism (%)
Plants	13,500	7,000	51.9
Mammals	433	73	16.9
Birds	1,266	64	5.1
Reptiles	522	204	39.1
Amphibians	286	154	53.8
Freshwater Fishes	1,262	553	43.8

Orchid

Red-headed vulture

Critically Endangered Sunda pangolin

the saola or "Asian unicorn"

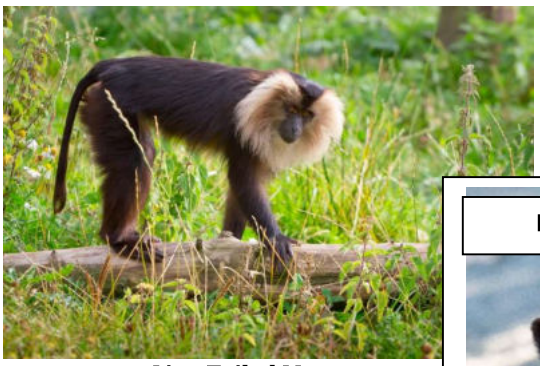
Cambodia's national bird, the giant ibis

3. Western Ghats and Sri Lanka

Taxonomic Group	Species	Endemic Species	Endemism (%)
Plants	5,916	3,049	51.5
Mammals	140	18	12.9
Birds	458	35	7.6
Reptiles	267	174	65.2
Amphibians	178	130	73.0
Freshwater Fishes	191	139	72.8

Hornbill

Gaur (Indian Bison)



Lion Tailed Macaque

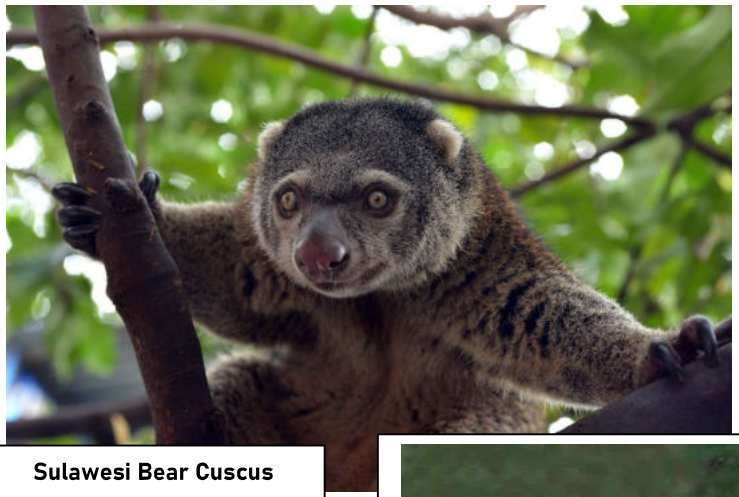
Nilgiri Marten

Baccaurea courtallensis

4. Sundaland

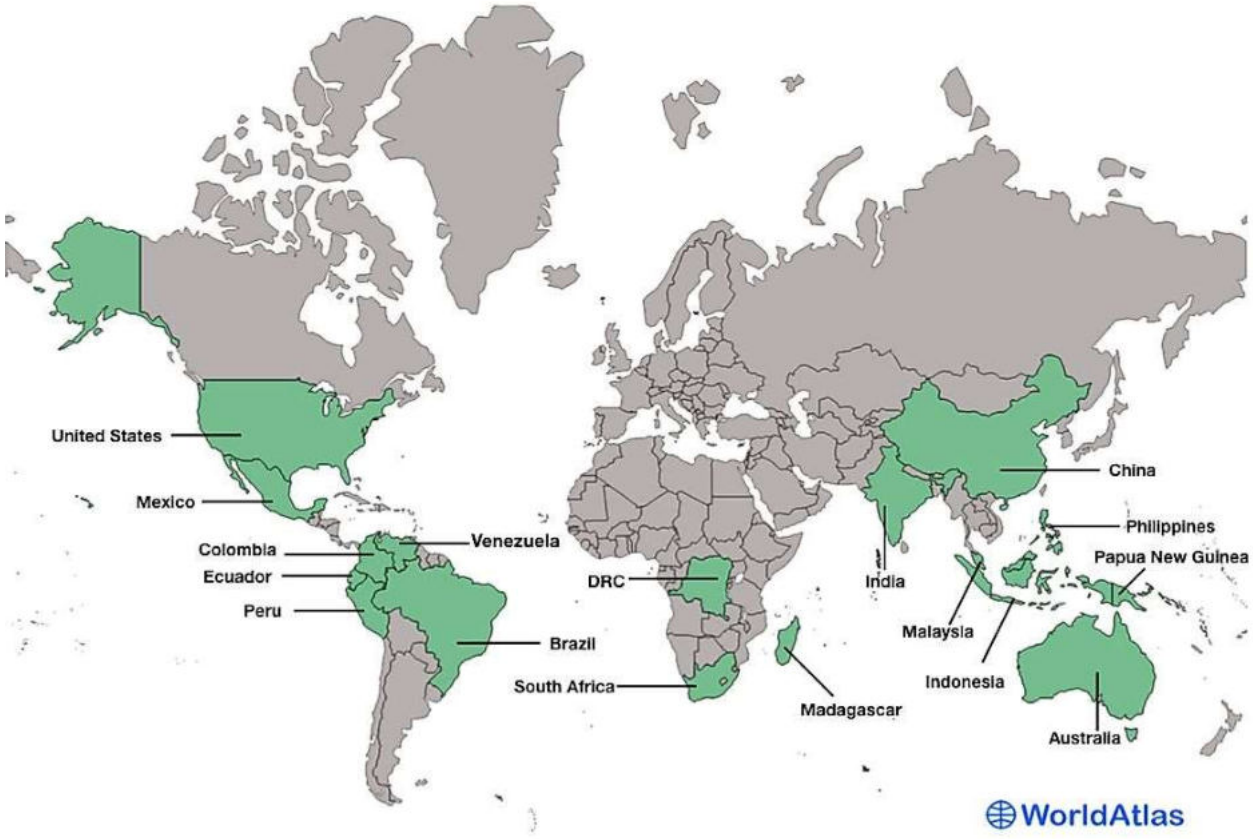
Taxonomic Group	Species	Endemic Species	Endemism (%
Plants	25,000	15,000	60.0
Mammals	380	172	45.3
Birds	769	142	18.5
Reptiles	452	243	53.8
Amphibians	244	196	80.3
Freshwater Fishes	950	350	36.8

Rat-Eating Pitcher plant



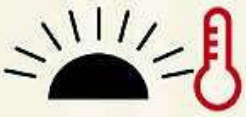
Sulawesi Bear Cuscus

Mega diversity countries



1. Australia
2. The Congo
3. Madagascar
4. South Africa
5. China
6. India
7. Indonesia
8. Malaysia
9. Papua New Guinea
10. Philippines
11. Brazil
12. Colombia
13. Ecuador
14. Mexico
15. Peru
16. United States
17. Venezuela

6 BIGGEST THREATS TO BIODIVERSITY



1. CLIMATE CHANGE

Increase in the temperature of the atmosphere has major effects on the environment such as the seasons, rising of the sea levels, and glacial retreats.

2. HABITAT LOSS & DEGRADATION

Habitat loss may either be caused by natural events like natural calamities and geological events or anthropogenic activities like deforestation and man-induced climate change.

3. POLLUTION

Be it water, air, or land pollution, all forms of pollution appear to be a threat to all life forms on Earth.

4. INVASIVE SPECIES

An exotic or unnatural species can be any kind of organism that has been introduced to a foreign habitat. This introduction can cause major threats to the native species.

5. OVEREXPLOITATION

Overexploitation refers to the act of over-harvesting species and natural resources at rates faster than they can actually sustain themselves in the wild.

6. OTHER POTENTIAL THREATS

Epidemics and infectious diseases of wildlife such as Ebola virus disease, infectious bursal disease, and flu affect wildlife and biodiversity.

Source: https://www.bioexplorer.net/threats-to-biodiversity.html/

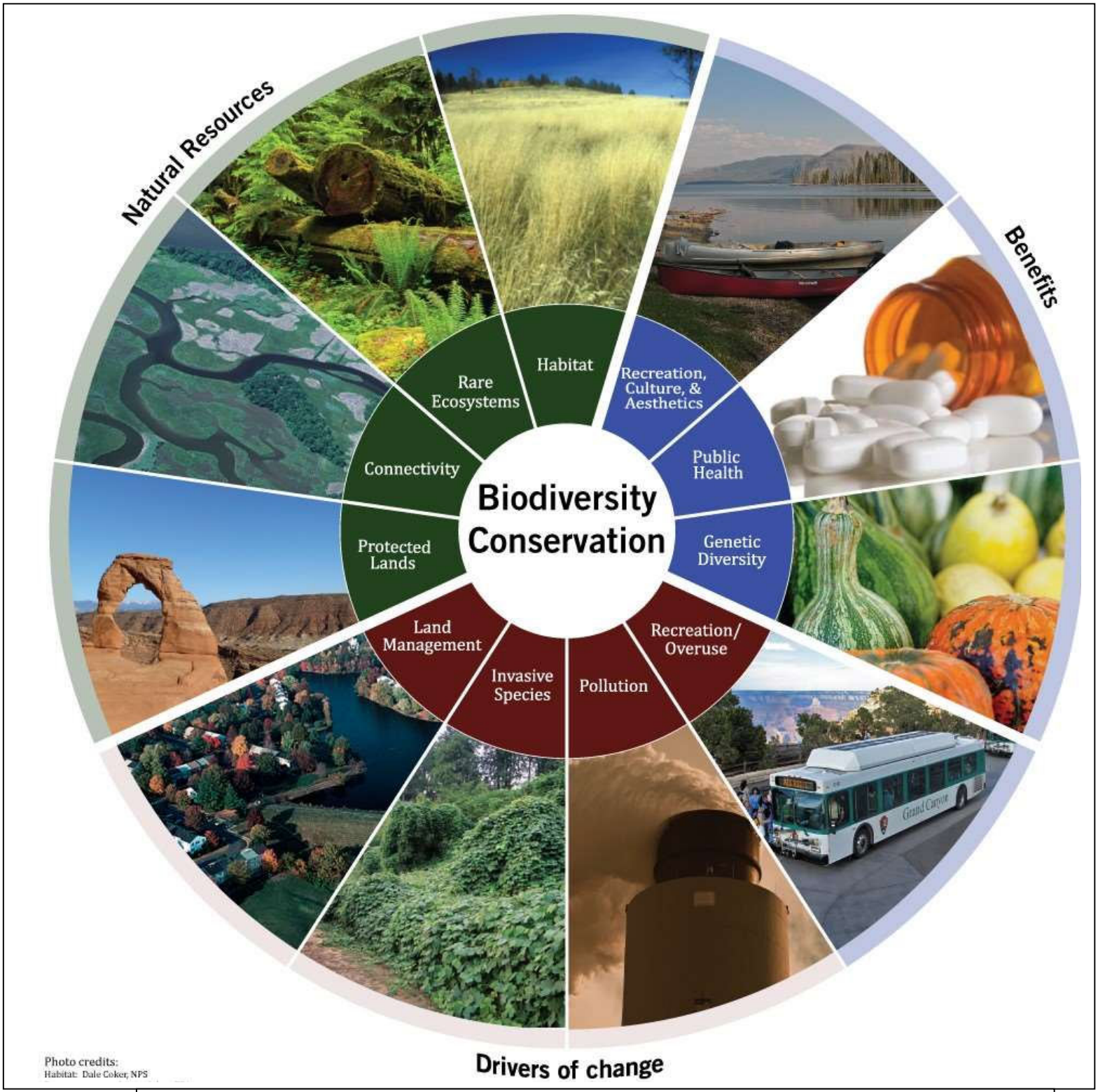
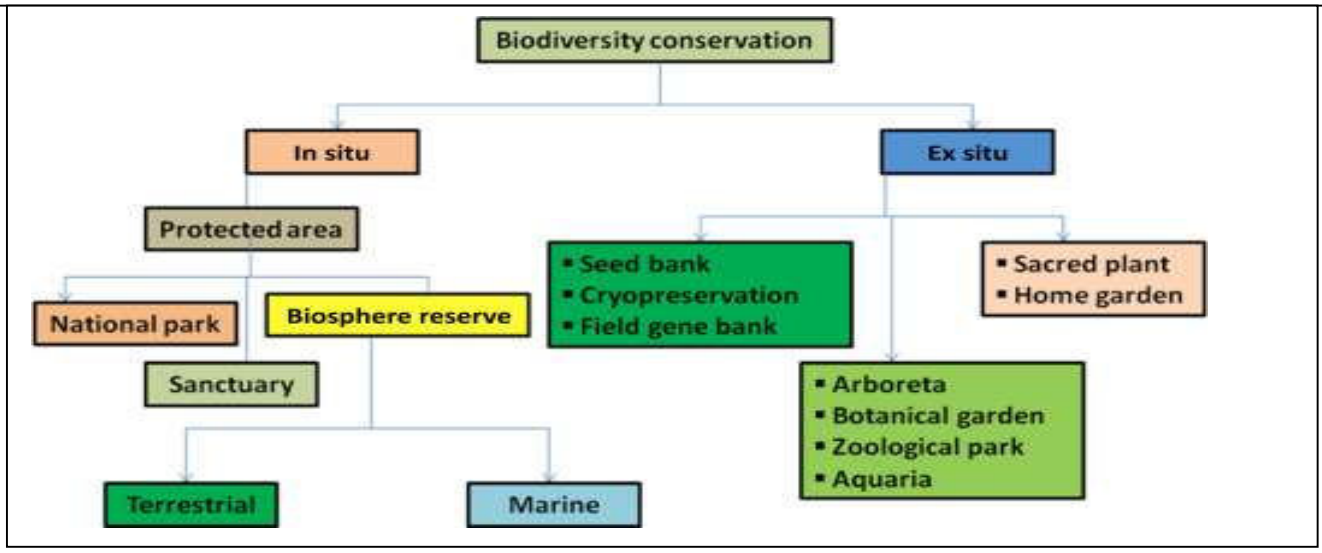


