Extension Topic B Impact of Climate Change on Animals and Plants Part 2

Learning Outcomes

(e) Explain how temperature changes impact insects, including increased temperature leading to increased metabolism and the narrow temperature tolerance of insects.

(f) Outline the life cycle of *Aedes aegypti* as an example of a typical mosquito vector.

(g) Outline the development of viral dengue disease in humans, including host-pathogen interactions, human susceptibility to the virus, pathogen virulence, transmission, and drug resistance.

(h) Explain how global warming affects the spread of mosquito-borne infectious diseases, including malaria and dengue, beyond the tropics.

(i) Discuss the effects of increased environmental stress (including increased temperatures and more extreme weather conditions) as a result of global climate change, on habitats, organisms, food chains and niche occupation.

(j) Discuss how climate change affects the rich biodiversity of the tropics including the potential loss of this rich reservoir for biomedicines and genetic diversity for food.

Lecture Outline

1) Impact of Temperature Change on Insects
1.1 Effect on Insects Growth, Development & Activity

2) Life cycle of a Mosquito Vector
2.1 The *Aedes aegypti* mosquito
2.2 Life cycle of *Aedes aegypti*

3) Viral Dengue Disease
3.1 Transmission
3.2 Pathogenesis
3.3 Treatment
3.4 Prevention

4) Effect of Global Warming on Mosquito-borne Diseases
4.1 Effect on Insect Distribution
4.2 Spread of Mosquito-borne Diseases

5) Effects of Environmental Stress
5.1 Impact on Distribution of Animals and Plants
5.2 Impact on Breeding Success of Animals and Plants

6) Effect on Biodiversity
6.1 Loss of Biomedicines
6.2 Loss of Genetic Diversity of Foods

Learning Outcome (e):

Explain how temperature changes impact insects, including increased temperature leading to increased metabolism and the narrow temperature tolerance of insects.

1 IMPACT OF TEMPERATURE CHANGE ON INSECTS

1.1 Effect on Insect Growth, Development & Activity

The **body temperature of ectotherms[1] fluctuates with environmental temperatures**, and basic physiological functions such as locomotion, growth and reproduction are strongly influenced by environmental temperature.

Growth and development are fuelled by metabolism. Metabolic rate is the result of many different biological reactions which are catalysed by enzymes. Thus, **temperature controls metabolism through its effect on the rates of these enzyme-catalysed biochemical reactions**.

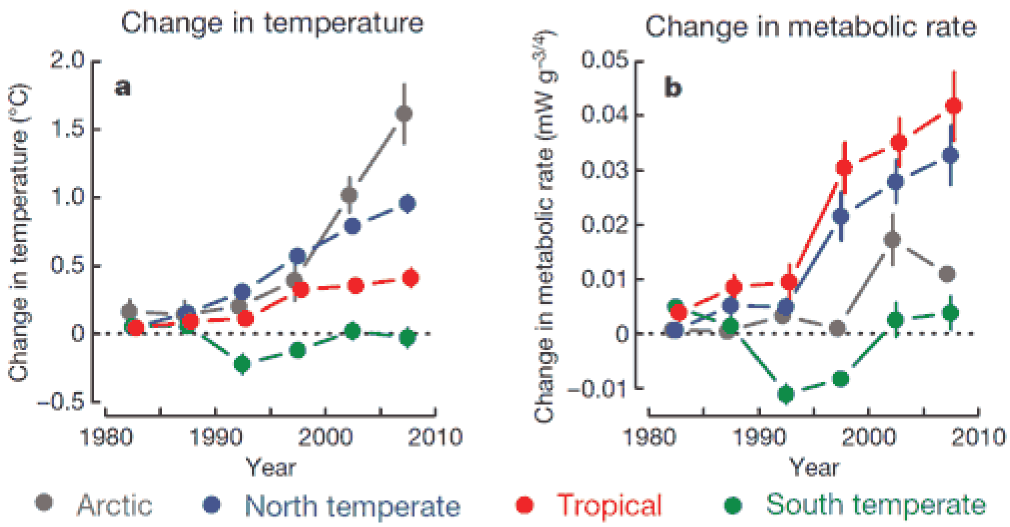


Fig. 1.1 Global changes in temperature and corresponding changes in metabolic rates of ectotherms

In ectotherms, rates of both somatic growth and ontogenic development[2] increase with increasing temperature. A 10°C temperature increase results in approximately 2.5-fold increase in developmental rate. Therefore, the **time to maturity is shorter at higher temperatures**. For insects, faster development of egg, larva and pupa stages implies a shorter time of exposure to adverse environmental conditions (e.g. low temperature, too low or too high humidity, attack by predators), hence resulting in **higher reproductive success**.

[1] Organisms whose regulation of blood temperature depends on external sources, such as sunlight/ a heated rock surface. Examples of organisms are fishes, reptiles, amphibians, invertebrates (such as insects).
[2] Development of organism from the time of fertilization of the egg to the organism's mature form.

However, insects have a **narrow temperature tolerance** for development, meaning that they can only develop within a limited range of temperatures. The difference between an organism's thermal optimum and its current climate is called the **thermal safety margin**. For insects, **thermal safety margins increase sharply with latitude**.

Insects in **tropic** environments:

- Species living in tropic environments that are already close to their physiological optimum have **small thermal safety margins**. Thus, tropical insects are more sensitive to climate changes, and even **small amounts of warming will likely decrease performance** since they will approach near-lethal temperatures much faster.

- Many species also have small dispersal ranges which increases their risk of extinction in the case of habitat or environmental changes.

Insects in **temperate** environments:

- Species living in temperate environments have **large thermal safety margins**. Their environments are on average cooler than their physiological optimal, and thus have broader thermal tolerance.

- **Warming may thus enhance the fitness** of insects in temperate environments. For example, with warmer winters, the insects will not all die out like they used to. In addition, since spring starts earlier, these insects also emerge earlier. With summer lasting longer, the insects also persist in the environment for a longer duration.

