

Country: _____

Student Code: _____

19th INTERNATIONAL BIOLOGY OLYMPIAD

13th – 20th July, 2008

Mumbai, INDIA



PRACTICAL TEST 4

ANIMAL BEHAVIOR

Total Points: 49

Duration: 60 minutes

Dear Participants,

- In this test, you have been given the following two tasks:

Task 1: Part A: Study of the olfactory response of *Drosophila melanogaster* larvae: Experimental design (7 points)

Part B: Study of the olfactory response of *Drosophila melanogaster* larvae: Larval plate test (18 points)

Part C: Study of olfactory adaptation in *Drosophila* larvae (11 points)

Task 2: Study of fish behavior (13 points)

- The duration of the test is **10 minutes** for Part A of Task 1 and **50 minutes** for the remaining paper.
- **You have to answer Part A in 10 minutes after which the buzzer will ring and the Answer Sheet for Part A will be collected from you. Only then will the Question Paper and Answer Sheet for Task 1 – Parts B and C, and Task 2 be given to you.**
- **Please do not switch on your computer before your Answer Sheet for Part A is collected.**
- **You have to write down your results and answers in the ANSWER SHEET. Answers written in the Question Paper will not be evaluated.**
- At the end of the test, put both the Question Papers as well as the Answer Sheet for Task 1 – Parts B and C, and Task 2 in the envelope. The supervisor will collect this envelope.

Good Luck!!

Country: _____

Country Code: _____

First Name: _____

Middle Name: _____

Family Name: _____

Student Code: _____

Practical Test 4

Animal Behavior

Task 1 – Part A (6 points)

Study of the olfactory response of *Drosophila melanogaster* larvae

Experimental design

You have been given Part A of this task. You have to answer this part in 10 minutes after which the buzzer will ring and the Answer Sheet for this part will be collected from you. Only then will the rest of the paper be given to you.

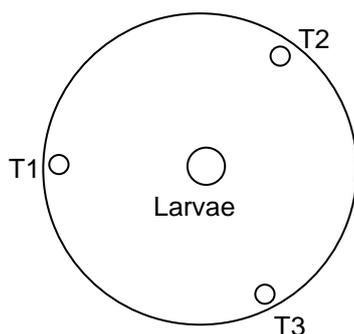
Introduction

Insects have a strong sense of smell. Adult moths, for example, can find their mates by smelling pheromone molecules at very low concentrations. The sense of smell is associated with a discriminatory behavior as well. This is evident from the fact that insects are able to choose their food by odor. The nature of an odor stimulus can be categorized into three types: (1) attractive, (2) repulsive, and (3) neutral.

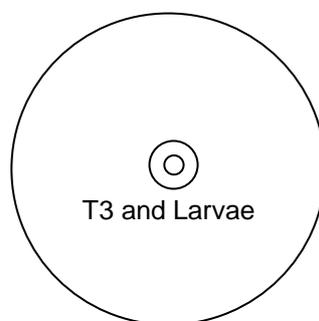
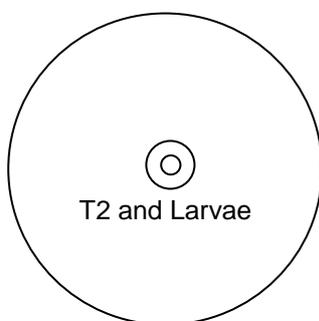
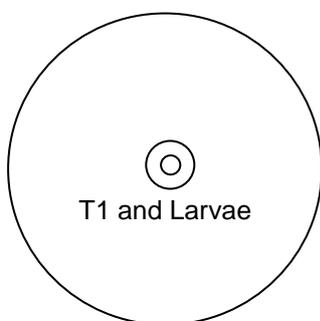
The odor discriminatory behavior of insects such as *Drosophila melanogaster*, the common fruit fly, can be assessed using either the adults or the larvae. *Drosophila* larvae respond to odor stimuli by crawling either towards or away from them. It is, therefore, possible to design an experiment to test the larval response towards different chemicals on a Petri plate.