Photo credits:
Habitat: Dale Coker, NPS



National park

S. No	Name of State	Name of Protected Area	Year of Notification	Area (in km^2)
1	**Andhra Pradesh**	Papikonda	2008	1012.8588
2		Rajiv Gandhi (Rameswaram)	2005	2.3952
3		Sri Venkateswara	1989	353.62
4	**Arunachal Pradesh**	Mouling	1986	483
5		Namdapha	1983	1807.82
6	**Assam**	Dibru-Saikhowa	1999	340
7		Kaziranga	1974	858.98
8		Manas	1990	500
9		Nameri	1998	200
10		Rajiv Gandhi (Orang)	1999	78.81
11	**Bihar**	Valmiki	1989	335.65
12	**Chhattisgarh**	Guru Ghasidas (Sanjay)	1981	1440.71
13		Indravati (Kutru)	1982	1258.37
14		Kanger Valley	1982	200
15	**Goa**	Mollem	1992	107
16	**Gujarat**	Blackbuck (Velavadar)	1976	34.53
17		Gir	1975	258.71
18		Marine (Gulf of Kachchh)	1982	162.89
19		Vansda	1979	23.99
20	**Haryana**	Kalesar	2003	46.82
21		Sultanpur	1989	1.43
22	**Himachal Pradesh**	Great Himalayan	1984	754.4
23		Inderkilla	2010	94
24		Khirganga	2010	705
25		Pin Valley	1987	675
26		Col. Sherjung Simbalbara	2010	27.88
27	**Jharkhand**	Betla	1986	226.33
28	**Karnataka**	Anshi	1987	417.34
29		Bandipur	1974	872.24
30		Bannerghatta	1974	260.51
31		Kudremukh	1987	600.57
32		Nagarahole (Rajiv Gandhi)	1988	643.39
33	**Kerala**	Anamudi Shola	2003	7.5
34		Eravikulam	1978	97
35		Mathikettan Shola	2003	12.82
36		Pambadum Shola	2003	1.32
37		Periyar	1982	350
38		Silent Valley	1984	89.52
39	**Madhya Pradesh**	Bandhavgarh	1968	448.842
40		Dinosaur Fossils	2011	0.897
41		Fossil	1983	0.27
42		Pench	1975	292.857
43		Kanha	1955	941.793
44		Kuno	2018	748.761
45		Madhav	1959	375.23
46		Panna	1981	542.66
47		Sanjay	1981	464.643
48		Satpura	1981	528.729
49		Van Vihar	1979	4.452
50	**Maharashtra**	Chandoli	2004	317.67

50	**Maharashtra**	Chandoli	2004	317.67
51		Gugamal	1975	361.28
52		Nawegaon	1975	133.88
53		Pench (Jawaharlal Nehru)	1975	257.26
54		Sanjay Gandhi (Borivilli)	1983	86.96
55		Tadoba	1955	116.55
56	**Manipur**	Keibul-Lamjao	1977	40
57		Shiroi	1982	100
58	**Meghalaya**	Balphakram	1986	220
59		Nokrek Ridge	1997	47.48
60	**Mizoram**	Murlen	1991	100
61		Phawngpui (Blue Mountain)	1992	50
62	**Nagaland**	Intanki	1993	202.02
63	**Odisha**	Bhitarkanika	1988	145
64		Simlipal	1980	845.7
65	**Rajasthan**	Desert	1992	3162
66		Keoladeo Ghana	1981	28.73
67		Mukundra Hills	2006	200.54
68		Ranthambhore	1980	282
69		Sariska	1992	273.8
70	**Sikkim**	Khangchendzonga	1977	1784
71	**Tamil Nadu**	Guindy	1976	2.7057
72		Gulf of Mannar Marine	1980	526.02
73		Indira Gandhi (Annamalai)	1989	117.1
74		Mudumalai	1990	103.23
75		Mukurthi	1990	78.46
76	**Telangana**	Kasu Brahmananda Reddy	1994	1.425
77		Mahaveer Harina Vanasthali	1994	14.59
78		Mrugavani	1994	3.6
79	**Tripura**	Clouded Leopard	2007	5.08
80		Bison (Rajbari)	2007	31.63
81	**Uttar Pradesh**	Dudhwa	1977	490
82	**Uttarakhand**	Corbett	1936	520.82
83		Gangotri	1989	2390.02
84		Govind	1990	472.08
85		Nanda Devi	1982	624.6
86		Rajaji	1983	820
87		Valley of Flowers	1982	87.5
88	**West Bengal**	Buxa	1992	117.1
89		Gorumara	1992	79.45
90		Jaldapara	2014	216.34
91		Neora Valley	1986	159.8917
92		Singalila	1986	78.6
93		Sunderban	1984	1330.1
94	**Andaman & Nicobar Islands**	Campbell Bay	1992	426.23
95		Galathea Bay	1992	110
96		Mahatama Gandhi Marine (Wandoor)	1983	281.5
97		Mount Harriett	1987	46.62
98		Rani Jhansi Marine	1996	320.06
99		Saddle Peak	1987	32.54
100	**Jammu &**	City Forest (Salim Ali)	1992	9.07

		Dachigam	1981	141
102		Kazinag	2000	90.88
103		Kishtwar High Altitute	1981	2191.5
104	**Ladakh**	Hemis	1981	3350

Source: National Wildlife Database, Wildlife Institute of India

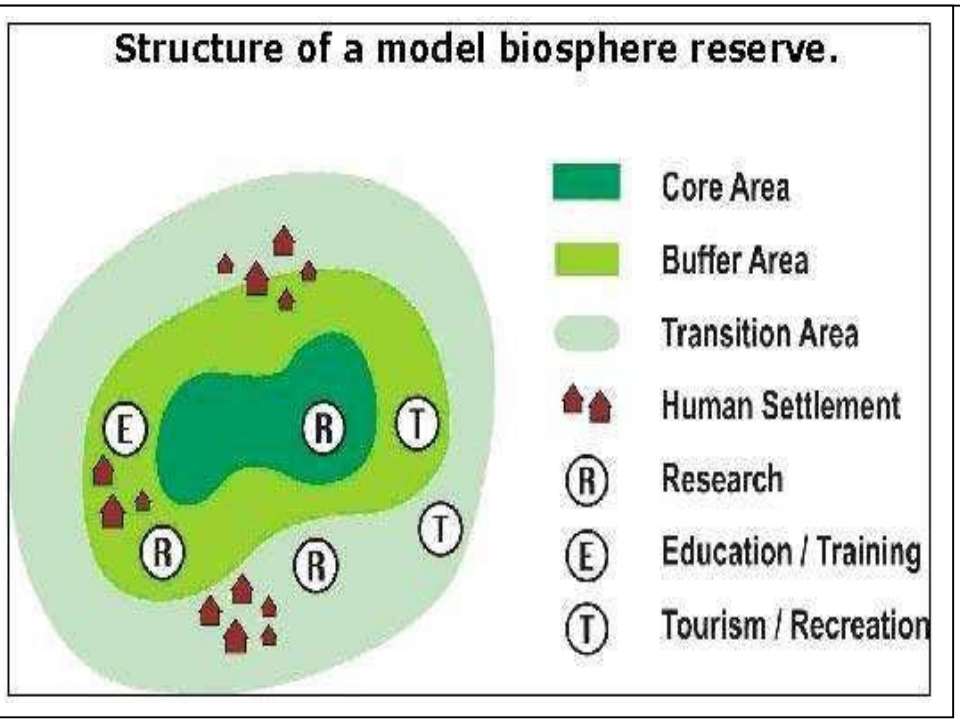
BIOSPHERE RESERVE

Biosphere reserves are sites established by countries and recognized under UNESCO's Man and the Biosphere (MAB) Programme to promote sustainable development based on local community efforts and sound science.The programme of Biosphere Reserve was initiated by UNESCO in 1971. The purpose of the formation of the biosphere reserve is to conserve in situ all forms of life, along with its support system, in its totality, so that it could serve as a referral system for monitoring and evaluating changes in natural ecosystems. The first biosphere reserve of the world was established in 1979, since then the network of biosphere reserves has increased to 631 in 119 countries across the world (Read more at https://en.unesco.org/biosphere).

Presently, there are 18 notified biosphere reserves in India.

S. No.	Name	Date of Notification	Area (in km^2)	Location (State)
1	Nilgiri	01.09.1986	5520 (Core 1240 & Buffer 4280)	Part of Wayanad, Nagarhole, Bandipur and Madumalai, Nilambur, Silent Valley and Siruvani hills (Tamil Nadu, Kerala and Karnataka).
2	Nanda Devi	18.01.1988	5860.69 (Core 712.12, Buffer 5,148.570) & T. 546.34)	Part of Chamoli, Pithoragarh, and Bageshwar districts (Uttarakhand).
3	Nokrek	01.09.1988	820 (Core 47.48 & Buffer 227.92, Transition Zone 544.60)	Part of Garo hills (Meghalaya).
4	Great Nicobar	06.01.1989	885 (Core 705 & Buffer 180)	Southern most islands of Andaman and Nicobar (A&N Islands).
5	Gulf of Mannar	18.02.1989	10,500 km2 Total Gulf area (area of Islands 5.55 km2)	Indian part of Gulf of Mannar between India and Sri Lanka (Tamil Nadu).
6	Manas	14.03.1989	2837 (Core 391 & Buffer 2,446)	Part of Kokrajhar, Bongaigaon, Barpeta, Nalbari, Kamprup and Darang districts (Assam)
7	Sunderbans	29.03.1989	9630 (Core 1700 & Buffer 7900)	Part of delta of Ganges and Brahamaputra river system (West Bengal).
8	Simlipal	21.06.1994	4374 (Core 845, Buffer 2129 & Transition 1400	Part of Mayurbhanj district (Orissa).
9	Dibru-Saikhowa	28.07.1997	765 (Core 340 & Buffer 425)	Part of Dibrugarh and Tinsukia Districts (Assam)
10	Dehang-Dibang	02.09.1998	5111.50 (Core 4094.80 &Buffer 1016.70)	Part of Siang and Dibang Valley in Arunachal Pradesh.
				Parts of Betul, Hoshangabad and

11	Pachmarhi	03.03.1999	4926	Parts of Betul, Hoshangabad and Chindwara districts of Madhya Pradesh.
12	Khangchendzonga	07.02.2000	2619.92 (Core 1819.34 & Buffer 835.92)	Parts of Khangchendzonga hills and Sikkim.
13	Agasthyamalai	12.11.2001	1828	Neyyar, Peppara and Shendurney Wildlife Sanctuaries and their adjoining areas in Kerala.
14	Achanakamar - Amarkantak	30.3.2005	3835.51 (Core 551.55 & Buffer 3283.86)	Covers parts of Anupur and Dindori districts of M.P. and parts of Bilaspur districts of Chhattishgarh State.
15	Kachchh	29.01.2008	12,454 km2	Part of Kachchh, Rajkot, Surendra Nagar and Patan Civil Districts of Gujarat State
16	Cold Desert	28.08.2009	7770	Pin Valley National Park and surroundings; Chandratal and Sarchu&Kibber Wildlife Sancturary in Himachal Pradesh
17	Seshachalam Hills	20.09.2010	4755.997	Seshachalam Hill Ranges covering parts of Chittoor and Kadapa districts of Andhra Pradesh
18	Panna	25.08.2011	2998.98	Part of Panna and Chhattarpur districts in Madhya Pradesh



Description of some National Parks in India

Bandipur & Nagarhole National Parks, Karnatak

- ✓ **Special Features:** Veritable paradise for wildlife. Moderate climate and diverse geographical features. Dry & tropical mixed deciduous forests
- ✓ **Location:** Situated within Chamarajanagar
- ✓ district of Karnataka, halfway down
- ✓ the Mysore-Ooty highway.
- ✓ **Prime Attractions:**
- ✓ **Year of Designation as National Park:**

In 1973, it became one of the first of India's Tiger Reserves.

Corbett National Park

- ✓ **Special Features:** First national park of India. Hilly & reverie , valleys, plateaus and ravines. Deciduous forests consisting 110 tree species. Lower areas are mainly populated by Sal, Pine, Shisham and Khair trees.
- ✓ **Year of Designation as National Park:**

  Formerly known as Hailey National Park ,

  established by Jim Corbett in 1936
- ✓ **Location :** 63 Kms southwest of Nainital, Uttaranchal
- ✓ **Area :** 52,082 hectares
- ✓ **Prime attractions :** Tigers, Leopards, Crocodiles,

Sloth Bear, Himalayan Black Bear, Dhole, Jackal,

Yellow Throated Martem, Himalayan Palm Civet, Indian Grey Mongoose.

Kanha National Park

- ✓ **Special Features:** The number of tigers has doubled and barasingha have crossed the 450 mark. Deciduous forests, surrounded by valley & plateaus.
- ✓ **Year of Designation as National Park:**

As National Park in 1955 &

as Tiger Reserve in 1975

- ✓ **Location :** Forests of the Central high

lands of Mandla and Balaghat districts

in Madhya Pradesh

- ✓ **Area :** 940 sq. kms.
- ✓ **Prime attractions :** Barasingha, cheetal sambar, black duck, barking deer, gaur, hog deer, chausingha, bison, boar, tiger, leopard, hyena and wild dog. Nearly two hundred bird species such as storks, teals, pintails, egrets, peafowl, partridges, doves, pigeons, cuckoos, eagles, kites, etc.

Kaziranga National Park

- ✓ **Special Features:** Listed in World Heritage Site
- ✓ **Year of Designation as National Park:** 1985
- ✓ **Location :** Districts of Golaghat & Nagaon in Assam
- ✓ **Area :** 429.93 sq.kms with an additional area of 429.40 sq.kms
- ✓ **Prime attractions :** One Horned Rhinoceros, Asiatic elepahnt, Asiatic wild buffalo & Royal Bengal Tiger.

Dudhwa National Park

- ✓ **Special Features:** Forty-seven species of mammals are

  found at Dudhwa; of these thirteen species are endangered.

  The only place in the world inhabited by 5 species of deer
- ✓ **Year of Designation as National Park:** 1st February 1977
- ✓ **Location :** Near Palia in Lakhimpur-Kheri District in foothills of Himalaya in Terai region in Uttar Pradesh

- ✓ **Area : 490 sq.kms**
- ✓ **Prime attractions :** Hog deer, swamp deer, barking deer, sambhar, wild boar, leopard, tiger, rhino.

Gir National Parks

- ✓ **Special Features:** It's ecosystem nurtures 450 species of plants, 350 species of birds, 32 mammal species & 24 reptiles
- ✓ **Year of Designation as National Park:** Established in 1965
- ✓ **Location :** Gir, 42 kms from Junagadh in Gujarat
- ✓ **Area :** 1421.13 sq. kms
- ✓ **Prime attractions :** Chital, chowsingha, Asiatic Lions, Leopard, Lion, sambhar, bluebul

Sunderbans National Park

- ✓ **Special Features:** A UNESCO World Heritage site, one of the world's largest delta & the mangrove
- ✓ **Year of Designation as National Park:**
- ✓ **Location :** Piyali, West Bengal
- ✓ **Area :** 1330.10 sq. kms
- ✓ **Prime attractions :** Bengal Tiger, Ridley Sea Turtle, porpoise, wild boar, pangolin, Gangetic dolphin.