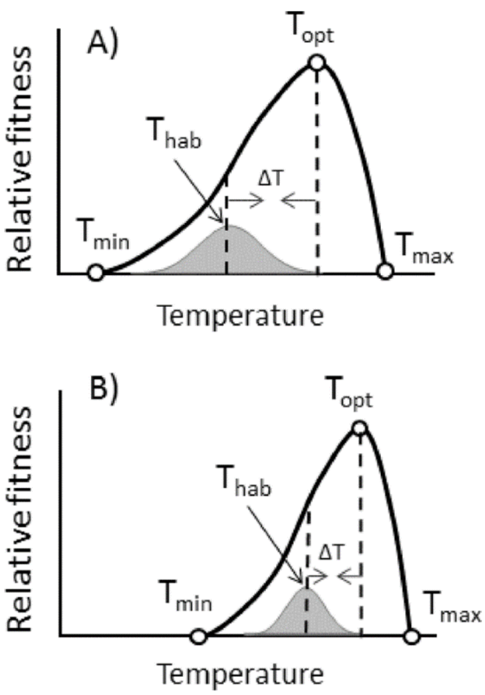


Fig. 1.2 Relative fitness curves of temperate (A) and a tropical (B) species of ectotherms

(T_{min} and T_{max} represent the minimum and maximum temperature at which organisms can perform, and T_{opt} is the optimal temperature for performance. The grey curve beneath the thermal performance curve is the temperature that the organism is exposed to during an average year and the average is depicted as T_{hab}. ΔT is the distance between T_{hab} and T_{opt}, which represents the organisms' thermal safety margin.)

Insects' physiological responses to warming temperatures can produce large and rapid effects causing **significant changes to ecological landscapes and patterns of infectious disease**, especially those caused by pathogens which require insects as vectors for transmission.

Learning Outcome (f):

Outline the life cycle of *Aedes aegypti* as an example of a typical mosquito vector.

2 LIFE CYCLE OF A MOSQUITO VECTOR

2.1 The *Aedes aegypti* mosquito

Aedes aegypti is a **vector of viral diseases such as dengue fever**, chikungunya, yellow fever and Zika fever. It is a small, dark mosquito with white lyre shaped markings and banded legs. It primarily bite humans.

Fig. 2.1.1 Body features of *Aedes aegypti*

Aedes aegypti dwell in tropical and subtropical regions (warmer climates) all around the world, where the winter temperature is no colder than 10°C, and at altitudes below 1000m. The adult life span can range from two weeks to a month depending on environmental conditions.

Fig. 2.1.2 Global distribution of *Aedes aegypti*

2.2 Life Cycle of *Aedes aegypti*

The life cycle of *Aedes aegypti* can be completed within one-and-a-half to three weeks depending on environmental conditions. There is an aquatic phase (larvae, pupae) and a terrestrial phase (eggs, adults) in the life cycle of the *A. aegypti* mosquito.

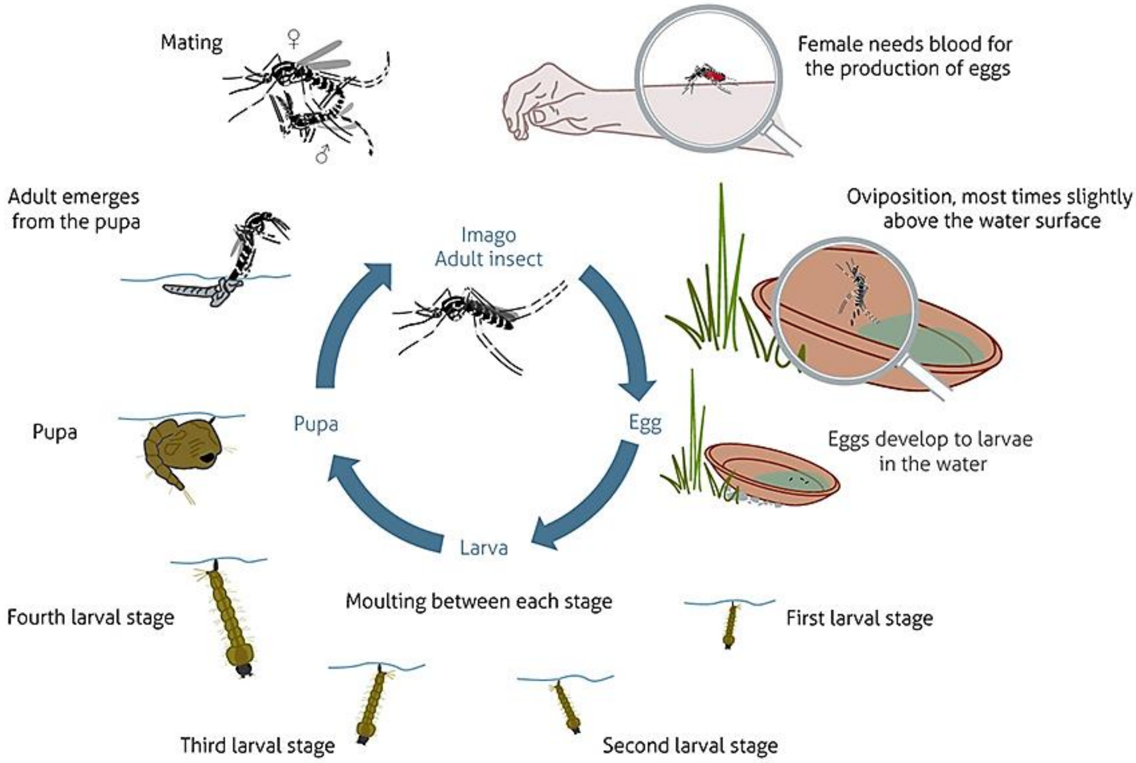


Fig. 2.2.1 Life cycle of *Aedes aegypti*

(i) Egg

- After a blood meal, adult female mosquitoes lay about 100 – 200 eggs on the inner, wet walls of containers with water.
- Eggs are very hardy and can survive drying out for up to 8 months and over very cold periods. In warm climates eggs may develop in as little as 2 days, whereas in cooler temperate climates, development can take up to a week.