Q. 1.A.1. (3 points) Suppose you want to determine the response of *Drosophila melanogaster* larvae to three chemical odorants T1, T2, and T3. Five possible experimental designs for this purpose are given below:

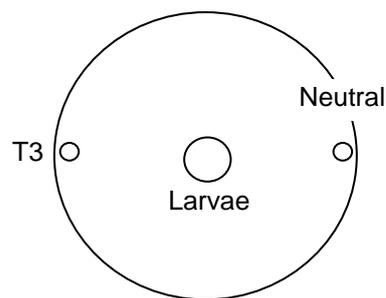
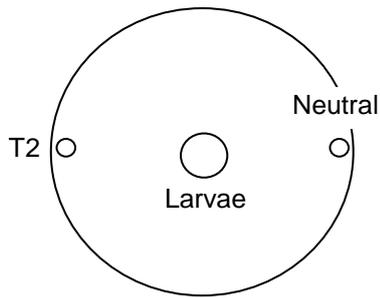
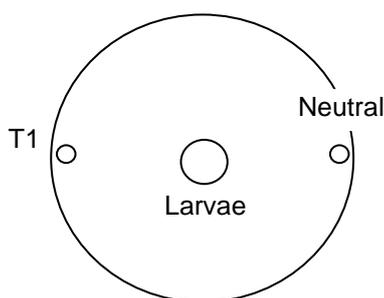
Design I: All the three chemicals are placed at equidistant positions on the periphery of a Petri plate and the larvae are introduced at the centre.



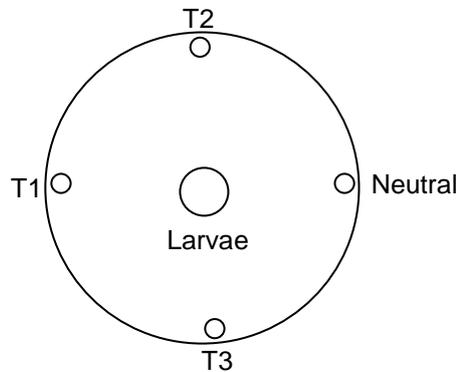
Design II: One of the chemicals and the larvae are placed together at the centre of a plate. Three such plates are set up for the three chemicals.



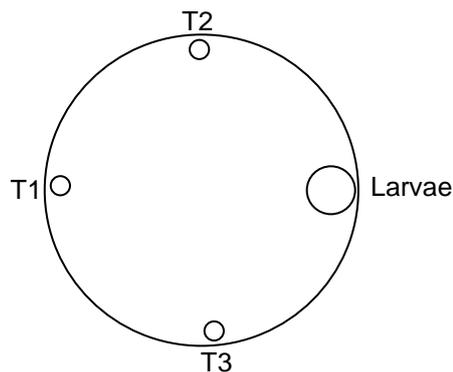
Design III: One of the chemicals and an odor-free (neutral) chemical are placed at two ends of a plate. Larvae are introduced in the centre. The test is repeated for the remaining chemicals.



Design IV: The three test chemicals and a neutral chemical are placed at equidistant positions on the periphery of a plate and the larvae are introduced at the centre.



Design V: The three test chemicals and the larvae are placed at equidistant positions on the periphery of a plate.



Choose the most appropriate experimental design and put a tick mark (✓) against it

in Q. 1.A.1. in the Answer Sheet.

Design I	
Design II	
Design III	
Design IV	
Design V	

Please note that the next question (Q. 1.A.2.) will be evaluated only if your answer to this question (Q. 1.A.1.) is correct.

Q. 1.A.2. (4 points) Mark the following statements as TRUE or FALSE in accordance with your choice of the experimental design.

STATEMENT	TRUE	FALSE
I. It allows the larvae to choose between two or more different chemicals presented simultaneously and thus acts as a discriminatory test.		
II. It can clearly distinguish between attractants and repellants by testing them one at a time against the neutral chemical.		
III. The entire experiment (i.e., testing all the chemicals) can be completed using a single test and thus inter-experimental variation can be avoided.		
IV. It can clearly distinguish the repulsive and attractive nature of the stimuli as each can enhance the response of the larvae to the other(s).		
V. There will not be any mixing of the odors and hence more reliable results will be obtained.		
VI. All the chemicals can be tested against the same control in a single plate.		
VII. Amongst the designs presented, it is the one in which the effect of the weakest odorants can be tested.		
VIII. Larvae can disperse in any direction without any hindrance.		