Some of the Bird Sancturies in India

- ❖ **Keoladeo National Park/Bharatpur Bird Sanctuary:**
- ✓ **Special Features:** Designated as UNESCO World Heritage Site in 1985
- ✓ **Location:** Bharatpur, Rajasthan
- ✓ **Area:** 29 sq. kms

Prime attractions: Sambar, Chital, Nilgai, Boar, Migratory Birds

- ❖ **Kumarakom Bird Sanctuary**

- ✓ **Special Features:** Situated on the banks of Vembanad Lake. Famous for its avian population. Known as an ornithologist's paradise.

- ✓ **Location :** 12 kms. from Kottayam, Kerala
- ✓ **Area:** 14 acres
- ✓ **Prime attractions:** Siberian Cranes, Waterfowls, Cuckoos

❖ **Sultanpur Bird Sanctuary**

- ✓ **Special Features:** Lush green lawns, trees, shrubs and masses of Bougainvillea.
- ✓ **Location:** Sultanpur, Haryana
- ✓ **Area:** 143 sq. kms
- ✓ **Prime attractions:** Migratory Birds, Kingfishers, Blue Bulls

❖ **Thattekad / Salim Ali Bird Sanctuary**

- ✓ **Special Features:** Situated on the banks of the Priver river
- ✓ **Location :** 13 kms. from Kothamangalam, Kerala
- ✓ **Area :** 25.16 sq. kms

Prime attractions : Rare Mottled Wood Owl, Spot-bellied Eagle Owl, Malayan Night Heron.

❖ **Kaundinya Bird Sanctuary**

- ✓ **Special Features:** Covered with rugged high hills and deep valleys. Two streams, the Kaigal and the Kaundinya flow through the sanctuary. The southern tropical type of forests.
- ✓ **Location :** 50 kms. from Chittor, Andhra Pradesh
- ✓ **Area :** 358sq. Kms
- ✓ **Prime attractions :** Cheetal, Four horned Antelope, Sambhar, Mouse Deer, Hare, Porcupine, Wild boar, Jungle Cat, Jackal, Sloth Bear, Panther, Jungle Fowl, Pea Fowls and Elephants.

❖ **Kawal Sanctuary**

- ✓ **Special Features:** Variety of flora including dry deciduous teak forest and bamboo trees.

- ✓ **Location :** 50 kms. from Mancherial, Andhra Pradesh
- ✓ **Area :** 893sq. Kms

Prime attractions : Sloth Bear, Panther, Tiger and variety of birds like Peacocks, Patridges, Quails, Vultures, Eagles, Kites, Owls, Mynas, Pigeons, Tree - pies, Kingfishers.

CONCLUSION

It will be worthwhile to mention that in the 31st meeting of the Standing Committee for National Board for Wildlife (NBWL), held between August 12-13,, 2014, as many as 173 projects were listed for clearance from 24 states of India. A total of 130 projects were cleared, but were eventually struck down by the Supreme Court of India on the grounds that the current constitution of NBWL is a violation of law (PA Update, 2014-15: 12-22). Again, in a single NBWL meeting, held on January 21, 2015, at least 34 project proposals, cutting across 12 states have been approved; including those for road, rail, oil drilling, pipeline, canal construction–all being within the declared boundary of 27 wildlife sanctuaries, four national parks, one tiger reserves and two bird sanctuaries, among others. All these projects involve diversion of forest land within 'Protected Area' for non-forestry purpose.

Besides, at least 15 proposals from 10 states got clearance for diversion of forest land within 10 km radius of national parks and wildlife sanctuaries, which according to EIA norm should not have been given permission. The range of projects included construction of jetty in water ways and highway on land, storage facilities, irrigation, canal construction, road, mining, thermal power, hydrocarbon exploration.

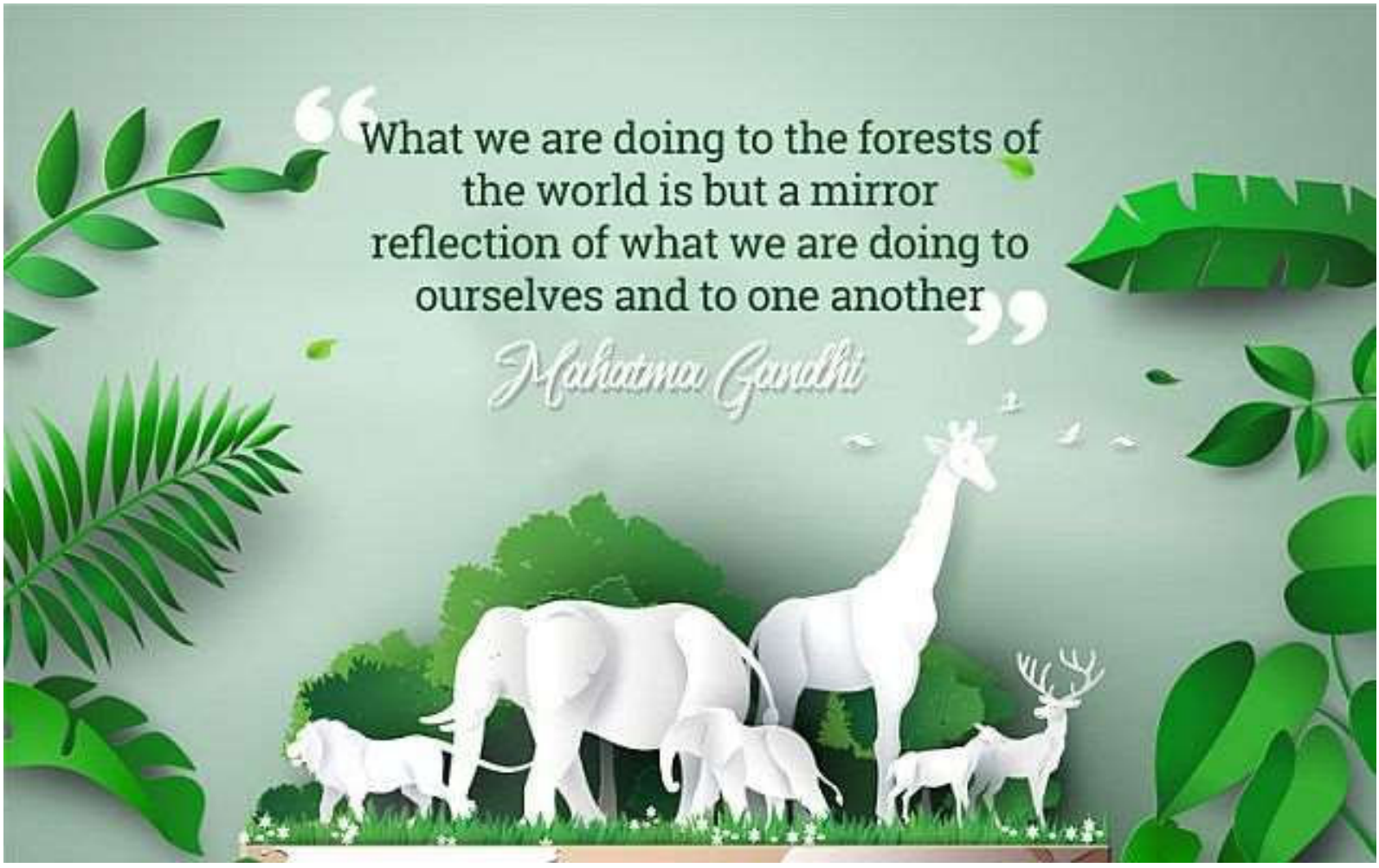
The gamut of development projects being cleared may be indicative of country's rapid growth, but it also poses a question—what is the future of wildlife in India? Thanks to illegal poaching, Sariskaand Panna tiger reserves in Rajasthan and Madhya Pradesh respectively were recently declared "tiger-less". Buxa Tiger Reserve in West Bengal also has no tiger now.A recent news on death of "lions" in large number in Gujarat attracted national media attention. Also the illegal poaching or human-induced deaths as witnessed in Manas Wildlife Sanctuary, causing decline in rhino population. But on the other hand, deliberate and predicted deaths of Indian Elephants on railway tracks in north Bengal. Stories of these deaths that were five times more since the railway line was broadened also attracted eyeballs. Has any action been taken to prevent such colossal loss of wildlife—legally or illegally? Very recently, Government of India had again cleared another railway project connecting North Bengal to Sikkim via Rongpo, diverting 86 ha of forests land. These wildlife species are all listed under Schedule I of Indian Wildlife Protection Act and should have been given highest protection status. Land is not the only place with wildlife crisis. Hundreds of dead sea turtles have recently been spotted on Odisha coast. It is alleged that uncontrolled trawling operation made the coast a cemetery for Olive Ridley turtles.

The 48 projects recommended for clearance in January 2015, if undertaken, will convert 2,144 ha of forest land within the Protected Area. But in some cases, forest area has not been clearly defined and maneuvered in such language as "afforestation of boundary of Protected Area for exclusion of part of limestone bearing mineral zone" in Kamur Wildlife Sanctuary, Bihar. The title at least does not indicate "what the limestone bearing area is" that is referred to within the sanctuary. In June 2015, NBWL had again cleared 18 new projects and deferred four projects without rejecting a single one. These include six projects within five tiger reserve areas (PA update, August, 2015). One can recall how years ago, dolomite mining was totally banned in Buxa Tiger Reserve, although mining history dates back 50 years before the tiger reserve was notified.

The forest cover in India has a target to reach 33 per cent of land area but forests within the Protected Areas have special significance in terms of biodiversity and wildlife conservation. Years back, a study by Zoological Survey of India on tiger reserves of India revealed how tiger reserves have contributed towards efforts of conservation of biological diversity in the country by protecting keystone species and forests. One has to remember that till date 70 per cent of biodiversity has been recorded from the forested area in the world.

International Biodiversity Day

22 May



Acknowledgement:

- I express my deep sense of gratitude to Dr. Sukhendu Roy, Assistant Professor, Head of the Department of Zoology, Gushkara Mahavidyalaya under whose direction this study was conducted.
- I am grateful to Principal, Dr. Swapan Kumar Pan, Gushkara Mahavidyalaya, Gushkara, Purba Burdwan, West Bengal.
- I owe a lot to my parents/Guardians, who encouraged and helped me at every stage of my personal and academic life.
- Above all, I owe it all to Almighty for granting me the wisdom, health and strength to undertake this project work and enabling me to its completion.

Project report on animal cell culture

Semester-V Honours (Practical) Examination, 2022

Roll no:- 200311000020

Registration no:- 202001004798 of 2020-21

Project submitted in partial fulfillment of the requirements for semester-V Examination of the degree of bachelor (science)

Dr. Sukhendu Roy
Assistant Professor
Department of Zoology
Gushkara Mahavidyalaya

Gushkara Mahavidyalaya
NAAC Accredited "A" Grade Degree College
P.O:Gushkara, Dist:Purba Burdwan,
Pin-713128, West Bengal.
Email: guskaramahavidyalaya@gmail.com
Phone: 03452-255105
Fax: 03452-257635

DATE: 09.02.2023

This is to certify that Md Riyajuddin bearing University Roll No 200311000020 with Registration number 202001004798 of 2020-21 students of B.Sc. Semester V Zoology Honours have worked out the Project work entitled "Animal Cell Culture" under my supervision and the entire work has been carried out by him/her in the Gushkara Mahavidyalaya, Department of Zoology, Guskara. The project work represents explanation of his/her own words. Due to lack of Infrastructure & proper instruments the whole work was not possible in practical based laboratory works. Most of the data were composed from someone's finding.

Sukhendu Roy.

Dr. Sukhendu Roy
Assistant Professor
Department of Zoology
Gushkara Mahavidyalaya

Introduction to Animal Cell Culture:

Animal cell culture basically involves the in vitro (in the laboratory) maintenance and propagation of animal cells in a suitable nutrient media. Thus, culturing is a process of growing cells artificially. Cell culture has become an indispensible technology in various branches of life sciences. Animal tissue culture technology is now becoming a significant model for many scientists in various fields of biology and medicine. Despite the various developments in animal cell and tissue culture since the late 1800s, until the early 1950s progress in animal tissue culture was stalled due to the non-availability of a suitable cell line. In the early 1950s, for the first time, successful growth of cells derived from the cervical cancer of Mrs Henrietta Lacks was demonstrated. This breakthrough using Mrs Henrietta Lacks's cells in culture successfully transformed medical and biological research, allowing numerous cellular, molecular and therapeutic discoveries, including the breakthrough of the first effective polio vaccine. This culture is now called HeLa, on which there were more than 60 000 publications by 2017, and which has been involved in numerous Nobel prize-winning innovations.

Animal cell culture is a significant tool for biological research. The importance of cell culture technology in biological science was realized a long time ago. Earlier dedifferentiation based experiments of cells due to selective overgrowth of fibroblasts resulted in the enhancement of culture techniques. Animal cell culture involves isolation of cells from a tissue before establishing a culture in a suitable artificial environment. Initial isolation of the cells from the tissues can be achieved by disaggregation using enzymatic or mechanical methods. The source of the isolated cells is usually an *in vivo* environment, but sometimes cells are also derived from an existing cell line or cell strain. Animal cell culture offers suitable model systems for investigating the following factors:

- Drug screening and development.
- Mutagenesis and carcinogenesis.
- Normal physiology and biochemistry of cells.
- Potential effects of drugs and toxic compounds on the cells.

In addition, it also permits reliable and reproducible results, and is thus considered as a significant model system in cellular and molecular biology. Mammalian cell culture requires an optimal environment for growth. Environmental conditions are divided into nutritional

requirements and physicochemical requirements. Nutritional requirements include a substrate or medium that provides support and essential nutrients such as amino acids, carbohydrates, vitamins, minerals, growth factors, hormones and gases (O_2, CO_2). All these factors control physical and chemical factors such as pH, osmotic pressure and temperature. In animal tissue culture the majority of cells are anchorage-dependent and therefore require a solid or semi-solid support in the form of a substrate (adherent or monolayer culture), whereas others can be cultured in the culture medium, called a suspension culture. Cell culture technologies have emerged as a tool to assess the efficacy and toxicity of new drugs, vaccines and biopharmaceuticals, and also play a major role in assisted reproductive technology. Animal cell culture is one of the more important and diverse techniques in current research streams. Animal, plant and microbial cells are always cultured in predetermined culture medium under controlled laboratory conditions. Animal cells are more complex than micro-organisms. Due to their genetic complexity it is difficult to determine the optimum nutrient requirements of animal cells cultured under *in vitro* conditions. Animal cells require additional nutrients compared to micro-organisms, and they usually grow only when attached to specially coated surfaces. Despite these challenges, different types of animal cells, including both undifferentiated and differentiated ones, can be cultured successfully (1,2).