(ii) Larva

- Larvae hatch from eggs and feed on organic matter in the water, such as algae and microscopic organisms. The larvae breathe oxygen through a posteriorly located siphon which is held above the water surface.
- The larvae will shed their skins three times to be able to grow from **first to fourth instars**. An instar refers to the developmental stage of an arthropod between moults until sexual maturity is reached.
- **Larval development** is **temperature dependent.** If temperatures are cool, *Aedes aegypti* can remain in the larval stage for months so long as the water supply is sufficient.
- When the larvae has acquired enough energy and size and is in the fourth instar, metamorphosis is triggered, changing the larva into a pupa.

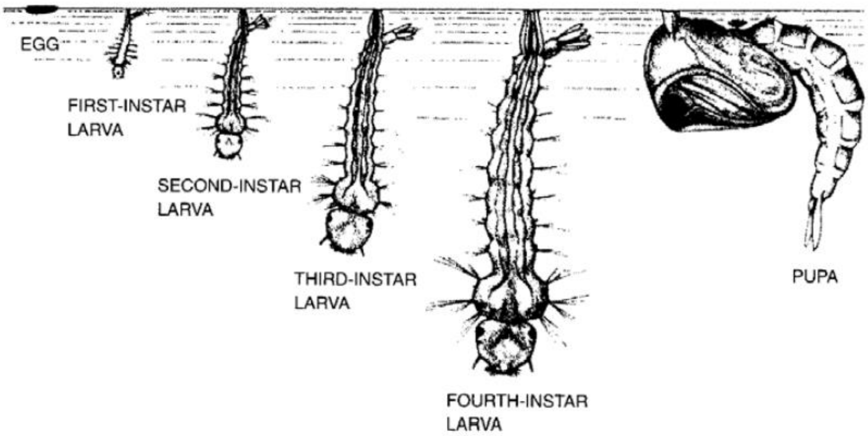


Fig. 2.2.2 Larvae development

(iii) Pupa

- Mosquito pupae do not feed. They are mobile and respond to stimuli. They take approximately 2 days to develop. Adults emerge by ingesting air to expand the abdomen thus splitting open the pupal case and emerge headfirst.

Fig. 2.2.3 Pupa stage

(iv) Adult

- Male mosquitoes feed on nectar from flowers.
- Female mosquitoes need a **blood meal** for maturation of their ovaries and must feed again between each egg batch.
    - Protein in blood from humans or animals is needed for eggs production. Unlike other mosquito species, *Aedes aegypti* mosquitoes prefer to bite people and live near people.

Learning Outcome (g):

Outline the development of viral dengue disease in humans, including host-pathogen interactions, human susceptibility to the virus, pathogen virulence, transmission and drug resistance.

3 VIRAL DENGUE DISEASE

The dengue virus (DENV) is transmitted between humans through the bite of infected female *Aedes aegypti* mosquitoes.

Dengue infections are caused by four closely related viruses (serotypes): DENV-1, DENV-2, DENV-3 and DENV-4. A **serotype** is a group of viruses classified together based on the antigens present on the surface (antigenicity) of the virus.

3.1 Transmission

Dengue cannot be spread directly from one person to another. Mosquitoes are needed for the transmission of the DENV; hence the DENV is transmitted through a **human-to-mosquito-to-human cycle of transmission**. Humans are the primary host of DENV. After a female mosquito feeds on the blood of an infected individual, the mosquito becomes a **dengue vector**.

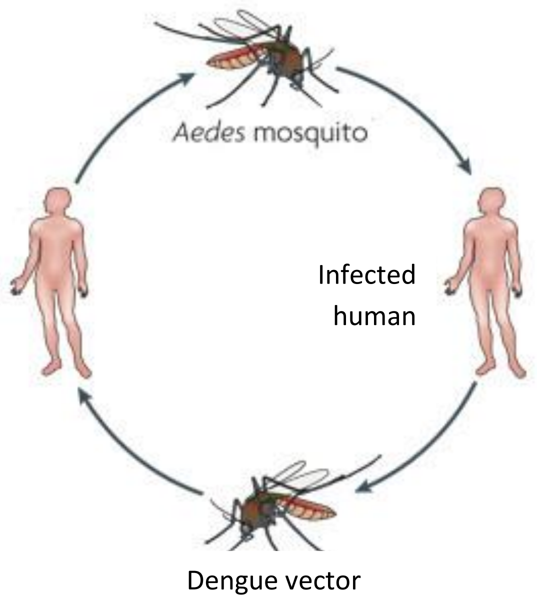


Fig. 3.1.1 Human-Human Transmission cycle

During the initial 2 – 10 days after feeding on the blood of the DENV-infected human, the mosquito becomes infected with the virus in the cells lining its gut.

About 8 – 10 days later, the virus spreads to other tissues including the mosquito's salivary glands and is subsequently released into its saliva. The virus seems to have no detrimental effect on the mosquito, which remains infected for life. The infected mosquito injects its saliva into the human to prevent the host's blood from clotting so as to ease feeding, thus infecting the human host with the virus.

About 4 days after being bitten by an infected mosquito, the person develops **viraemia** (high levels of virus in the blood). Viraemia lasts for approximately 5 – 12 days.