*****END OF PART A*****

Practical Test 4

Animal Behavior

Task 1 – Parts B and C

Study of the olfactory and phototactic responses of *Drosophila melanogaster*

larvae

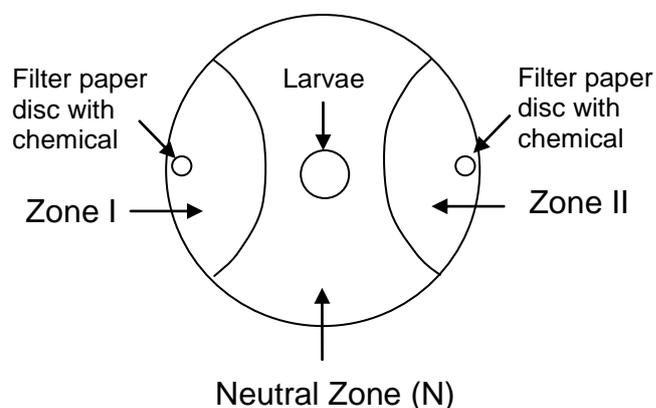
You should try and complete Parts B and C of this task in 35 minutes.

Part B (18 points)

Larval plate test

Design

Five experiments were conducted to test the response of *Drosophila melanogaster* larvae to chemical and light stimuli. Four chemical odorants, A, B, C, and D were used in the tests. Of these, D was known to be a neutral chemical while A, B, and C could be an attractant, repellent or a neutral chemical. The design for the experiment is as shown in the figure:



Method

Third instar larvae were used in these experiments. These larvae were obtained by washing the 6-day-old *Drosophila* culture with 15% sucrose solution. The larvae that float in this solution were washed free of sucrose and immediately used in the tests. The tests were performed in Petri plates containing a layer of 1% agarose.

In each experiment, two chemicals were spotted on separate filter paper discs that were placed in two Zones, Z I and Z II (marked as semicircular areas) at two diametrically opposite ends of a Petri plate. Approximately 30-40 larvae were placed at the centre of each plate and their movement over the next five minutes was recorded. Five such experiments were conducted. The recordings of these experiments have been provided to you as video films. Experiments No. 1, 2, and 4 were conducted under uniform light conditions. In Experiments No. 3 and 5, half the plate was covered with black paper and the remaining half was left exposed to light.

Q. 1.B.1. (10 points): Observation of video films

1. Double click on the video file labelled **1** on the computer monitor to observe the movement of larvae.
2. The duration of the video film is 5 minutes, compressed to 2.5 minutes. You may forward or rewind the video film, if required.
3. At the end of the experiment, count the number of larvae in Zone I (N_{ZI}) and Zone II (N_{ZII}).
4. Record your readings **in Table 1.B.1. in the Answer Sheet.**
5. Repeat steps 1 to 4 for the video files labelled **2 to 5.**

Table 1.B.1.

Experiment	Chemical in ZI	Number of larvae in ZI (N _{ZI})	Chemical in ZII	Number of larvae in ZII (N _{ZII})	$\frac{N_{ZI}}{N_{ZI} + N_{ZII}}$	$\frac{N_{ZII}}{N_{ZI} + N_{ZII}}$
1	B		C			
2	A		B			
3	A (in dark)		B			
4	B		D			
5	B (in dark)		C			

Q. 1.B.2. (3 points) What is the likely nature of the three chemicals A, B and C? Put a tick mark (✓) in the appropriate box **in the Answer Sheet.**

Chemical	Attractant	Repellant	Neutral chemical	Nature cannot be determined
A				
B				
C				

Q. 1.B.3. (5 points) Based on your observations, mark whether the following statements are true or false by putting a tick mark (✓) in the appropriate box **in the Answer Sheet.**

- a. Larvae exhibit a stronger positive movement towards the attractant odorant tested than towards darkness.
- b. Light is a stronger repelling stimulus for the larvae than the repellent odorant tested.
- c. The positive phototaxis shown by the larvae is stronger than the movement towards the attractant odorant.
- d. In the presence of light, the larvae do not exhibit chemotaxis.
- e. The repellent odorant has a stronger influence on the larvae than does darkness.

