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:

在此測驗，你要完成兩項工作(Task 1 and Task 2)

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

研究果蠅幼蟲嗅覺反應：實驗設計

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

研究果蠅幼蟲嗅覺反應：幼蟲培養皿測試

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

研究果蠅幼蟲嗅覺的適應

Task 2: Study of fish behavior (13 points)

魚行爲的研究

- The duration of the test is **10 minutes** for Part A of Task 1 and **50 minutes** for the remaining paper.

  此考試 Task 1 的 Part A 是 10 分鐘，其餘爲 50 分鐘

- **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 I – Parts B and C, and Task 2 be given to you.**

  Task 1 的 Part A 要在 10 分鐘後鈴響時交卷，然後再開始作答其他問題

- **Please do not switch on your computer before your Answer Sheet for Part A is collected.**

  Part A 答案卷未交卷前，不能開電腦

- **You have to write down your results and answers in the ANSWER SHEET. Answers written in the Question Paper will not be evaluated.**

  將答案寫在答案卷上，答在試卷紙上不計分

- 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.

  在考試結束後，將 Task 1 – Parts B 和 C, 和 Task 2 的答案卷放在信封內，監試人員會來收。

Good Luck!!

Country: _____

Country Code: _____

First Name: _____

Middle Name: _____

Family Name: _____

Student Code: _____

Practical Test 4

Animal Behavior

Task 1 – Part A (7 points)

Study of the olfactory response of *Drosophila melanogaster* larvae

Experimental design

研究果蠅的嗅覺反應的實驗設計

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.

現在你有 Part A 試卷，要在 10 分鐘內回答此問題，時間到鈴響時收答案卷，然後才會給你其他部分的試卷

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.

昆蟲嗅覺很強，例如雄蛾可以偵測到極低濃度的費洛蒙以找到雌蛾，不同嗅覺的感受會伴隨不同的行爲，此可由昆蟲藉由氣味來選擇食物獲得證實。氣味刺激造成的反應可分三類(1)吸引 (2)驅除 (3)中性(既不吸引也不驅除)

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.

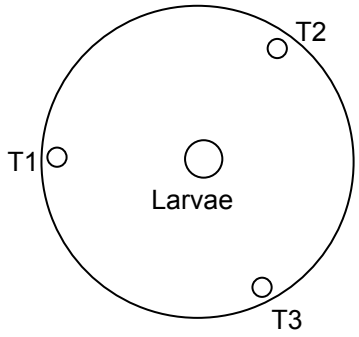
昆蟲如果蠅的成蟲或幼蟲皆可用來進行氣味分辨的行爲測試，所以可設計實驗來測試果蠅幼蟲在培養皿上對不同化學藥劑的反應。

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:

假如你想知道果蠅幼蟲對三種化學藥劑 T1，T2 和 T3 的反應，下列有五種可能的實驗設計。

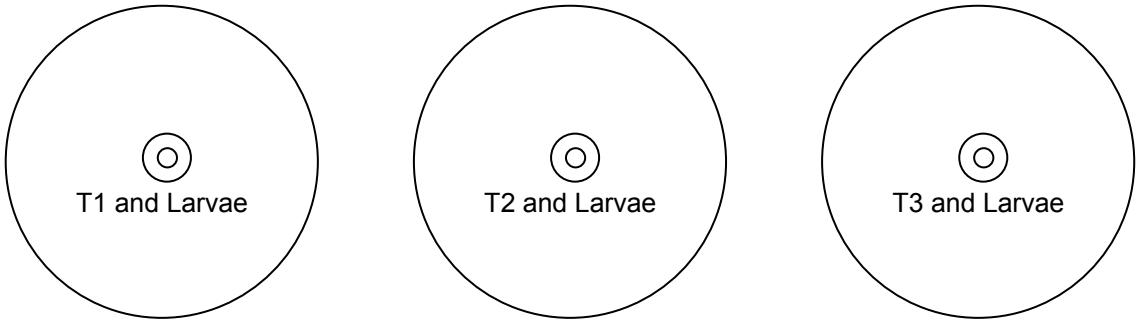
Design I: All the three chemicals are placed at equidistant positions on the periphery of a Petri plate and the larvae are introduced at the centre.