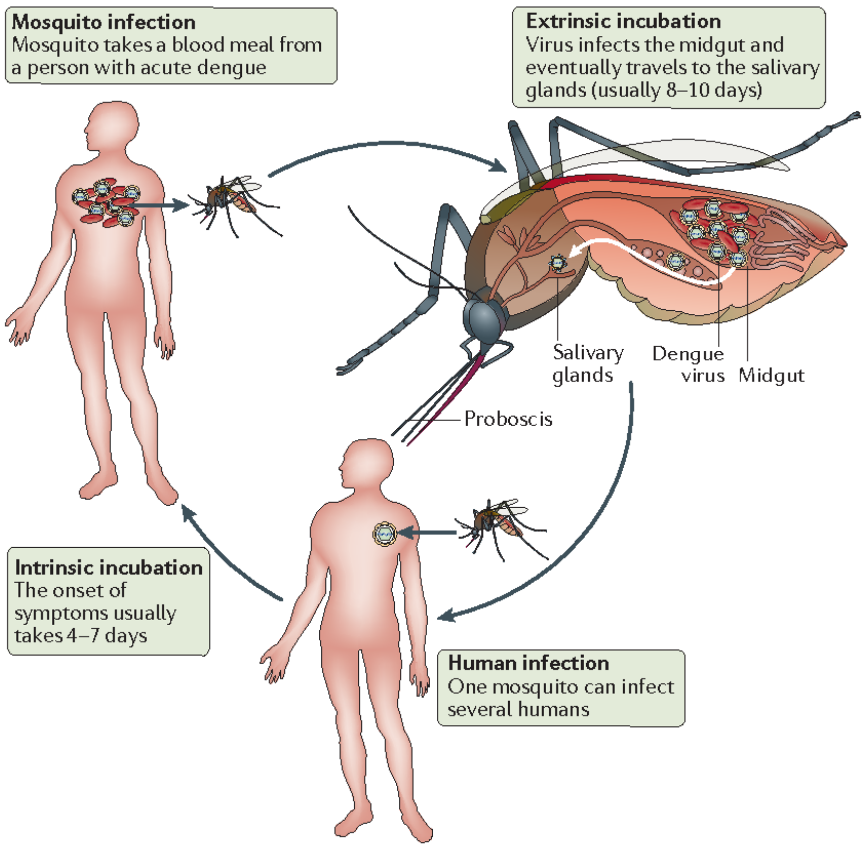


Fig. 3.1.2 Dengue transmission

The person develops symptoms of dengue fever within 5 days of being bitten, which can last for a week or longer. Usual symptoms experienced include:

- Fever
- Headache
- Muscle and joint aches
- Rash – different types of rashes, may be itchy and appears a few days after the onset of fever
- Bleeding tendency – from nose, gums, and other parts of the body due to low platelet[3] count.

Fig. 3.1.3 Dengue symptoms

[3] Platelets are one of the blood components which help to clot and prevent excessive bleeding.

3.2 Pathogenesis

When an infected mosquito feeds on a person, it injects the dengue virus into the bloodstream.

- The virus first infects skin cells nearby (**keratinocytes**) and specialised **dendritic** cells (Langerhans cells).
- Infected dendritic cells present dengue viral antigens on its surface to activate the **innate immune response**, resulting in the activation of monocytes and macrophages to fight the virus.
- Normally, monocytes and macrophages ingest and destroy pathogens, but instead of destroying the dengue virus, they are infected by the virus.
- As the infected monocytes and macrophages travel through the lymphatic system, the dengue virus spreads throughout the body and infects more cells. This includes cells in the lymph nodes and bone marrow, macrophages in both the spleen and liver, monocytes in the blood and endothelial cells.
- The spread and increase of the virus results in **viraemia**, a condition in which there is a **high level of DENV** in the bloodstream.

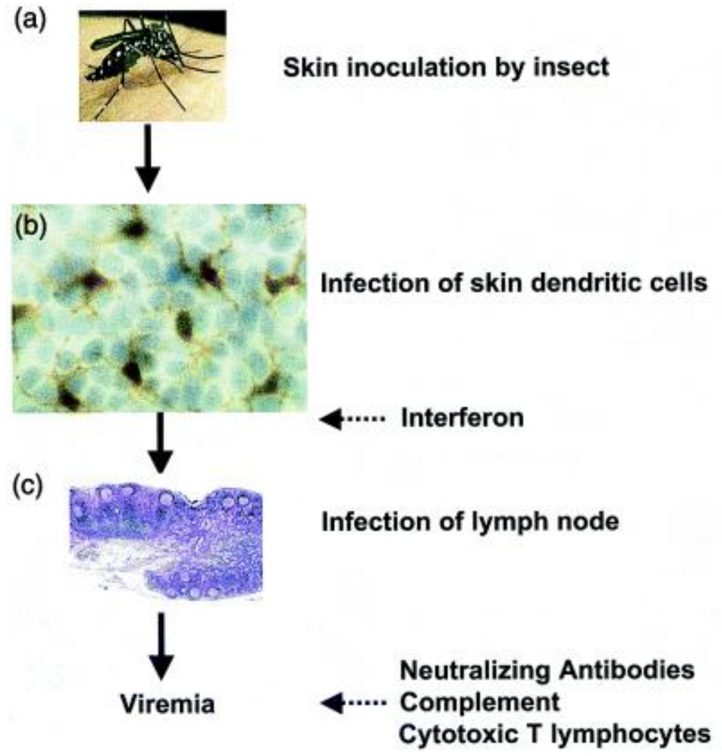


Fig. 3.2.1 Dengue viral infection

How the host immune system combats the dengue virus

- Infected cells produce and secrete small proteins called **interferons** that are part of a large group of proteins called cytokines. Interferons activate both the innate and adaptive immune system defences. They help the immune system recognise dengue-infected cells and help protect uninfected cells from infection.
- As part of the adaptive immune response, **plasma B cells secrete antibodies** called IgM and IgG that are released in the blood and lymph fluid, where they specifically recognise and

neutralise the dengue virus particles. **Cytotoxic T cells recognise and kill the cells which are infected by the virus**.

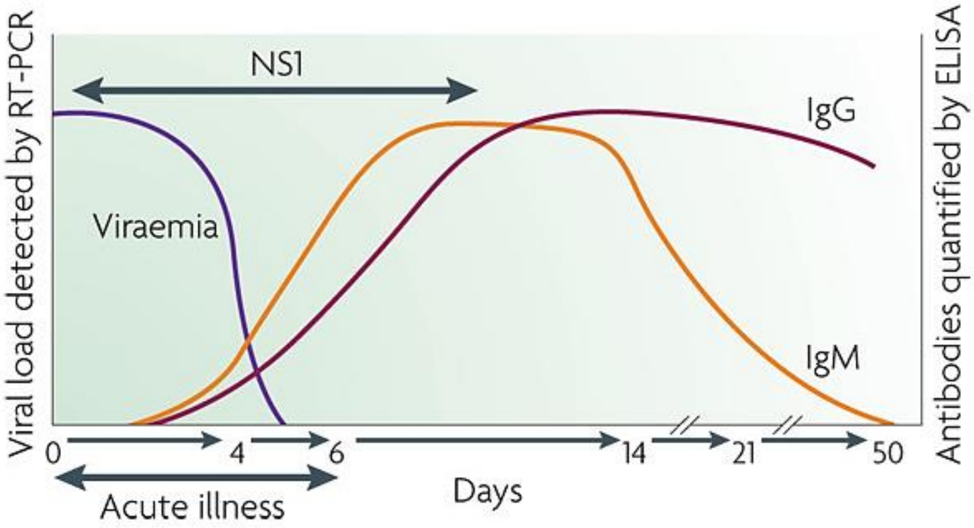


Fig. 3.2.2 Production of IgG and IgM that recognise and neutralise viral molecules