	True	False
a.		
b.		
c.		
d.		
e.		

Part C (11 points)

Study of olfactory adaptation in *Drosophila melanogaster* larvae

Continuous stimulation of the olfactory system with a given odor tends to result in adaptation, also known as desensitization. As a result, the larvae fail to respond to the odor to which they have been adapted. A researcher working on olfaction in *Drosophila melanogaster* larvae wanted to study adaptation in these larvae. She selected the following odorants for her study:

1. Ethyl acetate
2. Pentyl acetate
3. Hexyl acetate
4. Heptyl acetate

Pre-stimulation experiment: In each experiment (except in Experiment 1), the larvae were pre-stimulated by placing them in a Petri plate containing 40 microlitres of one of the above odorants for 25 min. The same larvae were then picked up from this plate and tested for their response to the same or different odorants using the protocol described earlier in Part B of Task 1.

The data obtained from these tests are tabulated below.

Data from the pre-stimulation experiment

Experiment	Pre-stimulation	Test odorant							
		Experiment A		Experiment B		Experiment C		Experiment D	
		Ethyl acetate	*	Pentyl acetate	*	Hexyl acetate	*	Heptyl acetate	*
		$N_{Z I}$	$N_{Z II}$	$N_{Z I}$	$N_{Z II}$	$N_{Z I}$	$N_{Z II}$	$N_{Z I}$	$N_{Z II}$
1	None	21	3	18	5	14	12	8	13
2	Ethyl acetate	14	11	15	11	13	10	9	15
3	Pentyl acetate	16	15	12	11	9	19	9	14
4	Hexyl acetate	17	9	17	14	16	13	8	13
5	Heptyl acetate	15	10	13	5	8	13	10	13

$N_{Z I}$ and $N_{Z II}$ are the number of larvae in Zone I and Zone II, respectively.

* Zone II in all the experiments contained a neutral chemical.

The data given in the table are a set of average responses. The actual numbers varied up to 10% on either side of the average.

Q. 1.C.1. (5 points) Calculate the Response Index (RI) for each experiment

according to the formula:

$$RI = \frac{N_{ZI} - N_{ZII}}{N_{ZI} + N_{ZII}} \times 100$$

Fill in the RI values **in Table 1.C.1. in the Answer Sheet.**

Table 1.C.1.

Experiment	Pre-stimulation	Test odorant			
		Experiment A	Experiment B	Experiment C	Experiment D
		Ethyl acetate	Pentyl acetate	Hexyl acetate	Heptyl acetate
		RI	RI	RI	RI
1	None				
2	Ethyl acetate				
3	Pentyl acetate				
4	Hexyl acetate				
5	Heptyl acetate				

Q. 1.C.2. (2 points) To which odorant have the larvae adapted the most?

Put a tick mark (✓) in the appropriate box **in the Answer Sheet.**

Ethyl acetate	
Pentyl acetate	
Hexyl acetate	
Heptyl acetate	

Q. 1.C.3. (2 points) To which odorant have the larvae adapted the least?

Put a tick mark (✓) in the appropriate box **in the Answer Sheet.**

Ethyl acetate	
Pentyl acetate	
Hexyl acetate	
Heptyl acetate	

Q.1.C.4. (2 points) In which one of the experiments do you find that larval sensitivity to the odorant has been reversed?