實驗設計 I： 三種化學藥劑 (T1, T2, T3) 放於培養皿周邊，彼此等距離，如圖所示。將幼蟲 (Larvae) 放於中央。



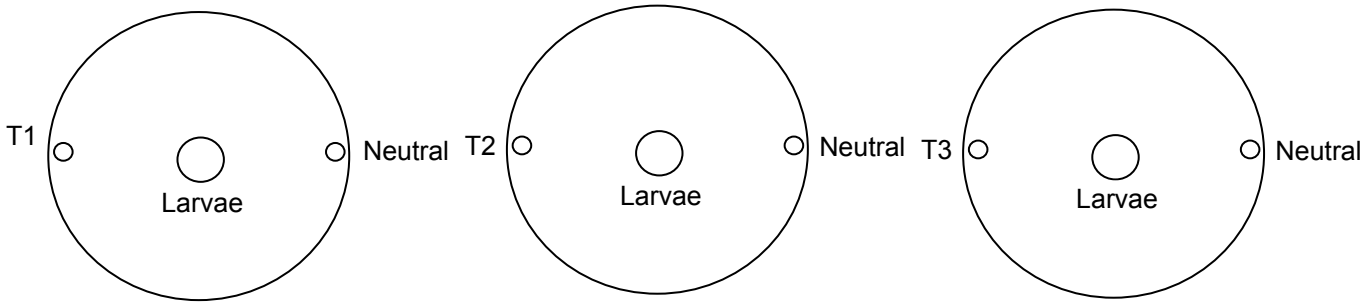
Design II: One of the chemicals and the larvae are placed together at the centre of a plate. Three such plates are set up for the three chemicals.

實驗設計 **II**：將幼蟲 (Larvae) 與任一種化學藥劑放於培養皿中央，以三種培養皿分別進行三種化學藥劑 (T1, T2, T3) 的測試。



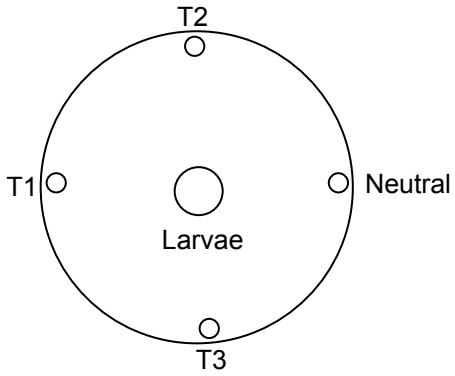
Design III: One of the chemicals and an odor-free (neutral) chemical are placed at two ends of a plate. Larvae are introduced in the centre. The test is repeated for the remaining chemicals.

實驗設計 **III:** 將其中一種化學藥劑與一種無氣味藥劑(中性 Neutral)分別置於培養皿兩側，將幼蟲 (Larvae) 置於中央進行測試，另以同法測試其它兩種藥劑。



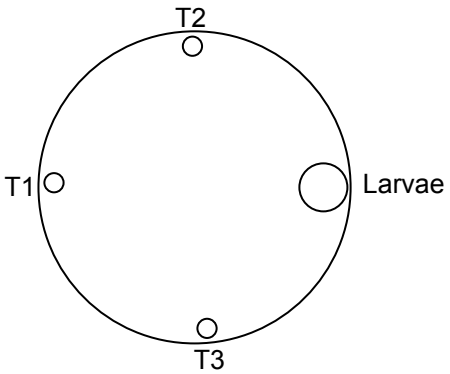
Design IV: The three test chemicals and a neutral chemical are placed at equidistant positions on the periphery of a plate and the larvae are introduced at the centre.

實驗設計 **IV:** 將三種化學藥劑與中性藥劑同時置於培養皿周圍，彼此等距。將幼蟲置於中央進行測試。



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

實驗設計 **V:** 將三種化學藥劑與幼蟲置於培養皿周圍，彼此等距，進行測試。



Choose the most appropriate experimental design and put a tick mark (√) against it **in Q. 1.A.1. in the Answer Sheet.** 選擇最適當的實驗設計在其旁打(√)

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.