Historical Background: Tissue culture involves the *in vitro* maintenance and propagation of cells in optimal conditions. Culturing animal cells, tissue or organs in a controlled artificial environment is called animal tissue culture. The importance of animal tissue culture was initially realized during the development of the polio vaccine using primary monkey kidney cells (the polio vaccine was the first commercial product generated using mammalian cell cultures). These primary monkey kidney cells were associated with many disadvantages such as:

- Chances of contamination with adventitious agents (risk of contamination by various monkey viruses is high).
- Most of the cells are anchorage-dependent and can be cultured efficiently only when they are attached to a solid or semi-solid substrate (obligatorily adherent cell growth).
- The cells are not well characterized for virus production.
- A scarcity of donor animals as they are on the verge of extinction.

The foundation of animal tissue culture can be considered to have occurred in 1880, when Arnold showed that leukocytes can divide outside the body. Then, in the beginning of the 19th century, Jolly investigated the behavior of animal cells in serum lymph. The development of animal tissue culture commenced after the breakthrough frog tissue culture technique, which was discovered by Harrison in 1907. Due to this effort Harrison is considered as the father of tissue culture. In his experiment he introduced tissue from frog embryos into frog lymph clots and showed that not only did the tissue survive, but nerve fibers grew out from the cells. During the mid-20th century, human diploid fibroblast cells were established by Hayflick and Moorhead (3). They named this cell line MRC-5 (a cell line of fibroblasts derived from lung tissue). Later, Wiktor *et al* (1964) explored the utilization of this cell line in the production of rabies virus for vaccine production. After a couple of years they suggested a large-scale production protocol along with a method for the assessment of purified rabies vaccine immunogenicity. During the same time, BHK-21 (C13) cells (baby hamster kidney cells) were established. These cells are susceptible to human adenovirus D, reovirus 3 and vesicular stomatitis virus. The commercial production of inactivated foot and mouth disease (a viral disease that causes sores in the mouth and a rash on the hands and feet of children) vaccine began using a suspension process. Back in 1914, Losee and Ebeling cultured the first cancer cells and after a few decades the first continuous rodent cell line was established by Earle (1943) (7). In 1951, Gay established that human tumor cells can give rise to continuous cell lines. The cell line considered as the first human continuous cell line was derived from a cancer patient, Henrietta Lacks, as mentioned above, and HeLa cells are still used very widely. Continuous cell lines derived from human cancers are the most extensively used resource in the modern laboratory. The HeLa discovery was followed by FDA approval for the production of interferon from HeLa cell lines. In addition to the progress in the field of cell culture, different media have been explored, which are typically based on specific cell nutritional requirements, such as serum-free media, starting with Ham's fully defined medium in 1965. In the 1970s, serum-free media were optimized by the addition of hormones and growth factors. Currently, thousands of cell lines are available and for the establishment and maintenance of these cell lines many media are available.

Organ culture: The culture of native tissue (i.e. un-disaggregated tissue) that retains most of the in vivo histological features is regarded as organ culture.

Cell culture: This refers to the culture of dispersed (or disaggregated) cells obtained from the original tissue, or from a cell line.

Equipment: Laminar-flow, sterilizer, incubator, refrigerator and freezer (-20°C), balance, C02 cylinder, centrifuge, inverted microscope, water purifier, hemocytometer, liquid nitrogen freezer, slow- cooling device (for freezing cells), pipette washer, deep washing sink. Besides the basic and minimal requirements listed above, there are many more facilities that may be beneficial or useful for tissue cultures. These include air-conditioned rooms, containment room for biohazard work, phase-contrast microscope, fluorescence microscope, confocal microscope, osmometer, and high capacity centrifuge and time lapse video equipment.

Culture Vessels: In the tissue culture technology, the cells attach to the surface of a vessel which serves as the substrate, and grow. Hence there is a lot of importance attached to the nature of the materials used and the quality of the culture vessels. The term anchorage dependent cells is used when the cells require an attachment for their growth. On the other hand, some cells undergo transformation, and become anchorage independent.

Materials used for culture vessels: Glass: Although glass was the original substrate used for culturing, its use is almost discontinued now. This is mainly because of the availability of more suitable and alternate substrates.

Disposable plastics: Synthetic plastic materials with good consistency and optical properties are now in use to provide uniform and reproducible cultures. The most commonly used plastics are polystyrene, polyvinyl chloride (PVC), polycarbonate, metinex and thermonex (TPX).

Types of culture vessels: The following are the common types of culture vessels.

i. Multiwall plates

ii. Petridishes

iii. Flasks

iv. Stirrer bottles.

Limitations of Tissue Culture: There are several limitations of tissue culture; some of them are given below.

1. Need of expertise and technical skill for the development, and regular use of tissue culture.

2. Cost factor is a major limitation. Establishment of infrastructure, equipment and other facilities are expensive.

3. It is estimated that the cost of production of cells is about 10 times higher than direct use of animal tissues.

4. Control of the environmental factors (pH, temperature, dissolved gases, disposal of biohazards) is not easy.

5. The native in vivo cells exist in a three- dimensional geometry while in in vitro tissue culture, the propagation of cells occurs on a two dimensional substrate. Due to this, the cell to cell interactive characters are lost.

Risks in a Tissue Culture Laboratory and Safety: There are several risks associated with tissue culture technology. Most of the accidents that occur in culture laboratories are due to negligence and casual approach while dealing with biological and radiological samples, besides improper maintenance of the laboratory.

Primary culture broadly involves the culturing techniques carried following the isolation of the cells, but before the first subculture (3, 4). Primary cultures are usually prepared from large tissue masses. Thus, these cultures may contain a variety of differentiated cells e.g. fibroblasts, lymphocytes, macrophages, epithelial cells.

With the experiences of the personnel working in tissue culture laboratories, the following criteria/ characteristics are considered for efficient development of primary cultures:

a. Embryonic tissues rather than adult tissues are preferred for primary cultures. This is due to the fact that the embryonic cells can be disaggregated easily and yield more viable cells, besides rapidly proliferating in vitro.

b. The quantity of cells used in the primary culture should be higher since their survival rate is substantially lower (when compared to subcultures).

c. The tissues should be processed with minimum damage to cells for use in primary culture. Further, the dead cells should be removed.

d. Selection of an appropriate medium (preferably a nutrient rich one) is advisable. For the addition of serum, fetal bovine source is preferred rather than calf or horse serum.

e. It is necessary to remove the enzymes used for disaggregation of cells by centrifugation.

Culture Media for Cells and Tissues: Culture medium is the most important factor for culturing cells and tissues. It provides optimum conditions of factors like pH, osmotic pressure etc. It also provides all nutritional requirements needed by growing cells in the culture.

The media used in animal cell and tissue culture are of two types:

(i) Natural media: The natural media include fluids of biological origin, such as plasma clots and serum. Plasma clot is prepared by treating the blood of an animal with an anticoagulant such as heparin. Serum is the clear fluid part of the blood, formed after blood coagulation when fibrin separates from the plasma. It is considered as an ideal growth medium for animal cells as if is formed of hundreds of proteins and hormones.

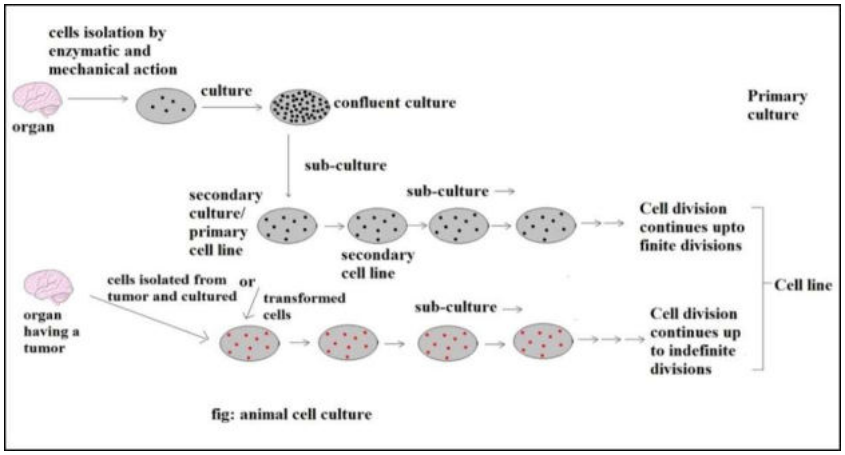


fig: animal cell culture

(ii) Artificial media: The artificial culture media primarily consist of balanced salt solution (BSS) which provide essential inorganic ions, correct osmolarity, required pH (7.0-7.3), energy (= glucose) and a pH indicator (such as phenol red). However, BSS lacks essential amino acids and vitamins. Earle's balanced salt solution and Hanks' balanced salt solution come under this category. These media cannot support growth of cells and tissues but can keep them alive for a period of 12 hours.

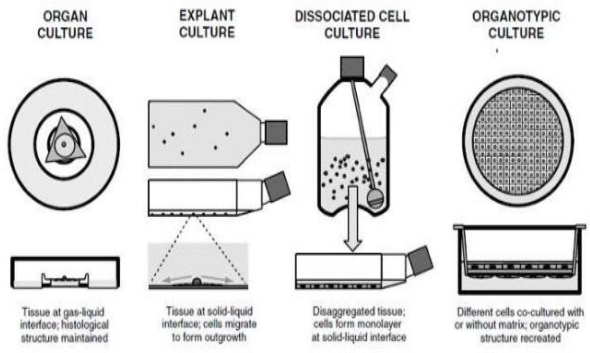
For longer survival of the culture, serum may be used or the balanced salt solution may be supplemented with amino acids, oxygen, vitamins and serum protein (7, 8).

Culture Procedures: The procedure for tissue culture and cell culture involve preparation and sterilization of glassware, equipment, reagents and media. As the tissue culture is being carried out in highly aseptic condition, all the usable, media and solutions are required to be sterilized thoroughly. Some of the methods of sterilization include swabbing, capping, flaming, dry at, wet heat, radiation, filter sterilization etc.

The work bench, reagent bottles and growth media are swabbed (= cleaned) with 70% ethanol before and after operation. Culture and reagent bottles are capped with deep screw caps. The reagents bottles are capped immediately alter using the reagent. The necks of all bottles and the screw caps are flamed before and after opening and closing. This is a common practice to prevent infection and maintaining aseptic condition.

Glassware, stainless steel instruments, plastic containers, distilled water, phosphate buffered saline and growth medium are sterilized in autoclave at 121 °C and under pressure 15 lb/square inch for 20 minutes. This process of sterilization by means of an autoclave is called as wet heat. It is followed by dry heat. During dry heating, glass ware and dissecting instruments are placed within a hot air oven at 160°C for one hour. The culture room or laminar How cabinet is fitted with a UV lamp.

The lamp is switched on half an hour before the operation of the work for the purpose of sterilization. Some heat labile constituents of the growth medium (such as polypeptides, hormones etc.) are sterilized not by heating method but by another method called filter sterilization. In this method liquid substances are passed through a micro filter (0.2 μm) which



removes everything except mycoplasma (50%) and bacterial endotoxins.

Preparation of Starting Material: The starting material is an isolated tissue or a body part. The isolated tissue or the body part consists of numerous cells cemented together by proteinous substances. To start the cell culture, the starting tissue is to be dissociated into cells by two methods: (i) mechanical method, (ii) enzymatic method.

In mechanical method the isolated tissue is cut into small pieces in BSS (Balanced Salt Solution) and these are then cultured in suitable vessels. In enzymatic method, the enzyme trypsin is generally used to dissociate cells present in the tissue by digesting the proteinous cementing material.

Following dissociation of starting tissue into cells by the mechanical or enzymatic method, the dissociated cells are placed in flat bottomed culture vessel (either made of glass or high grade plastic) containing culture medium. The culture vessel should have optimum number of cells and it should be incubated at 37°C. The inner surface of the culture vessel should have negatively charged (SO_3) group.

The transfer of dissociated cells into the culture medium is called as inoculation. A culture established directly from differentiated tissue (isolated tissue or body part) is known as primary culture. After sometime the bottom of the culture vessel will be covered by a continuous layer of cells, often one cell thick. This layer of cells is known as monolayer.

The cells from the primary culture can be detached from the culture vessel by trypsin or EDTA (Ethylene Diamine Tetraacetic Acid) treatment and transferred to fresh media. The culture so obtained is called cell lines. The process of transferring cultured cells into fresh culture vessels is termed as sub culturing.

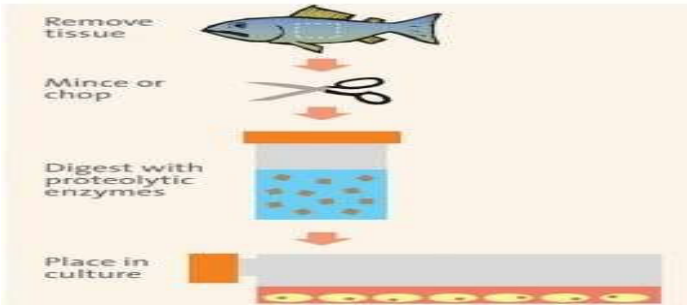
The cells divide at a constant rate over successive transfers. Such cells comprise a cell strain. Cell strains do not have an infinite life. They divide 50-100 times before dying. For long term culture the cell lines are preserved in frozen state in liquid nitrogen in presence of cryo preservative agents and foetal calf serum. Small-scale cultures are generally carried out either in plastic petriplates or plastic T-flasks. Large-scale cultures involving mammalian cells are carried out in bioreactors or fermenters (9).

Types of Animal Cell Culture:

i. Primary Culture:

When cells are surgically removed from an organism and placed into a suitable culture environment, they will attach, divide and grow. This is called a Primary Culture.

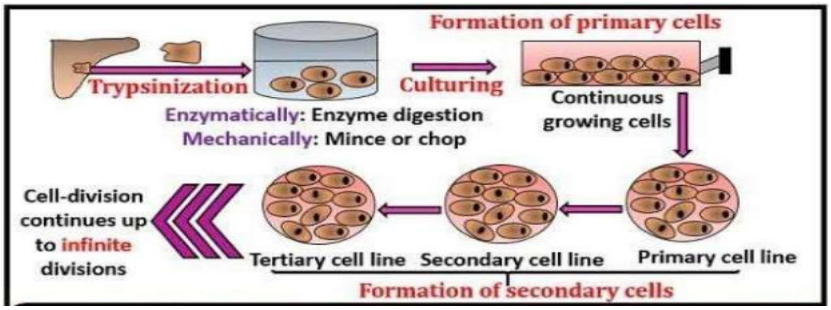
ii. Sub-Culturing:



When the cells in the primary culture vessel have grown and filled up all of the available culture substrate, they must be Sub-cultured to give them room for continued growth. This is usually done by removing them as gently as possible from the substrate with enzymes. Some cell lines can be harvested by gently scraping the cells off the bottom of the culture vessel. Once released, the cell sus-pension can then be subdivided and placed into new culture vessels.

ii. Functional Characteristics:

The characteristics of cultured cells result from both their origin (liver, heart, etc.) and how well they adapt to the culture conditions. Frequently, these characteristics are either lost or changed as a result of being placed in an artificial environment. Some cell lines will eventually stop dividing and show signs of aging. These lines are called Finite. Other lines which become immortal can continue to divide indefinitely and are called Continuous cell lines. When a "normal" finite cell line becomes immortal, it undergoes a fundamental irreversible change or "transformation". This can occur spontaneously or be brought about intentionally using drugs, radiation or viruses.