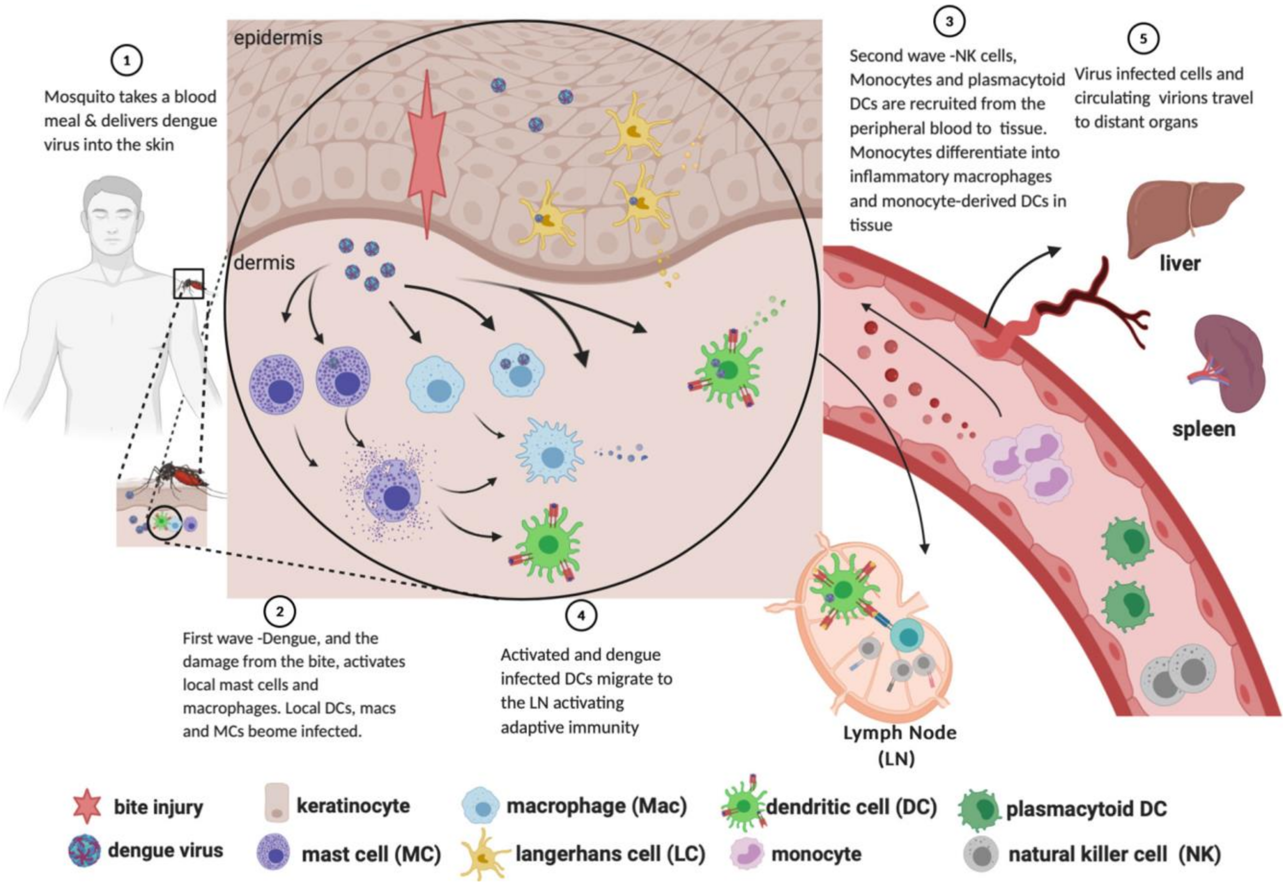


Fig. 3.2.3 Immune responses to DENV infection

Secondary Exposure to another DENV serotype

- After recovery from an infection by a DENV serotype, the immune system develops immunological memory due to presence of memory B and memory T cells. These memory cells can result in a rapid adaptive response in a secondary infection.
- These memory cells however, are **specific to the DENV serotype from the first infection**. If an individual is **infected with a different DENV serotype**, the antibodies produced by the memory cells are **ineffective in inactivating and neutralising** the virus.
- Instead, in a phenomenon known as **<u>antibody-dependent enhancement (ADE)</u>**, antibodies from the first infection actually help the virus infect host cells more efficiently. This occurs when pre-existing antibodies present in the body from a primary DENV infection bind to an infecting DENV particle during a secondary infection with a different DENV serotype but does not effectively neutralise the virus. This allows the virus to replicate in the host cell, leading to increased viraemia and an **increased risk of severe dengue**.