注意：除非此題**(Q. 1.A.1.)**答對，下一題**(Q. 1.A.2.)**才會計分

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

根據你所選擇的實驗設計，判斷下列各項敘述之真僞。

Put a tick mark (√) in the appropriate box **in Q. 1.A.2. in the Answer Sheet.**在答案紙上勾選適當的選項

	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. 此設計允許幼蟲同時具有 2 或 2 種以上化學藥劑的選擇，故有分辨的功效。		
II	It can clearly distinguish between attractants and repellants by testing them one at a time against the neutral chemical. 藉由用中性藥劑同時與任一種測試藥劑同時存在的測試，可清楚的分辨出吸引與驅除的藥劑爲何。		
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. 藉由一個簡單的試驗即可完成所有化學藥劑的測試，且可避免實驗間的變異。		
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). 此設計是藉由一種藥劑的存在能加強昆蟲對其它藥劑的反應，故而能更清楚的分辨出藥物刺激所具有的吸引與驅除本質。		
V	There will not be any mixing of the odors and hence more reliable results will be obtained. 此實驗無任何氣味的混合，故可獲得較可信的結果。		
VI	All the chemicals can be tested against the same control in a single plate.在單一培養皿內，所有的藥劑可以與相同的控制組進行比對。		
VII	Amongst the designs presented, it is the one in which the effect of the weakest odorants can be tested. 對於氣味效果微弱的藥劑，此實驗仍可有效分辨其功能。		
VIII	Larvae can disperse in any direction without any hindrance.幼蟲不受任何限制可向任何方向擴散。		

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

Practical Test 4

Animal Behavior

Task 1 – Parts B and C

Study of the olfactory and phototatic response of *Drosophila melanogaster* larvae 研究果蠅幼蟲嗅覺及趨光反應

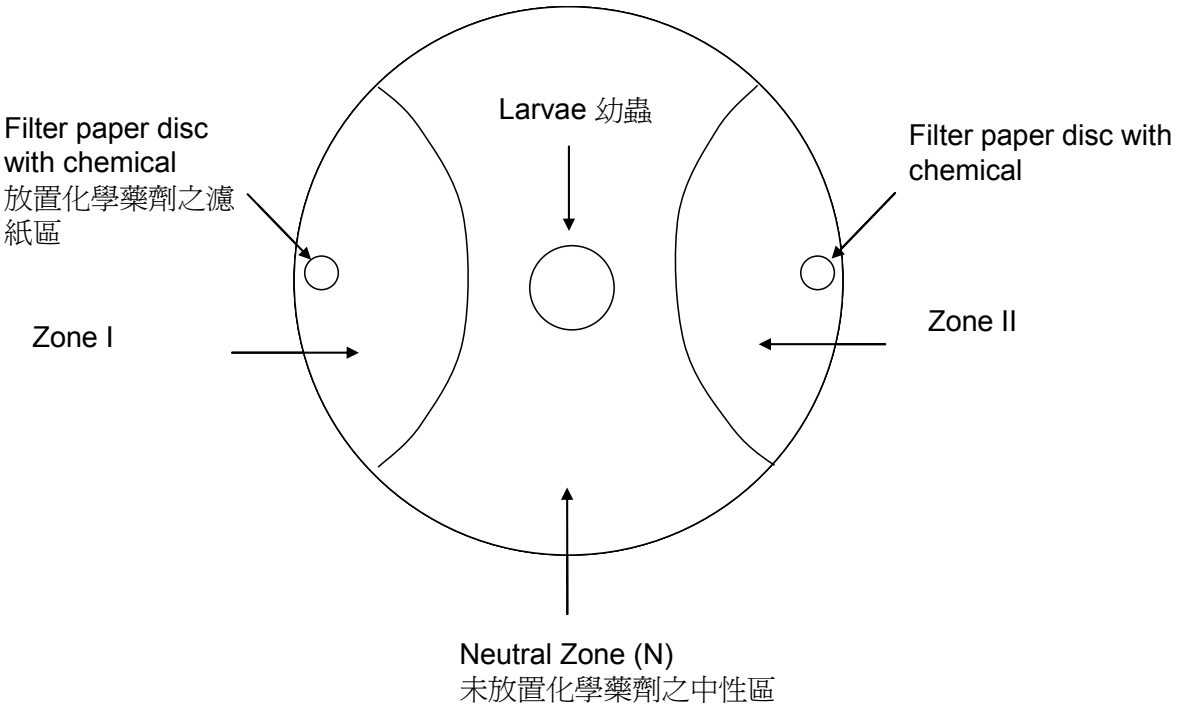
You should try and complete Parts B and C of this task in 35 minutes.本部分限 35 分鐘內完成

Part B (18 points)

Larval plate test 幼蟲培養皿測試

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:利用 5 個實驗進行果蠅幼蟲對化學與光的刺激，四種化學藥劑 A，B， C，及 D 用於測試，其中 D 爲中性藥劑，A，B，C 可能爲吸引、驅除、或中性藥劑。實驗設計如下圖：