Put a tick mark (✓) the appropriate box **in the Answer Sheet.**

Experiment	Experiment			
	A	B	C	D
1				
2				
3				
4				
5				

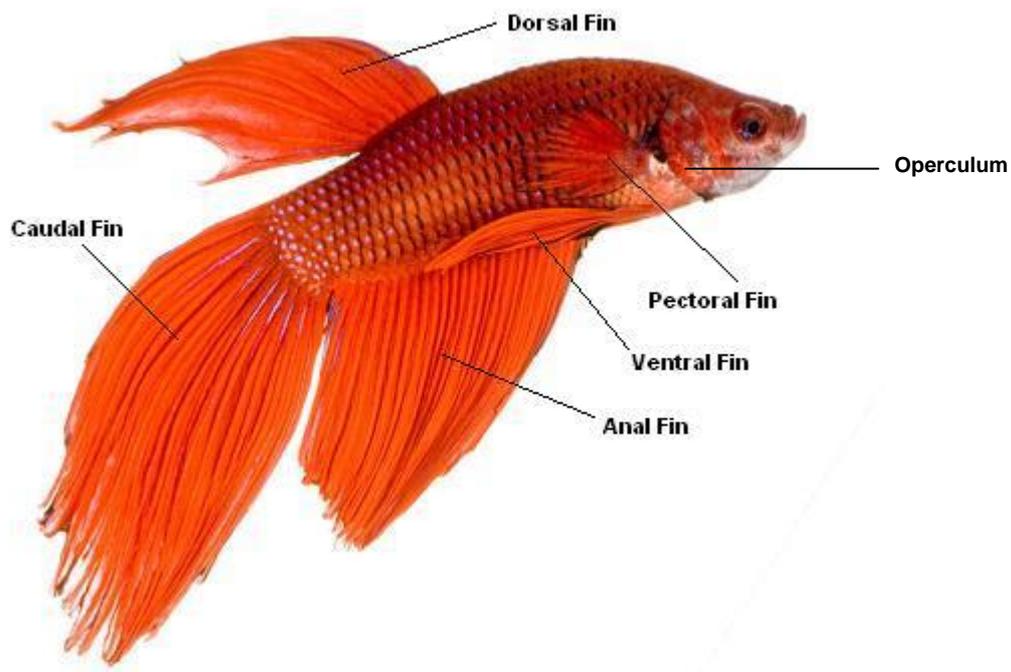
Task 2 (13 points)

Study of fish behavior

You should try and complete this task in 15 minutes.

Introduction

The Siamese fighting fish, *Betta splendens*, is one of the most popular species of freshwater aquarium fish. This fish shows varied responses when exposed to different stimuli.



You have been provided with a video recording of an experiment using a male Siamese fighting fish.

Q. 2.1. (11 points) Double click on the video file **6** on the computer monitor and observe the behavior of the fish before and after introduction of a mirror.

After observing the film, write a '+' for the particular behaviors that were displayed by the fish and '-' for those that were not displayed. Record your observations **in Table**

2.1. in the Answer Sheet.

Table 2.1.

Number	Behavior	Before the introduction of the mirror	After the introduction of the mirror
1.	Pectoral fin beating		
2.	Rapid zigzag movement of the body and the caudal fin		
3.	Appearance of horizontal stripes on body		
4.	Pecking at the base of the aquarium		
5.	Brightening of body coloration		
6.	Erection of dorsal, anal and caudal fin		
7.	Appearance of vertical stripes on body		
8.	Operculum display (opening of the operculum)		
9.	Bleaching of body color		
10.	Lateral display*		
11.	Gasping for air		

*The lateral display is a behavior where the fish exhibits the lateral surface of its body, expands its dorsal and caudal fins and vibrates/quivers its body.

Q. 2.2. (1 point) The differences in behavior of the fish that you observed before and after the mirror was introduced into the aquarium could be due to:

- a. an apparent increase in the size of the territory that the fish now has to defend.
- b. an urge to display courtship behavior towards a conspecific individual that the fish now perceives in its territory.
- c. an urge to establish dominance over a conspecific individual that the fish now perceives in its territory.
- d. a startle response displayed by the fish when confronted with a mirror.

Put a tick mark (✓) in the appropriate box **in Q. 2.2. in the Answer Sheet.**

a.	b.	c.	d.