Techniques for Primary Culture: Among the various techniques devised for the primary culture of isolated tissues, three techniques are most commonly used:

1. Mechanical disaggregation.

2. Enzymatic disaggregation.

3. Primary explant technique.

Technique # 1. Mechanical Disaggregation: For the disaggregation of soft tissues (e.g. spleen, brain, embryonic liver, soft tumors), mechanical technique is usually employed. This technique basically involves careful chopping or slicing of tissue into pieces and collection of spill out cells.

The cells can be collected by two ways:

i. Pressing the tissue pieces through a series of sieves with a gradual reduction in the mesh size.

ii. Forcing the tissue fragments through a syringe and needle.

Although mechanical disaggregation involves the risk of cell damage, the procedure is less expensive, quick and simple. This technique is particularly useful when the availability of the tissue is in plenty, and the efficiency of the yield is not very crucial.

Technique # 2. Enzymatic Disaggregation: Enzymatic disaggregation is mostly used when high recovery of cells is required from a tissue. Disaggregation of embryonic tissues is more efficient with higher yield of cells by use of enzymes. This is due to the presence of less fibrous connective tissue and extracellular matrix. Enzymatic disaggregation can be carried out by using trypsin, collagenase or some other enzymes.

Disaggregation by trypsin: The term trypsinization is commonly used for disaggregation of tissues by the enzyme, trypsin.

Many workers prefer to use crude trypsin rather than pure trypsin for the following reasons:

i. The crude trypsin is more effective due to the presence of other proteases

ii. Cells can tolerate crude trypsin better.

iii. The residual activity of crude trypsin can be easily neutralized by the serum of the culture media (when serum-free media are used, a trypsin inhibitor can be used for neutralization).

Disaggregation of cells can also be carried out by using pure trypsin which is less toxic and more specific in its action. The desired tissue is chopped to 2-3 mm pieces and then subjected to disaggregation by trypsin. There are two techniques of trypsinization-warm trypsinization and cold trypsinization.

Warm trypsinization: This method is widely used for disaggregation of cells. The chopped tissue is washed with dissection basal salt solution (DBSS), and then transferred to a flask containing warm trypsin (37° C). The contents are stirred, and at an interval of every thirty minutes, the supernatant containing the dissociated cells can be collected. After removal of trypsin, the cells are dispersed in a suitable medium and preserved (by keeping the vial on ice).

The process of addition of fresh trypsin (to the tissue pieces), incubation and collection of dissociated cells (at 30 minutes intervals) is carried out for about 4 hours. The disaggregated cells are pooled, counted, appropriately diluted and then incubated.

Cold trypsinization: This technique is more appropriately referred to as trypsinization with cold pre-exposure. The risk of damage to the cells by prolonged exposure to trypsin at 37°C (in warm trypsinization) can be minimized in this technique (8).

After chopping and washing, the tissue pieces are kept in a vial (on ice) and soaked with cold trypsin for about 6-24 hours. The trypsin is removed and discarded. However, the tissue pieces contain residual trypsin. These tissue pieces in a medium are incubated at 37°C for 20-30 minutes. The cells get dispersed by repeated pi-pettings. The dissociated cells can be counted, appropriately diluted and then used.

The cold trypsinization method usually results in a higher yield of viable cells with an improved survival of cells after 24 hours of incubation. This method does not involve stirring or centrifugation, and can be conveniently adopted in a laboratory. The major limitation of cold trypsinization is that it is not suitable for disaggregation of cells from large quantities of tissues.

Limitations of trypsin disaggregation: Disaggregation by trypsin may damage some cells (e.g. epithelial cells) or it may be almost ineffective for certain tissues (e.g. fibrous connective tissue). Hence other enzymes are also in use for dissociation of cells.

Disaggregation by collagenase: Collagen is the most abundant structural protein in higher animals. It is mainly present in the extracellular matrix of connective tissue and muscle. The enzyme collagenase (usually a crude one contaminated with non-specific proteases) can be effectively used for the disaggregation of several tissues (normal or malignant) that may be sensitive to trypsin.

Highly purified grades of collagenase have been tried, but they are less effective when compared to crude collagenase. Collagenase in combination with hyaluronidase is found to be very effective for dissociating rat or rabbit liver. This can be done by per-fusing the whole organ in situ. Some workers use collagenase in conjunction with trypsin, a formulation developed in chick serum, for disaggregation of certain tissues (5, 6, 8).

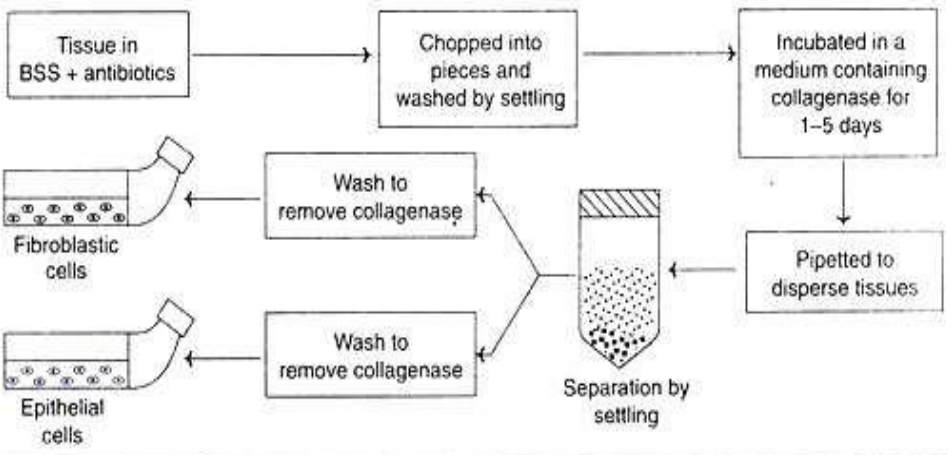


Fig. 36.3 : Important stages in collagenase disaggregation of tissue for primary culture (BSS–Basal salt solution).

Technique # 3. Primary Explant Technique: The primary explant technique was, in fact the original method, developed by Harrison in 1907. This technique has undergone several modifications, and is still in use. The tissue in basal salt solution is finely chopped, and washed by settlings. The basal salt solution is then removed. The tissue pieces are spread evenly over the growth surface. After addition of appropriate medium, incubation is carried out for 3-5

days. Then the medium is changed at weekly intervals until a substantial outgrowth of cells is observed. Now, the explants are removed and transferred to a fresh culture vessel.

Separation of Viable and Non-Viable Cells: It is a common practice to remove the nonviable cells while the primary culture is prepared from the disaggregated cells. This is usually done when the first change of the medium is carried out. The very few left over non-viable cells get diluted and gradually disappear as the proliferation of viable cells commences.

Advantages of Animal Cell Culture:

A. Toxicity Testing: Cultured cells are widely used alone or in conjunction with animal tests to study the effects of new drugs, cosmetics and chemicals on survival and growth in a wide variety of cell types. Especially important are liver and kidney derived cell cultures.

C. Cancer Research: Since both normal cells and cancer cells can be grown in culture, the basic differences between them can be closely studied. In addition, it is possible, by the use of chemicals, viruses and radiation, to convert normal cultured cells to cancer causing cells.

D. Virology: One of the earliest and major uses of cell culture is the replication of viruses in cell cultures (in place of animals) for use in vaccine production. Cell cultures are also widely used in the clinical detection and isolation of viruses, as well as basic research into how they grow and infect organisms.

E. Cell-Based Manufacturing: While cultured cells can be used to produce many important products, three areas are generating the most interest.

The first is the large-scale production of viruses for use in vaccine production. These include vaccines for polio, rabies, chicken pox, hepatitis B and measles.

The second is the large-scale production of cells that have been genetically engineered to produce proteins that have medicinal or commercial value. These include monoclonal antibodies, insulin, hormones, etc. The third is the use of cells as replacement tissues and organs. Artificial skin for use in treating burns and ulcers is the first commercially available product (9,10).

F. Genetic Counselling: Amniocentesis, a diagnostic technique that enables doctors to remove and culture fetal cells from pregnant women, has given doctors an important tool for the early diagnosis of fetal disorders. These cells can then be examined for abnormalities in their chromosomes and genes using karyotyping, chromosome painting and other molecular techniques.

G. Genetic Engineering: The ability to transfect or reprogram cultured cells with new genetic material (DNA and genes) has provided a major tool to molecular biologists wishing to study the cellular effects of the expression of these genes (new proteins).

H. Drug Screening and Development: Cell-based assays have become increasingly important for the pharmaceutical industry, not just for cytotoxicity testing but also for high throughput screening of compounds that may have potential use as drugs. Originally, these cell culture tests were done in 96 well plates, but increasing use is now being made of 384 and 1536 well plates.

I. Gene Therapy: In modern molecular biology, Gene Therapy is an experimental technique that involves insertion of cloned/altered genes into cells using r-DNA technology to replace defective genes causing genetic abnormalities or to prevent potential disorders.

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ACKNOWLEDGEMENT

I express my deep sense of gratitude to Dr. Sukhendu Roy,Assistant Professor, Head of the Department of Zoology, GushkaraMahavidyalaya under whose direction this study was conducted.

I am ever grateful to Dr. Saurabh Sarkar, Assistant Professor, Department of Zoology, GushkaraMahavidyalaya, for his continuous encouragement.

I am thankful to Aparnita Nandi Roy, Lecturer, Department of Zoology, GushkaraMahavidyalaya, for her valuable suggestions.

I am also thankful to Dr. NabyenduRakshit& Miss Poulomi Roy, SACT-I , Department of Zoology, GushkaraMahavidyalaya, for their continuous support.

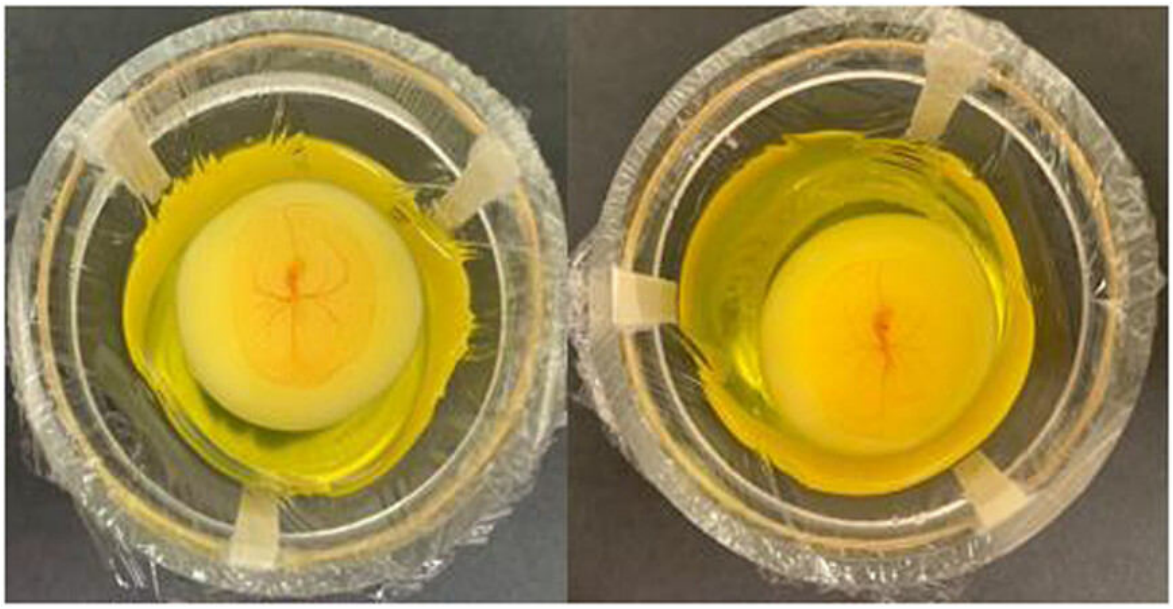
I am grateful to Principal, Dr. Swapan Kumar Pan, GushkaraMahavidyalaya, Gushkara, PurbaBurdwan, West Bengal.

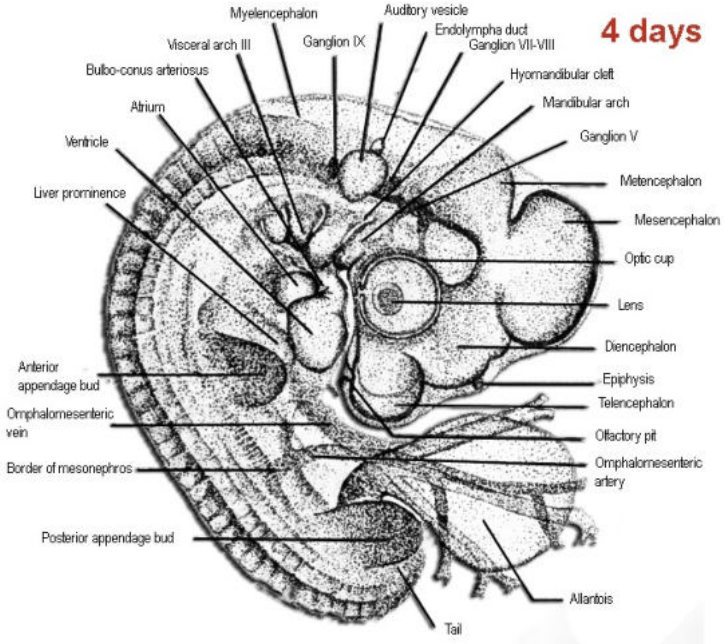
My appreciation goes to Mr. Kalyan Kr. Mukherjee and Mr. Uday Chand Mete, Laboratory Attendant for technical works, whose active involvement smoothened the entire tedious venture.

I owe a lot to my parents, who encouraged and helped me at every stage of my personal and academic life.

Above all, I owe it all to Almighty God for granting me the wisdom, health and strength to undertake this project work and enabling me to its completion.

Project work entitled "Whole Mounting & Identification of specific stages of Chick Embryos"





SUBMITTED BY

Name: Seheli Pervin

University Roll No: 200311000032

Registration number 202001004811 of 2020-21

Dr. Sukhendu Roy
Assistant Professor
Department of Zoology
Gushkara Mahavidyalaya

Gushkara Mahavidyalaya
NAAC Accredited "A" Grade Degree College
P.O:Gushkara, Dist:Purba Burdwan,
Pin-713128, West Bengal.
Email: guskaramahavidyalaya@gmail.com
Phone: 03452-255105
Fax: 03452-257635

DATE: 21.06.2023

This is to certify that Seheli Pervin bearing University Roll No 200311000032 with Registration number 202001004811 of 2020-21 students of B.Sc. Semester VI Zoology Honours have worked out the Project work entitled " Whole Mounting & Identification of specific stages of Chick Embryos" under my supervision and the entire work has been carried out by him/her in the Gushkara Mahavidyalaya, Department of Zoology, Guskara. The project work represents explanation of his/her own words. Due to lack of Infrastructure & proper instruments the whole work was not possible in practical based laboratory works.