Method 方法

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. 實驗選用三齡幼蟲，此幼蟲是利用 15%的蔗糖溶液沖洗 6 天的果蠅培養基以取得，將浮在蔗糖溶液的幼蟲洗淨後利用。這些實驗是在含有 1%洋菜膠的培養皿上進行。

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. 每一實驗，在培養皿水平中軸兩側分別置放含有不同藥劑的濾紙，將 30-40 隻幼蟲置於培養皿的中央後，記錄它們 5 分鐘內的活動，進行 5 種這樣的實驗，此實驗的歷程及結果可由提供的記錄影片獲得，實驗 1、2、4 是在固定的光源下進行的，實驗 3、5 則在培養皿一半覆蓋黑紙，一半透光的環境下進行。

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

1. Double click on the video file labelled **1** on the computer monitor to observe the movement of larvae.操作電腦，在檔案 1 處按兩下，觀察影片中幼蟲活動。
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. 影片原長 5 分鐘，壓縮成 2.5 分鐘，如有必要，你可用快轉鈕前後快轉選擇所需。
3. At the end of the experiment, count the number of larvae in Zone I (N_{ZI}) and Zone II (N_{ZII}). 實驗結束，計算 Zone I (N_{ZI}) 及 Zone II (N_{ZII}).的幼蟲數目

4. Record your readings **in Table 1.B.1. in the Answer Sheet**. 將你的記錄寫在答案紙的 **Table 1.B.1.**

5. Repeat steps 1 to 4 for the video files labelled **2** to **5**.針對檔案 2 到 5 重覆 1 到 4 的步驟。

Table 1.B.1.

Experiment 實驗	Chemical in ZI ZI 的化學藥劑	Number of larvae in ZI (N_{ZI}) ZI 的幼蟲數目(N_{ZI})	Chemical in ZII ZII 的化學藥劑	Number of larvae in ZII (N_{ZII}) ZII 的幼蟲數目(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**.試問 ABC 三種化學藥劑的特性爲何？於答案紙勾選適合的答案。

Chemical 化學物質	Attractant 吸引	Repellant 驅除	Neutral chemical 中性反應	Nature cannot be determined 無法決定
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**.根據你的觀察，判斷下列敘述何者爲真，何者爲錯，並在答案紙上勾選。

a. Larvae exhibit a stronger positive movement towards the attractant odorant tested than towards darkness.幼蟲在有氣味吸引的情況下比在黑暗情況下，顯現出更強的正向反應(朝刺激方向移動)
b. Light is a stronger repelling stimulus for the larvae than the repellant odorant tested. 光對幼蟲的驅除效果比具有驅除效應的化學藥劑更強。

c. The positive phototaxis shown by the larvae is stronger than the movement towards the attractant odorant.幼蟲所顯示的正趨光性比具有吸引力的化學藥劑所產生的正向移動效果更強

d. In the presence of light, the larvae do not exhibit chemotaxis.在光照下，幼蟲不顯示趨化性。

e. The repellant odorant has a stronger influence on the larvae than does darkness.驅除性藥劑對幼蟲的影響較黑暗的影響強

	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:用同一味道對嗅覺系統持續刺激，會造成適應現象，又稱爲去敏感化。此結果使幼蟲對已適應氣味失去反應。有人利用果蠅幼蟲進行嗅覺適應的研究，她選擇下列的藥劑。

1. Ethyl acetate 乙基乙酸
2. Pentyl acetate 戊基乙酸
3. Hexyl acetate 己基乙酸
4. Heptyl acetate 庚基乙酸

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.刺激前的實驗：在每一實驗(除實驗 1 外)，果蠅幼蟲於實驗前，先放置於含 40 microlitres 的上述藥劑之一的培養皿上，進行 25 分鐘前置刺激。將幼蟲取出後，再用與原來相同或不同的化學藥劑進行測試，其實驗流程與 Task 1 的 Part B 相同。

The data obtained from these tests are tabulated below. 這些實驗所得資料列於下表

Data from the pre-stimulation experiment 前置刺激實驗所得資料

Experiment 實驗	Pre-stimulation 前置刺激	Test odorant 測試的化學藥劑							
		Experiment A		Experiment B		Experiment C		Experiment D	
		Ethyl acetate 乙基乙酸	*	Pentyl acetate 戊基乙酸	*	Hexyl acetate 己基乙酸	*	Heptyl acetate 庚基乙酸	*
		$N_{Z\,I}$	$N_{Z\,II}$	$N_{Z\,I}$	$N_{Z\,II}$	$N_{Z\,I}$	$N_{Z\,II}$	$N_{Z\,I}$	$N_{Z\,II}$
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_{ZI} and N_{ZII} are the number of larvae in Zone I and Zone II, respectively.
N_{ZI} 和 N_{ZII} 分別代表 Zone I 及 Zone II 幼蟲數