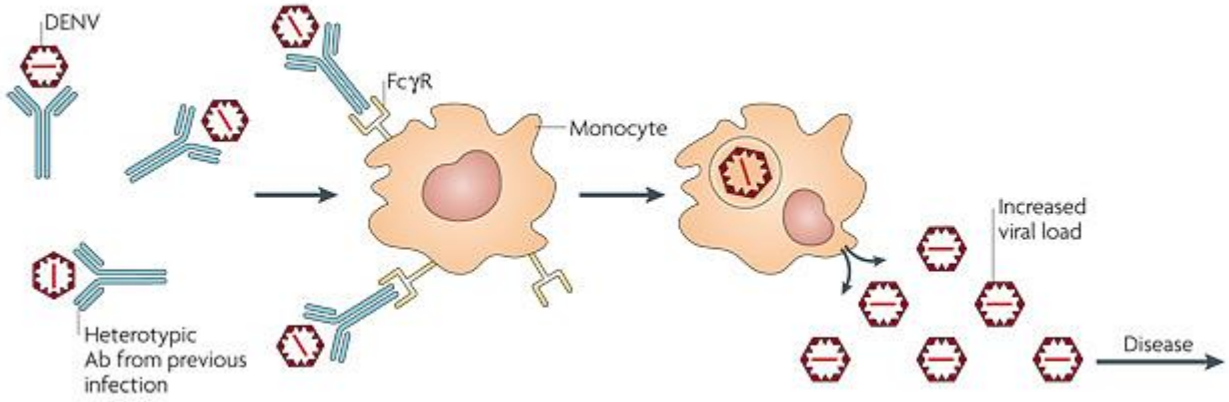
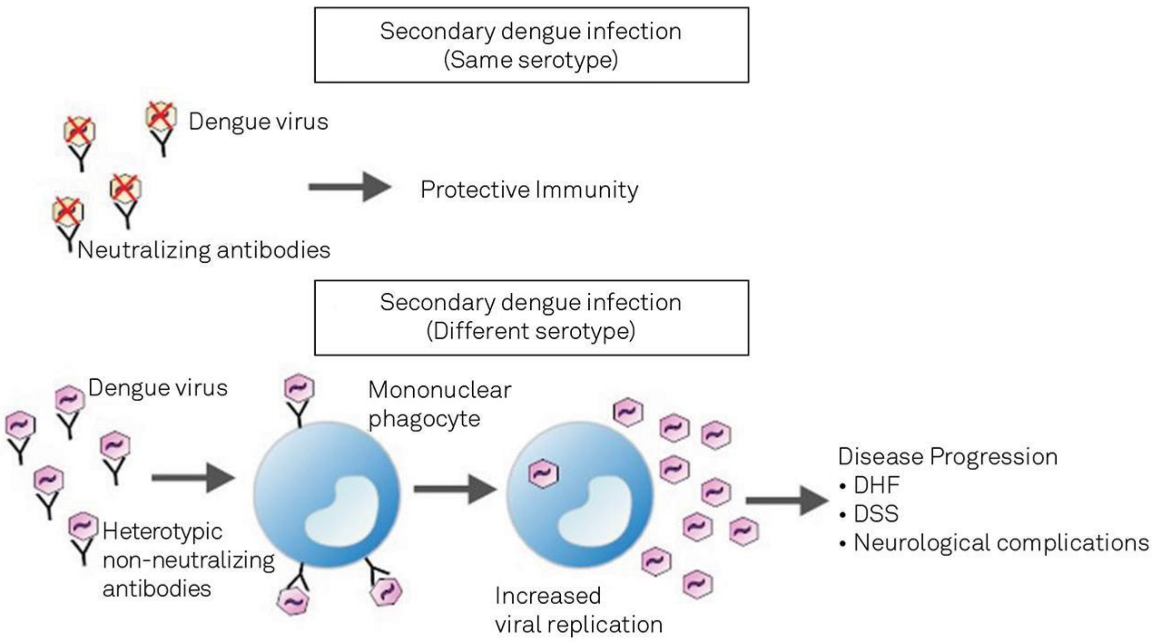


Fig. 3.2.4 Antibody-dependent enhancement (ADE)



DENV: dengue virus; DHF: dengue severe hemorrhagic syndrome; DSS: dengue severe shock syndrome.

Fig. 3.2.5 Immune responses to secondary infection with the same/different serotypes

- During a secondary infection with dengue, the cytotoxic T cells produced by the immune response provide only partial immunity against the new dengue serotype. The cytotoxic T cells do not effectively clear the virus from the body, and they release **excess quantities of molecules called cytokines**. In normal quantities, cytokines aid the immune response; however, **in high quantities, cytokines can result in serious inflammation and tissue damage** such as leakage form the capillaries, possibly contributing to the **development of severe dengue**.
- Symptoms of severe dengue include:
    - severe abdominal pain
    - persistent vomiting
    - rapid breathing
    - bleeding gums or nose and blood in vomit or stool
    - dengue shock syndrome, in which patient shows signs of circulatory failure when blood pressure plummets.

Fig. 3.2.6 Symptoms of mild and severe dengue fever

3.3 Treatment

Currently, treatment of dengue patients depends on symptoms developed. There are no specific antiviral drugs for dengue.

Management of symptoms

Primarily, maintaining proper fluid balance is important. Anti-pyretic drugs like paracetamol are given to help reduce fever. Blood transfusion is initiated for patients with unstable vital signs like decreasing haemoglobin levels.

Anti-viral drugs are currently being developed and target the viral proteins e.g. inhibitors for viral proteins used during attachment, replication of genome and biosynthesis of the viral proteins.

Vaccine Development

Dengue vaccine development has been in progress for many years. However, the complex pathology of the illness, the need to control 4 virus serotypes simultaneously and insufficient investment by vaccine developers have hampered the progress.

DENV secondary infection can result in severe dengue and poses a special challenge to the development of a dengue vaccine. An effective vaccine should induce a robust immune response against the 4 serotypes in naïve as well as previous immune individuals without causing severe dengue.

3.4 Prevention

(i) Eliminating breeding grounds

The spread of dengue can be prevented through the eradication of vector breeding grounds. An example would be the 5-Step Mozzie Wipe out from the National Environmental Agency (NEA), Singapore.

Fig. 3.4.1 NEA's 5 Step Mozzie Wipe Out for dengue prevention

(ii) Controlling vector reproduction

From 2016, the National Environment Agency (NEA) has released male *Aedes aegypti* mosquitoes carrying the naturally occurring *Wolbachia* bacteria in housing estates to control the mosquito population.