Sukhendu Roy.

Dr. Sukhendu Roy
Assistant Professor
Department of Zoology
Gushkara Mahavidyalaya

Introduction:

The chicken (taxon -*Gallus gallus*) embryo develops and hatches in 20 to 21 days and has been extensively used in embryology studies. Historically, the chicken embryo was one of the first embryos studied, readily available and easy to incubate, embryo development can be directly observed by cutting a small window in the egg shell. A key to this model organism study was the establishment of a staging atlas by Hamburger & Hamilton in 1951[1], which allowed specific developmental landmarks to be seen and correlated with experimental manipulations of development. This much cited paper included images of all key stages and was more recently republished in the journal Developmental Dynamics (1993), for a new generation of avian researchers. Probably just as important has been the recent chicken genome sequencing, providing a resource to extend our knowledge of this excellent developmental model.

Fertilized eggs can be easily maintained in humidified incubators and during early stages of development the embryo floats on to the egg yolk that it is using for nutrition. As the embryo grows it sinks into, or below the, yolk. The regular appearance of somites allowed early experimenters to accurately stage the embryo. The embryo was accessible and easy to manipulate (limb grafts/removal etc) that were informative about developmental processes. Chicken cells and tissues (neural ganglia/fragments) are also easy to grow in tissue culture. The discovery that quail cells have a different nuclear appearance meant that transplanted cells (chick/quail chimeras) could be tracked during development. For example, LeDourian's studies showed how neural crest cells migrate widely throughout the embryo.

The chicken (*Gallus gallus*) embryo is an excellent model for the study of early vertebrate embryogenesis and later organogenesis. The embryo is encased within a hardened eggshell which provides a natural incubator or culture dish. Through a hole in the eggshell, the embryo

can be visualized and easily manipulated with microsurgical tools or gene constructs, then allowed to continue development in ovum to determine the consequence of the experimental manipulation. Fertilized chicken eggs are readily available anywhere in the world and the equipment needed is minimal – a humidified incubator (39°C, no CO2 required), a dissecting microscope, microsurgical tools that can be prepared in the lab or purchased, and either a hand held mouth pipette or a manufactured micromanipulator. Fertilized eggs can be held at ~13-16ºC for up to 1 week prior to incubation. They are incubated at 38ºC39ºC to the desired stage in a humidified incubator with the eggs placed on their side (horizontal). For long term post-operative survival, it is best that the eggs be left in the incubator until experimental manipulation. However, eggs can be removed from the incubator and held at room temperature to slow development.

Taxonomic Hierarchy

Kingdom Animalia

Subkingdom Bilateria

Infrakingdom Deuterostomia

Phylum Chordata

Subphylum Vertebrata

Infraphylum Gnathostomata

Superclass Tetrapoda

Class Aves

Order Galliformes

Family Phasianidae

Subfamily Phasianinae

Genus Gallus Brisson, 1760

Species Gallus gallus (Linnaeus, 1758)

Aims

- To investigate the effects of temperature on heart rate
- To observe embryonic structures, particularly the somites

Learning outcomes

By the end of the practical, students will hopefully:

1. Appreciate the utility of embryos for the study of anatomy and physiology
2. Recognise the advantage of having an accessible embryo in an egg for studies of development
3. Appreciate the dynamic nature of development
4. Understand how heart rate is regulated and how external factors can affect it

SAFE WORKING PROCEDURES AND ANIMAL ETHICS

Risks Associated with Practical

Eggs have the potential to be contaminated with *Salmonella*. Wear gloves throughout and students should wash their hands before leaving the lab. Dissection implements are sharp, so students should take care not to cut themselves or other students. Students should wear a labcoat, gloves and enclosed shoes to protect themselves from egg splatter.

Animal Ethics Compliance

The procedures used in this practicum are in compliance with the Animal Care and Ethics Committee and the National Health and Medical Research Council 'India code of practice (9th edition, 2017).

MATERIAL REQUIRED

1. Fertilized hen's egg,
2. Incubator (38°C, preferably with automatic egg turning facility),
3. Coarse forceps,
4. Watchmaker's forceps,
5. Fine scissors,
6. Petri dishes

EXPERIMENTAL PROCEDURES

1. Take fertilized hen's egg.
2. 1. Eggs were incubated for 1 up to 8 days at 38.5°C to generate embryos of several developmental stages (16 hrs/18 hrs/24hrs/33hrs/48 hrs/72 hrs/ 96 hrs etc.)

A. Opening of the egg

1. Each student pair should have one egg and an egg holder, a pair of blunt forceps and a pair of scissors,

2. Petri dishes, PBS, disposable pipet, syringe and 23G needle, Indian ink solution, dissection microscope.

3. Put the egg into the holder with the blunt end up (pointed end down).

4. Use the pointy end of the scissors to tap a hole in the top of the egg into the air chamber, be very careful not to push the scissors too far into the egg.

5. Use the forceps to pick bits of shell out. Do not remove egg shell beyond the air chamber. You will see the air chamber and the vitelline membrane.

6. Carefully remove the vitelline membrane at the top.

7. If you do not see the embryo (a ring of blood vessels should be visible) then gently swirl the egg so that it floats up to the top of the yolk. If this doesn't work you will need to take another egg if available.

8. Use the forceps to break off pieces of shell down to the yolk so that the embryo (visible as a ring of blood vessels) is exposed and the rim of the shell is just above the surface of the egg white or albumen.

9. You may be able to see the heart beating without magnification. If not, then put the egg under the dissection microscope.

B. Early stage somitogenesis embryos

1. Draw Indian ink solution (if available) up into a 1 ml syringe fitted with a 23G syringe needle.

2. Open an egg as described above. Once the shell has been removed down to the level of the yolk and the vitelline membrane has been removed, slide the syringe needle under the embryo.

It is easiest to insert the syringe needle vertically at the edge of the egg initially and then rotate the needle until it is almost horizontal using the edge of the egg shell as a support. The tip of the needle should end up just below the embryo in the centre of the egg.

3. Slowly inject the Indian ink solution to reveal the embryo and view under a dissecting microscope.

4. Identify the HH stage and the diverse embryonic structures.

5. Draw your embryo and annotate the embryonic structures that you have identified.

6. Hand your drawing in at the end of the practical.

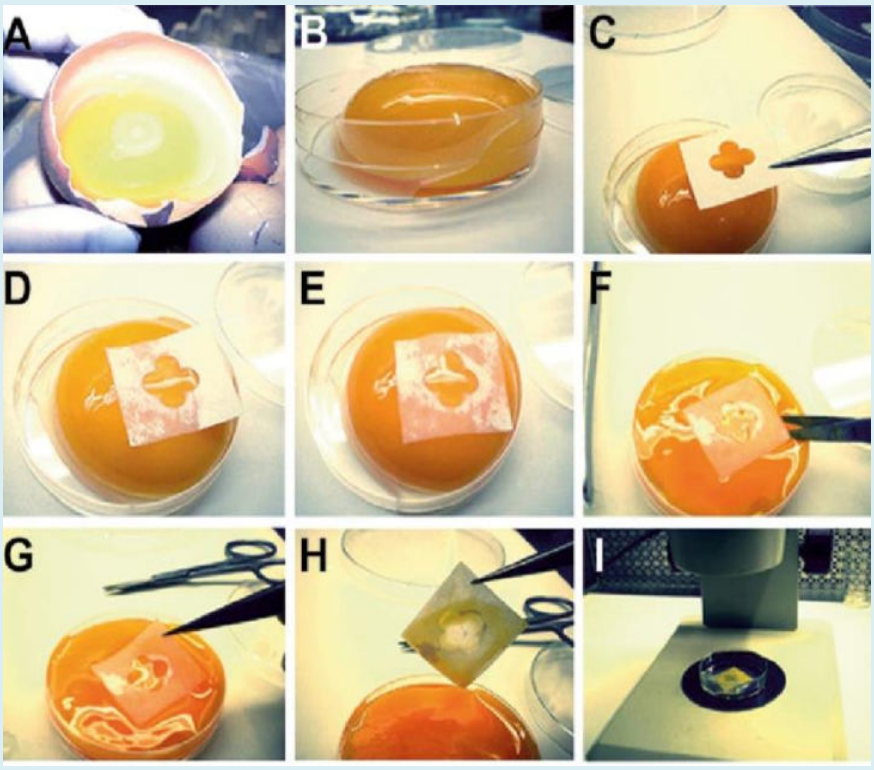


Fig-1. Early Chick (EC) procedure for preparing whole mounts from chick embryos to be used in ISH. (a) After opening the egg shell, pour off the albumin. (b) Place the yolk in a dry Petri dish with the blastoderm faceup. (c–e) Place upon the blastoderm a piece of filter paper pre-prepared with a central hole (note the particular tetra-lobulated

cloverleaf shape of the hole), centering the hole on the embryo, and slightly press it down with forceps to make it adhere to the blastoderm (it becomes transparent). (f) The vitelline membrane is trimmed around the filter paper. (g) Detach the filter plus blastoderm from the yolk in an angle, holding it with forceps. (h) Remove excess yolk adhering to the underside of the filter paper and blastoderm, and transfer it to a Petri dish with clean PBS. (i) Remove traces of yolk under a dissecting microscope and transfer to another Petri dish with cold fixative solution

C. Late stage organogenesis embryos

1. Carefully tear a hole in the vitelline membrane.

2. Very carefully tilt egg contents in a large Petri dish, holding the egg very close to the dish.

3. Find and dissect the embryo, transfer it to a clean Petri dish and cover it with PBS.

4. Study embryo under a dissection microscope, determine the developmental HH stage (see last page of the manual or link below), and identify the embryonic structures.

5. Draw your embryo, indicate the developmental stage (HH), and annotate the embryonic structures that you have identified.

6. Hand your drawing in at the end of the practical.

Fig-1.1 Mounting of the embryo to a slide

D. Observation of developmental stages

Move around the class to study the embryonic chicken stages of your colleagues. Identify the following structures:

- Amniotic sac
- Hensen's node
- Neural groove and folds
- Head ectoderm
- Somites
- Brain vesicles
- Cardiogenic mesoderm
- Heart and vasculature
- Optic vesicles
- Branchial arches
- Nasal pits and nasal processes
- Otic vesicle
- Limb buds

Study of Chick Embryo at 4 Hours

Features of whole mount chick embryo at 4 hours.

i. At 4 hours of incubation the differentiation of the blastodisc into area pellucida and area opaca can be observed in the chick embryo.

ii. One quadrant of the area pellucida appears thickened which is the region of the future caudal end of embryo (Fig. 7.1). After 7 to 8 hours, the thickening will become more elongated and then represents the start of primitive streak.

Study of Chick Embryo at 16 Hours

I. Features of whole mount of chick embryo at 16 hours of incubation.

i) In the whole mount of a 16 hour chick embryo (Fig. 1.1.1) you will observe the distinct primitive streak. The embryo at this stage is characterized as being in the primitive streak stage.

ii) In the whole mount, the embryo is seen to have a central furrow, called the primitive groove which appears lined by thickened primitive ridges.

iii) At the cephalic end (head end) of the embryo, closely packed cells form a thickened area, called as Hensen's node.

iv) Part of area pellucida adjacent to the primitive streak shows increased thickness and forms the embryonic area or embryonic shield.

v) The area of pellucida at this stage assumes an elliptical shape.

vi) An elongated primitive streak is visible which represents the long axis of the future embryonic body. vii) The end diametrically opposite to the Hensen's node is the caudal end of the embryo.

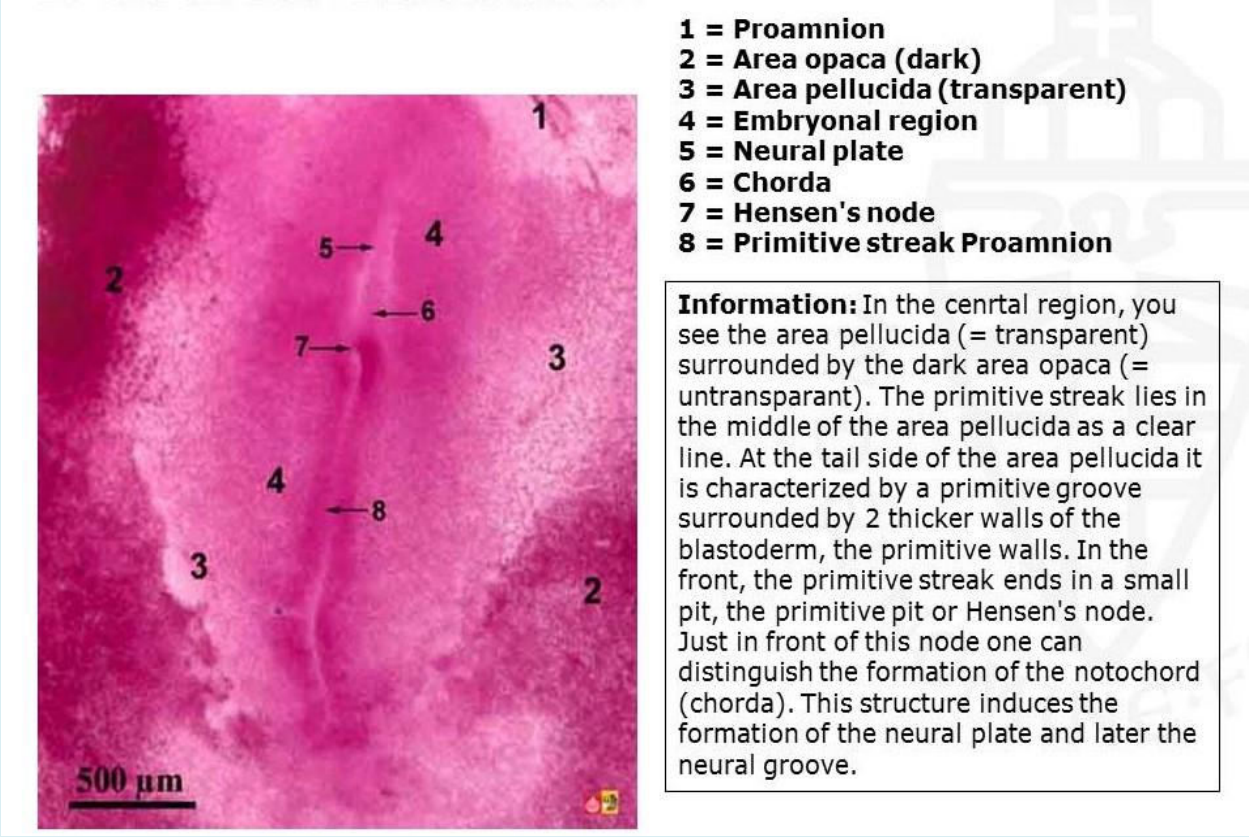


Fig-1.1.1: chick embryo at 16 hours of incubation

II. Features of chick embryo in longitudinal section (LS) at 16 hours after incubation

i) The LS through the 16 hour chick embryo represents the embryonic chick stage shortly after primitive streak formation. It also marks the beginning of morphogenetic movement of cells to form the notochord.

ii) The LS shows the ectoderm, Hensen's node, primitive pit, primitive groove, notochord and primitive gut. The mesoderm extends on either side between ectoderm and endoderm.