*** Zone II in all the experiments contained a neutral chemical**.
在所有實驗的 Zone II 中含有一種中性藥劑

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.此表所列的資料是平均反應，實際數字的變異是此平均數值的上下 10%

Q. 1.C.1. (5 points) Calculate the Response Index (RI) for each experiment according to the formula:根據下列公式計算每一實驗的反應指數(RI)

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

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

將反應指數填於答案紙的 **Table 1.C.1.**

Table 1.C.1.

Experiment	Pre-stimulation	Test odorant 測試的化學藥劑			
		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?

幼蟲對那一種藥劑最具適應性？

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

於答案紙上勾選正確選項。

Ethyl acetate	
Pentyl acetate	
Hexyl acetate	
Heptyl acetate	

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

幼蟲對那一種藥劑最不具適應性

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

在答案紙上勾選正確選項。

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? 於那一實驗可發現幼蟲對藥劑敏感性產生反轉 。

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

在答案紙上勾選正確選項。

Experiment	Experiment			
	A	B	C	D
1				
2				
3				
4				
5				

Task 2 (13 points)

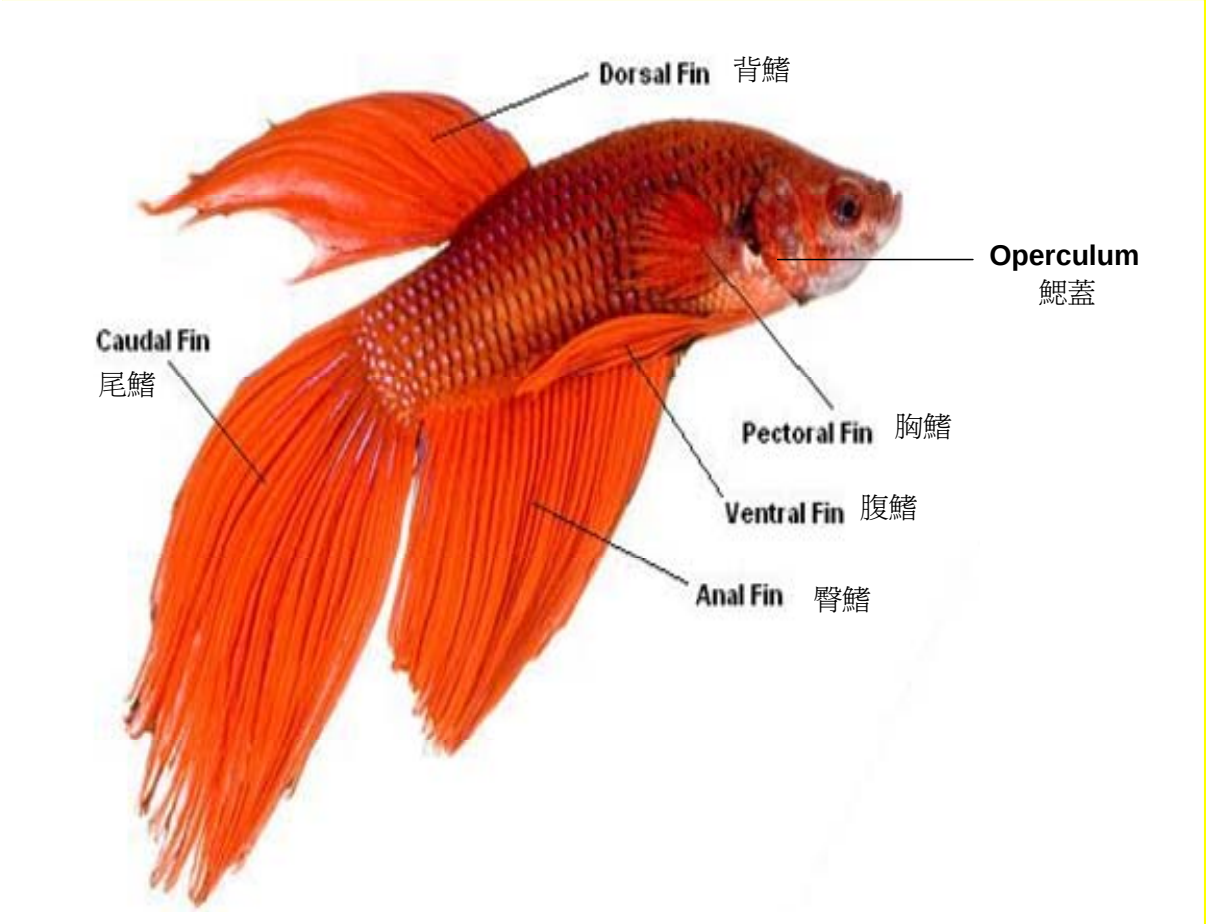
Study of fish behavior

You should try and complete this task in 15 minutes.