Q. 2.3. (1 point) Different behaviors in animals have certain benefits and costs associated with them. For example, prolonged extension of the gill cover or operculum display may indicate its physical strength but may also severely limit the ability of the fish to ventilate its gills. In the light of your observation, what could be the rationale for the experimental fish displaying or not displaying this particular behavior?

- a. Fish always prefer to maintain regular opercular movement without any display, independent of the presence or absence of another conspecific individual, to maintain the oxygen supply for the body at its optimum.
- b. Fish will exhibit the operculum display advertising its ability to tolerate oxygen stress in presence of another conspecific individual to establish its dominance.
- c. Operculum display, being an energetically costly behavior, is usually not exhibited by a fish under most circumstances. Males of this species, however, may display this behavior in the presence of a conspecific female because the potential reproductive success that it will acquire will more than compensate for the energetic cost of the display.
- d. Operculum display is likely to be determined only by abiotic environmental factors such as level of dissolved oxygen in the water. Thus, fish in sufficiently aerated water will always show this response so as to declare its territory and maintain its dominance.

Put a tick mark (✓) in the appropriate box **in Q. 2.3. in the Answer Sheet.**

a.	b.	c.	d.

***** END OF PRACTICAL TEST 4 *****

STUDENT CODE:

Student Code: _____

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PRACTICAL TEST 4

ANIMAL BEHAVIOR

Total Points: 49

Duration: 60 minutes

ANSWER SHEET

Task 1 – Part A (7 points)

Study of the olfactory response of *Drosophila melanogaster* larvae

Experimental design

Q. 1.A.1. (3 points)

Design I	
Design II	
Design III	
Design IV	
Design V	

Q. 1.A.2. (4 points)

STATEMENT	TRUE	FALSE
I		
II		
III		
IV		
V		
VI		
VII		
VIII		

*****END OF PART A*****

Task 1 – Parts B and C

Study of the olfactory response of *Drosophila melanogaster* larvae

Part B (18 points)

Larval plate test

Q.1.B.1 (10 points)

Table 1.B.1.

Experiment	Chemical in ZI	Number of larvae in ZI (N _{ZI})	Chemical in ZII	Number of larvae in ZII (N _{ZII})	$\frac{N_{ZI}}{N_{ZI} + N_{ZII}}$	$\frac{N_{ZII}}{N_{ZI} + N_{ZII}}$
1	B		C			
2	A		B			
3	A (in dark)		B			
4	B		D			
5	B (in dark)		C			

Q.1.B.2. (3 points)

Chemical	Attractant	Repellant	Neutral chemical	Nature cannot be determined
A				
B				
C				

STUDENT CODE:

Q.1.B.3. (5 points)

	True	False
a.		
b.		
c.		
d.		
e.		

Part C (11 points)

Study of olfactory adaptation in *Drosophila melanogaster* larvae

Q.1.C.1. (5 points)

Table 1.C.1.

Experiment	Pre-stimulation	Test odorant			
		Experiment A	Experiment B	Experiment C	Experiment D
		Ethyl acetate	Pentyl acetate	Hexyl acetate	Heptyl acetate
		RI	RI	RI	RI
1	None				
2	Ethyl acetate				
3	Pentyl acetate				
4	Hexyl acetate				
5	Heptyl acetate				

Q. 1.C.2. (2 points)

Ethyl acetate	
Pentyl acetate	
Hexyl acetate	
Heptyl acetate	

Q. 1.C.3. (2 points)

Ethyl acetate	
Pentyl acetate	
Hexyl acetate	
Heptyl acetate	

Q. 1.C.4. (2 points)

Experiment	Experiment			
	A	B	C	D
1				
2				
3				
4				
5				

STUDENT CODE:

Task 2

Study of fish behavior

Q. 2.1. (11 points)

Table 2.1.

Number	Before the introduction of the mirror	After the introduction of the mirror
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		

STUDENT CODE:

Q. 2.2. (1 point)

a.	b.	c.	d.

Q. 2.3. (1 point)

a.	b.	c.	d.

*****END OF PRACTICAL TEST 4*****