Study of Chick Embryo at 18 Hours

I. Features of whole mount of chick embryo at 18 hours of incubation.

i) In the 18 hour whole embryo you will observe that the notochord has become markedly elongated to form a conspicuous structure (Fig 2).

ii) Notochord is seen to extend from the Hensen's node towards the cephalic region present in the middle.

iii) Embryo at this stage of development is said to be in the head process stage.

iv) Neural plate develops around the notochord.

v) The dark peripheral area opaca, the inner translucent area pellucida and the central embryonal area are clearly visible.

vi) In the anterior region a small and more translucent portion of area pellucida, known as proamnion can be observed.

vii) The primitive streak lies in the middle of the area pellucida in the posterior half.

viii) The neural plate and primitive streak can be seen to be separated by Hensen's node.

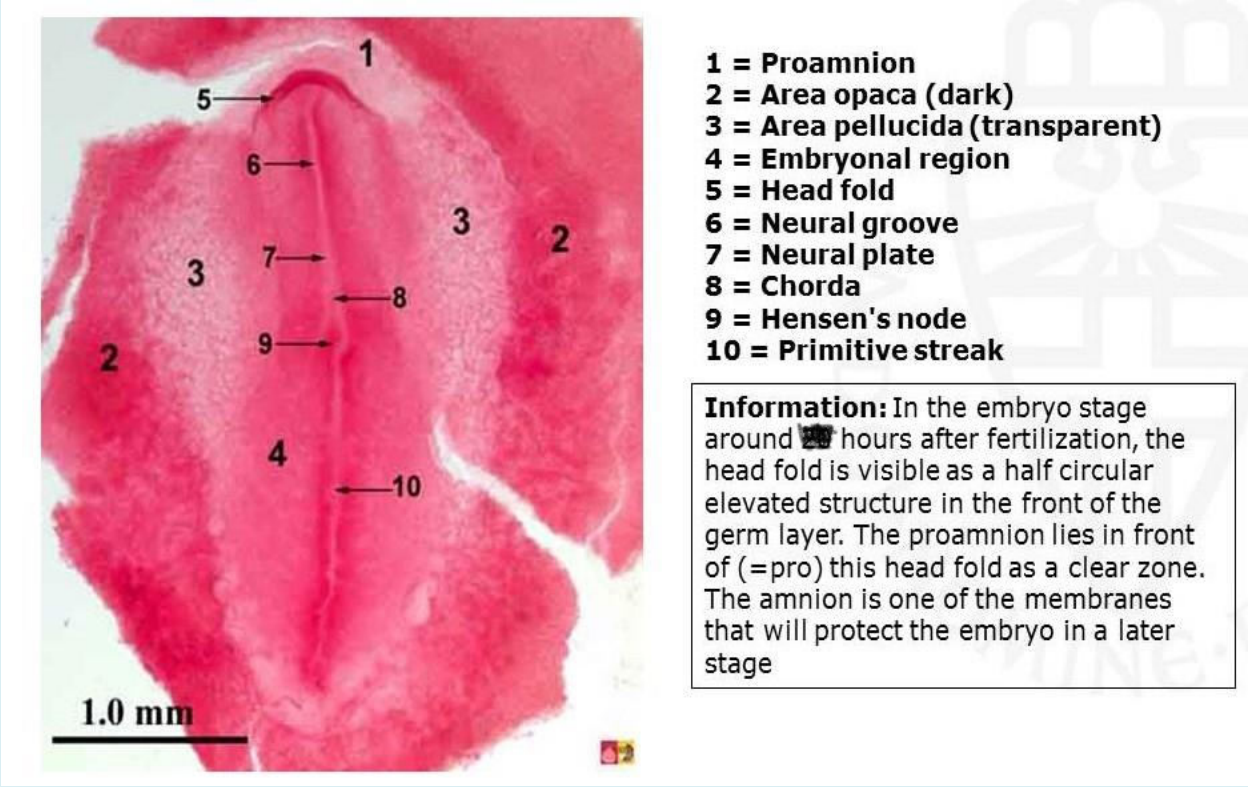


Fig-2: chick embryo at 18 hours of incubation

II. Features of chick embryo in longitudinal section (LS) at 18 hours after incubation

i) The LS of the 18 hours incubated chick embryo shows advanced inner structures of the germ layers.

ii) Ectoderm is seen to have vertical cells while the cells of the mesoderm are represented by heavy angular dots. Endoderm is represented by stippling backed by a single line.

iii) Yolk, ectoderm of neural plate, notochord, mesodem, ectoderm and endoderm of blastoderm can be observed in the section of the slide.

iv) You will also observe the primitive pit, primitive ridge, and primitive gut.

Study of Chick Embryo at 24 Hours

I. Features of whole mount of chick embryo at 24 hours of incubation.

i) In the whole mount of a 24 hour old embryo (Fig 3) the cephalic region is prominently visible because of rapid growth in this region.

ii) The cephalic region extends anteriorly and overhangs the proamnion region. The cephalic region which projects free from the blastoderm can now be properly termed as the head of embryo.

iii) A space between the head and the blastoderm is formed and is visible and is called the sub-cephalic pocket.

iv) In the mid-line of the embryo the notochord is visible. It is larger at the caudal area near its point of origin than at the cephalic area.

v) The neural plate is much more clearly visible. The neural folds appear as a pair of dark bands.

vi) At its cephalic end, the neural groove is deeper. The neural folds are correspondingly more prominent at the cephalic end than they are caudally.

vii) Four pairs of somites are seen in the mid-line.

viii) Primitive streak is seen to gradually decrease in size.

ix) Foregut is also formed and is visible.

x) The part of the gut, caudal to the foregut is termed the midgut and the opening of the midgut into the foregut is called the anterior intestinal portal.

xi) In addition to the structures, mentioned above the area opaca vitellina, area pellucida, proamnion, Hensen's node, area vasculosa, blood islands and unsegmented mesoderm are also seen (Fig. 3).

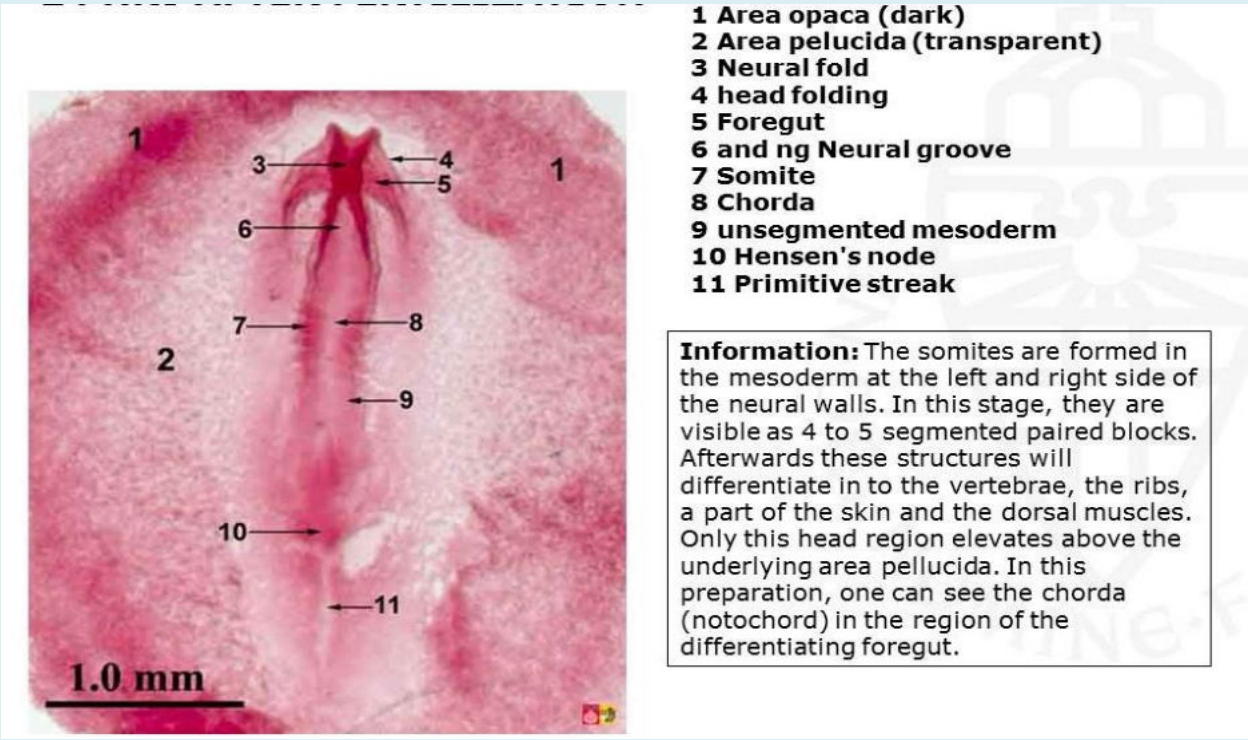


Fig-3: chick embryo at 24 hours of incubation

II. Features of chick embryo in transverse section (T.S) at 24 hours after incubation

i) The transverse section passing through head region (Fig. 7.7a) shows the folded neural plate forming a complete tube.

ii) The notochord can be seen beneath the neural fold.

iii) Mesenchyme, foregut, ectoderm of head, mesoderm and endoderm can be seen in this section.

iv) The transverse section passing through mid-body of the chick embryo (Fig. 7.7b) shows formation of somites and changes in the mesoderm.

v) Mesoderm is seen to be differentiated into: i) dorsal mesoderm, ii) intermediate mesoderm and iii) lateral mesoderm.

vi) Other structures seen in the slide are ectododerm, endoderm, lateral margin of anterior intestinal portal, midgut and pericardial coelom.

Study of Chick Embryo at *33* Hours

I. Features of whole mount of chick embryo at *33* hours of incubation

i) The 33 hour old embryo of chick (Fig. 4) shows some of the fundamental structures which will be involved in the formation of central nervous system and circulatory system.

ii) You will notice remarkable changes in the development of the brain. Observe that the brain is differentiated into prosencephalon (fore-brain), mesencephalon (mid-brain), and rhombencephalon (hind- brain).

iii) The optic vesicles have been established and can be seen in the whole mount as paired lateral outgrowths of the prosencephalon. The optic vesicles will later extend to occupy the full width of the head. Infundibulum is seen to have formed in the floor of the prosencephalon.

iv) Mid-region of the heart is considerably dilated and is bent to the right.

v) Twelve pairs of somites can be observed.

vi) Anterior omphalomesentric veins have developed and can be seen.

vii) The primitive streak becomes shorter because of the lengthening of the neural tube.

viii) Proamnion, neural tube, notochord, sinus rhomboidalis and sinus terminalis are also present and can be seen.

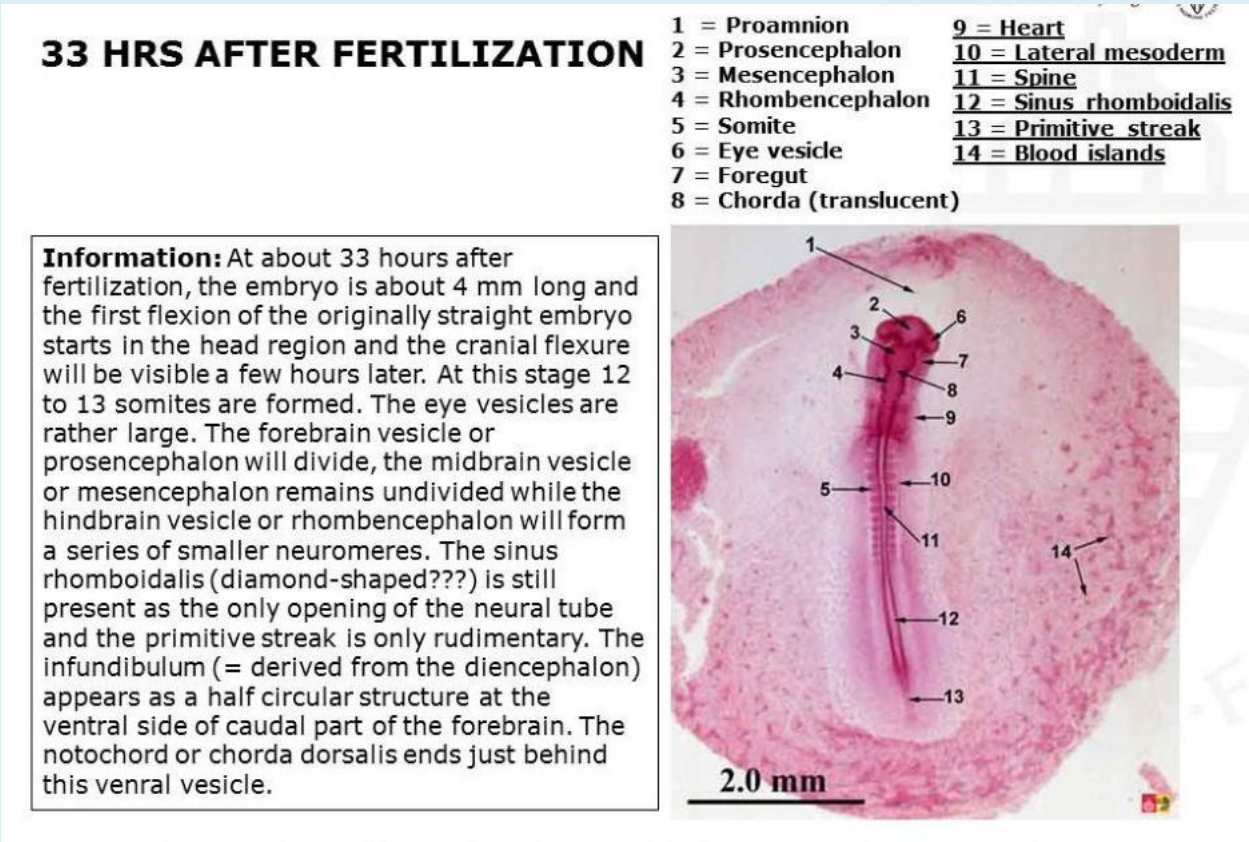


Fig-4: chick embryo at 33 hours of incubation

II. Features of chick embryo in transverse section (T.S) at 33 hours after incubation

i) The transverse section of the 33 hour old embryo chick shows the following structures ectoderm, prosocoel, opticoel, mesenchyme, somatic mesoderm, splanchnic mesoderm and endoderm.

ii) The section shows mid- structures namely, mesocoel, anterior cardinal vein, dorsal aortic root, somatopleure, extraembryonic coelom, splanchnopleure, foregut, notochord and ventral aortic root.