儘量於 15 鐘內完成

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.暹邏鬥魚是水族箱中最常見的淡水魚，此種魚在不同的刺激下有不同的反應



You have been provided with a video recording of an experiment using a male Siamese fighting fish.此處提供你有關一隻雄性暹邏鬥魚的實驗影片

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.操作電腦，利用檔案 6 來觀察暹邏鬥魚於置放鏡子前與置放鏡子後的行爲。

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.** 觀察影片後，於表中以'**+**'表示魚曾經出現的行爲，以'**-**'表示魚未曾出現的行爲

Table 2.1.

Number	Behavior 行爲	Before the introduction of the mirror 置放鏡子前	After the introduction of the mirror 置放鏡子後
1.	Pectoral fin beating 擺動胸鰭		
2.	Rapid zigzag movement of the body and the tail (caudal) fin 魚體及尾鰭快速左右變向前進(呈 z 形)		
3.	Appearance of horizontal stripes on body 體表顯示平行條紋		
4.	Pecking at the base of the aquarium 啄咬水族箱基部		
5.	Brightening of body coloration 體色變亮而鮮艷		
6.	Erection of dorsal, anal and caudal fin 背鰭、尾鰭、及臀鰭豎立		
7.	Appearance of vertical stripes on body 體表顯示垂直條紋		
8.	Operculum display (opening of the operculum)鰓蓋展示(鰓蓋開張)		
9.	Bleaching of body color 體色變淡或變白		
10.	Lateral display*側面展示		
11.	Gasping for air 吞嚥空氣		

*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.

側面展示是魚將其體側顯示於刺激之來源，此時尾鰭及背鰭擴張且魚體快速擺動

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:

水族箱內，置放鏡子前與置放鏡子後，魚行爲的改變是由下列何者原因所造成

a. an apparent increase in the size of the territory that the fish now has to defend.
一個明顯的領域空間的增加，使魚現在需要防禦。

b. an urge to display courtship behavior towards a conspecific individual that the fish now perceives in its territory.魚對於牠認爲在領域中出現的個體，表現出的求偶行爲

c. an urge to establish dominance over a conspecific individual that the fish now perceives in its territory. 魚對於牠認爲在領域中出現的個體，爲維繫原有的優勢所表現的行爲

d. a startle response displayed by the fish when confronted with a mirror.
魚遇到鏡子時所表現出的驚嚇反應

Put a tick mark (√) in the appropriate box **in Q. 2.2. in the Answer Sheet.**
在答案紙上的 **Q. 2.2.**勾選正確選項。

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?動物所表現出的不同行爲皆有利弊，例如魚擴展它的鰓蓋來顯示牠的體能時，其嚴重的限制魚氣體交換的能力，在你觀察期間，這隻實驗魚表現這種行爲或不表現這種行爲的理由爲何？

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.
   在沒有任何展示時，魚總是維持正常的鰓蓋運動，使牠的身體獲得足夠的氧以維持最佳的身體狀態，而與同種其他個體的存在無關
b. Fish will exhibit the operculum display advertising its ability to tolerate oxygen stress in presence of another conspecific individual to establish its dominance.當魚面對一個同類的對手時，其表現出鰓蓋擴張行爲以顯示牠能容忍缺氧的能耐，以表示牠優於對方

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. 鰓蓋展示是一種個耗能行爲，故於大多數時不會採用，但本種雄魚在同種雌性個體出現時會展示此種行爲，因爲牠由與雌性個體所獲得的潛在繁殖好處遠大於其在行爲展示上的能量耗費

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. 鰓蓋展示很可能只受到非生物環境因子的影響，例如水的溶氧程度。是以魚在充氣十足的環境裡將總是展示此種行爲以顯示它的領域及其優勢地位

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

在答案紙上的 **Q. 2.3.**勾選正確選項

a.	b.	c.	d.

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