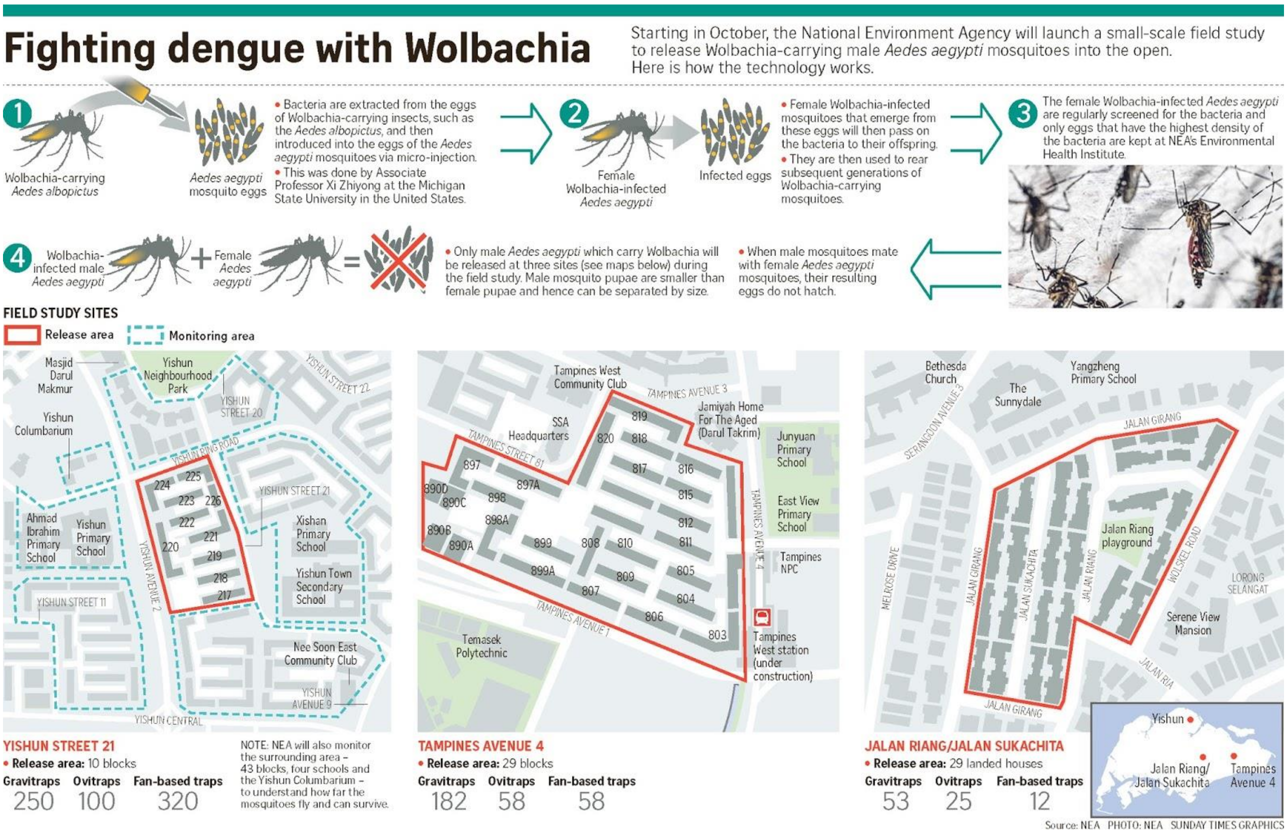


Fig. 3.4.2 Project Wolbachia Singapore

Wolbachia is extracted from eggs of *Wolbachia*-carrying insects and introduced into *Aedes aegypti* eggs via microinjection. Female *Wolbachia-Aedes aegypti* mosquitoes that emerge from these eggs will then pass *Wolbachia* on to their offspring (maternal transmission). When male *Wolbachia*-carrying *Aedes aegypti* mosquitoes mate with female wild-type *Aedes aegypti* mosquitoes that do not carry *Wolbachia*, their **resulting eggs do not hatch due to cytoplasmic incompatibility**. Male *Wolbachia-Aedes aegypti* mosquitoes released in the field will compete with wild-type males for wild-type females, leading to a **reduction in the population of *Aedes aegypti* over time**.

Scan these QR codes/ click on the links for an Singapore's Wolbachia Project:
https://www.youtube.com/watch?v=HBE10y5kv1c
https://www.youtube.com/watch?v=GGiCO_4EqoU

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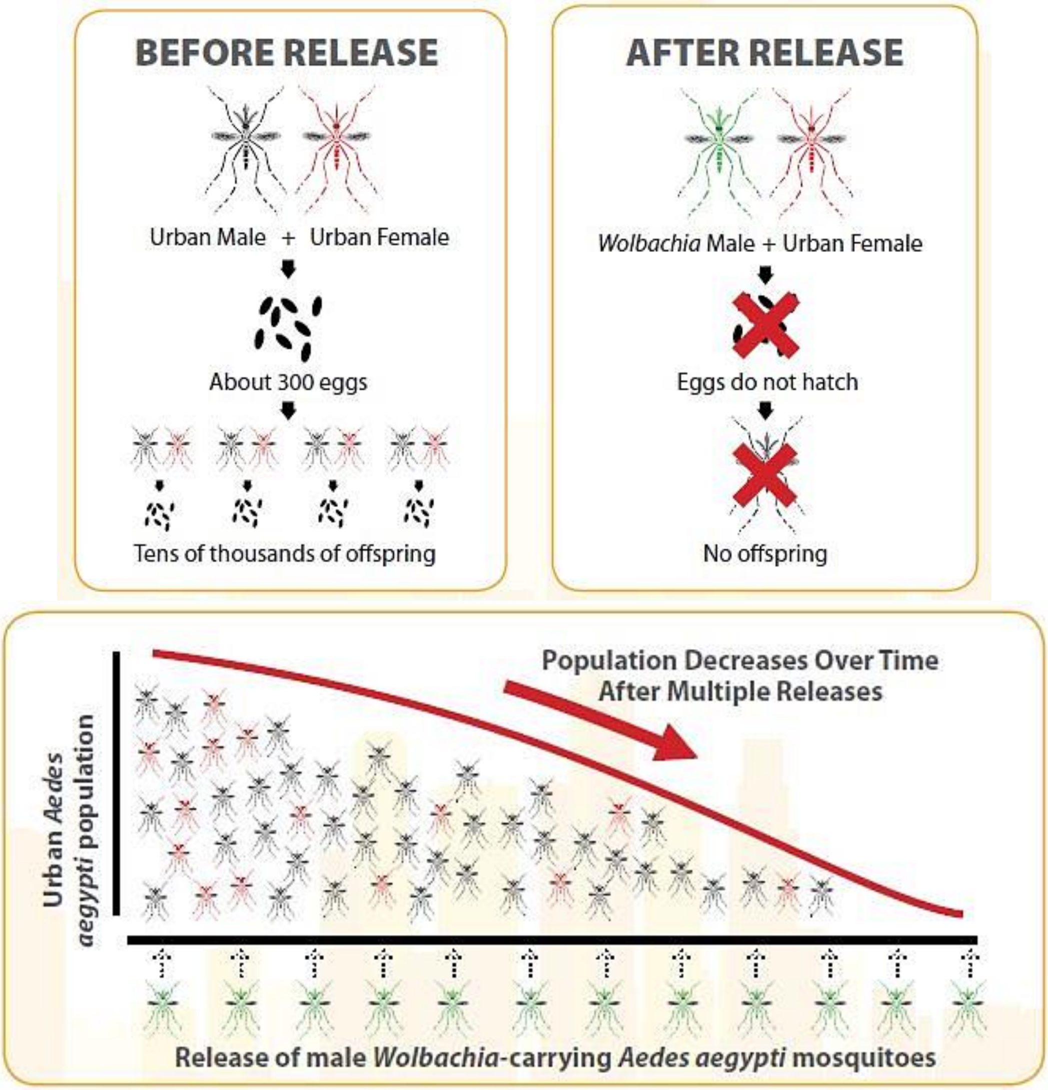