Study of Chick Embryo at 48 Hours

I. Features of whole mount of chick embryo at 48 hours of incubation

i. Head is turning onto the left side with distinct cranial flexure.

ii. Anterior neuropore closed.

iii. Telencephalon indicated.

iv. Primary optic vessel and optic stalk well established.

v. Auditory pit is deep but wide opened.

vi. Heart is slightly S-shaped.

vii. Headfold of amnion covers entire region of forebrain.

viii. Presence of 16-19 pairs of somites (Fig. 5).

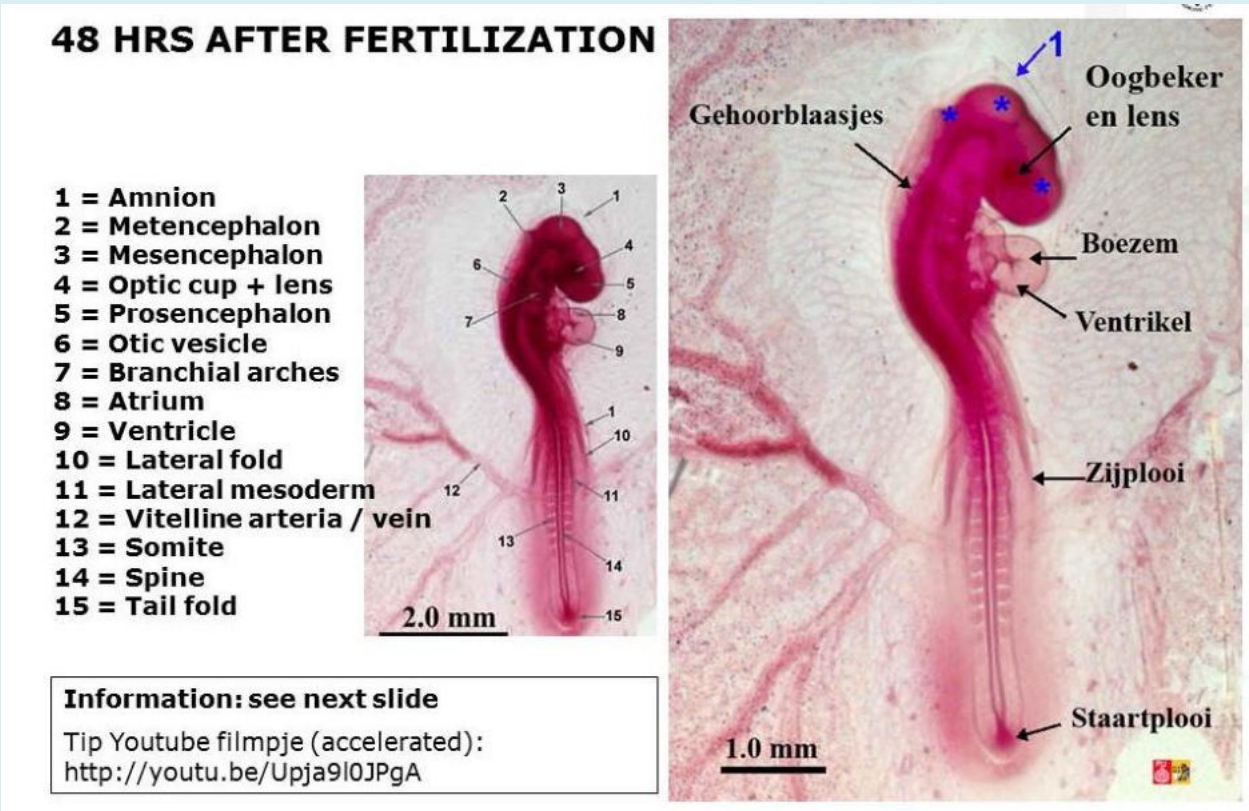


Fig-5: chick embryo at 48 hours of incubation

Study of Whole Mount of Chick Embryo at 72 Hours of Incubation

I. Features of whole mount of chick embryo at 72 hours of incubation

i) The 72 hours old chick embryo (Fig. 6) as observed in the whole mount is seen to be affected throughout by torsion and the entire body is seen to have turned through 90". Torsion is

complete in the chick embryo posterior to the level of heart, but the caudal portion of embryo is not turned on its side.

ii) The long axis of the embryo due to the presence of cranial and cervical flexures, shows nearly right angled bends in the mid brain and neck region. The mid body appears concave.

iii) Visceral arches have developed and can be seen.

iv) Mandibular arch is visible and has formed the caudal boundary of oral depression and become more distinct.

v) Nasal pits appear as shallow depressions.

vi) Cephalization can be seen to be in process. Telencephalon has developed and is visible.

vii) In the eye, the lens, sensory and pigmented layers can be seen to have differentiated.

viii) Number of somites have increased to 36 pairs.

ix) Vitelline arteries and vitelline veins also make their appearance and are visible.

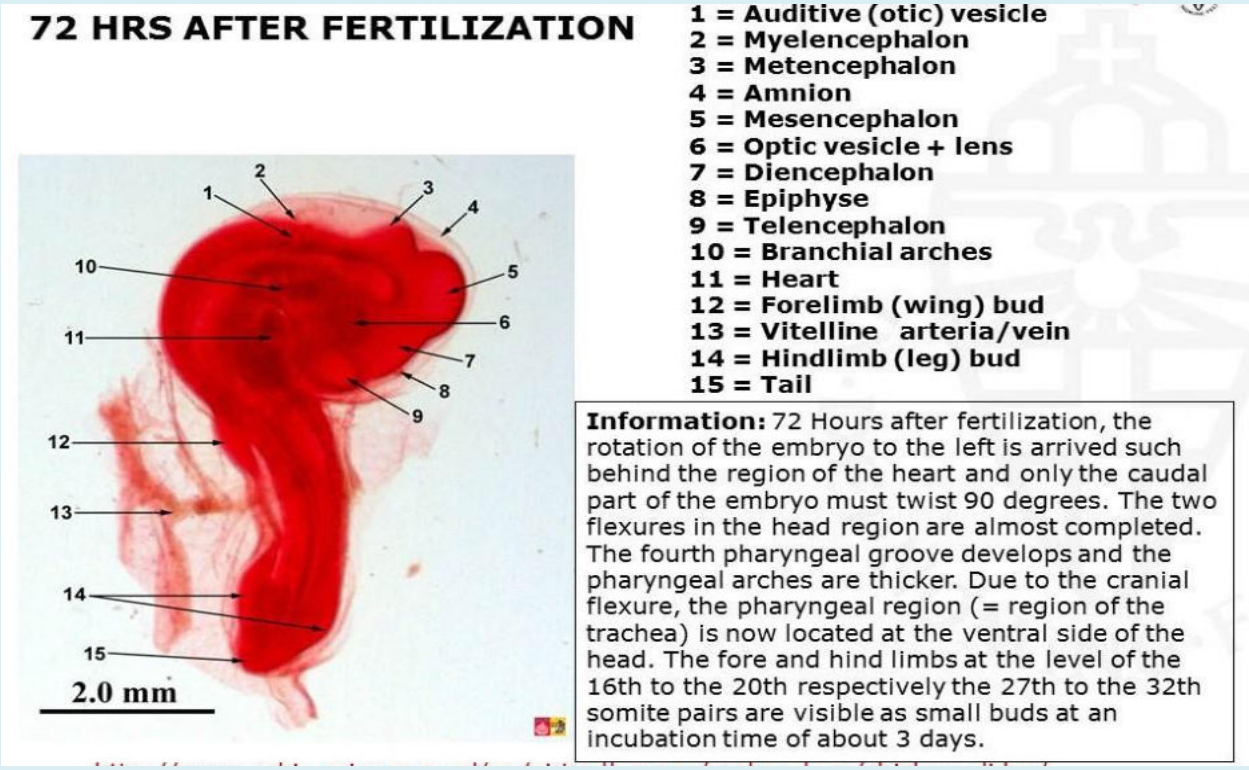


Fig-6: chick embryo at 72 hours of incubation

Study of Whole Mount of Chick Embryo at 96 Hours of Incubation

I. Features of whole mount of chick embryo at 96 hours of incubation

i) At 96 hours of incubation the entire body of the chick embryo has been turned through 90" and the embryo lies with its left side on the yolk (Fig. 7).

ii) At the end of 96 hours the body folds have undercut the embryo so that the embryo is seen to be attached to the yolk only by a slender stalk.

iii) The yolk stalk soon becomes elongated, allowing the embryo to become first straight in the mid-dorsal region and then in the dorsal region.

iv) The progressive increase in the cranial, cervical, dorsal and caudal flexures results in the bending of the embryo on itself so that its originally straight long axis becomes C shaped and its head and tail lie close together.

v) Paired optic cups show more developed lens.

vi) Endolymphatic ducts arise from the paired auditory vesicle.

vii) Visceral arches appear to have thickened.

viii) Appendage buds appear increased in size and can be seen to have become elongated.

ix) The number of somites are seen to have increased to 41 pairs.

x) Allantois has also made an appearance and is visible.

xi) Omphalomesentric artery and omphalomesentric vein have also developed and can be observed.

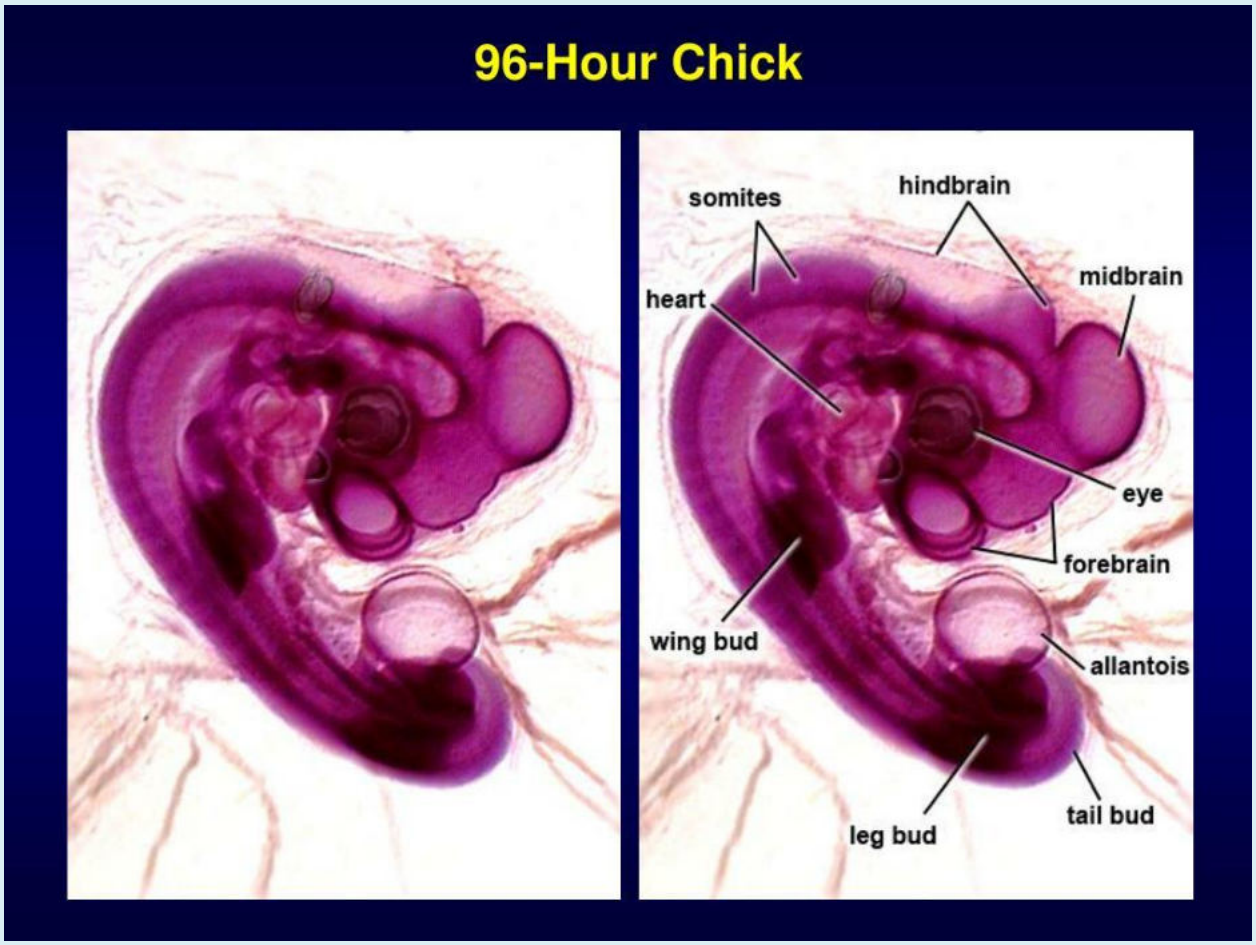


Fig-7: chick embryo at 96 hours of incubation

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Acknowledgement:

- I express my deep sense of gratitude to Dr. Sukhendu Roy, Assistant Professor, Head of the Department of Zoology, Gushkara Mahavidyalaya under whose direction this study was conducted.
- I am ever grateful to Dr. Saurabh Sarkar, Assistant Professor, Department of Zoology, Gushkara Mahavidyalaya, for his continuous encouragement.
- I am thankful to Aparnita Nandi Roy, Lecturer, Department of Zoology, Gushkara Mahavidyalaya, for her valuable suggestions.
- I am also thankful to Dr. Nabyendu Rakshit & Miss Poulomi Roy, SACT-I , Department of Zoology, Gushkara Mahavidyalaya, for their continuous support.
- I am grateful to Principal, Dr. Swapan Kumar Pan, Gushkara Mahavidyalaya, Gushkara, Purba Burdwan, West Bengal.
- My appreciation goes to Mr. Kalyan Kr. Mukherjee and Mr. Uday Chand Mete, Laboratory Attendant for technical works, whose active involvement smoothened the entire tedious venture.

- I owe a lot to my parents, who encouraged and helped me at every stage of my personal and academic life.
- Above all, I owe it all to Almighty God for granting me the wisdom, health and strength to undertake this project work and enabling me to its completion.