Fig. 3.4.3 Use of *Wolbachia* to control *Ades* populations

Learning Outcome (h):

Explain how global warming affects the spread of mosquito-borne infectious diseases, including malaria and dengue, beyond the tropics.

4 EFFECT OF GLOBAL WARMING ON MOSQUITO-BORNE DISEASES

4.1 Effect on Insect Distribution

Global patterns of change in temperature, precipitation, relative humidity, wind and solar radiation **set the physiological limits** determining which insect species can exist in certain habitats.

With warmer climates, many species of insects would **migrate northward or expand their range upwards** to find a climate within their temperature tolerance range. Inability of insects to adapt to the warmer temperatures could lead to extinction, or a sharp drop in total population.

Climate change has a large and rapid effect on insect species population dynamics because insects have relatively short life cycle, high reproductive capacity and high degree of mobility.

4.2 Spread of Mosquito-borne Diseases

Temperature is an important factor in the spread of malaria and other mosquito-borne diseases. Research shows that mosquitoes that can carry malaria, dengue fever and other tropical diseases have expanded their ranges towards the northern latitudes in recent decades. **With warming temperatures, mosquito-borne diseases like malaria and dengue fever may become increasingly common in more northerly regions**.

(i) Dengue

Increase in temperature and precipitation can lead to increased abundance of the mosquito vector of dengue, *Aedes aegypti*, by increasing their development rate, decreasing the length of reproductive cycles, stimulating egg-hatching, and providing sites for egg deposition. Higher temperature further abets transmission by shortening incubation period of the dengue virus in the mosquito, allowing the virus to reproduce faster in the mosquito vector.

(ii) Malaria

The mosquito vector of the malaria parasite, *Anopheles* mosquito, thrives in regions with warm temperatures, humid conditions, and high rainfall. Warm temperatures are also required for malaria parasites (*Plasmodium*) to complete their growth cycle within the *Anopheles* mosquitoes. With global warming, malaria may expand into new regions.

The changes in malaria risk are also affected by local environment conditions, socioeconomic development, government policies and malaria control programs or capabilities of countries to handle a pandemic.

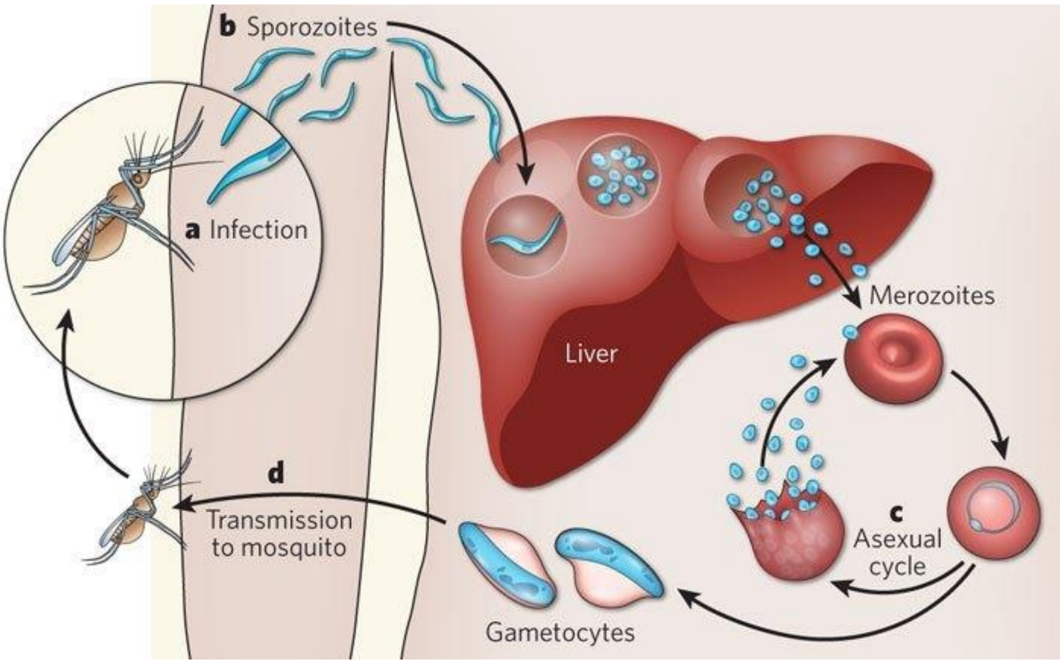


Fig. 4.2.1 Malaria transmission

The **predicted increase is most pronounced at the borders of endemic malaria regions** and **at higher altitudes within these regions**.

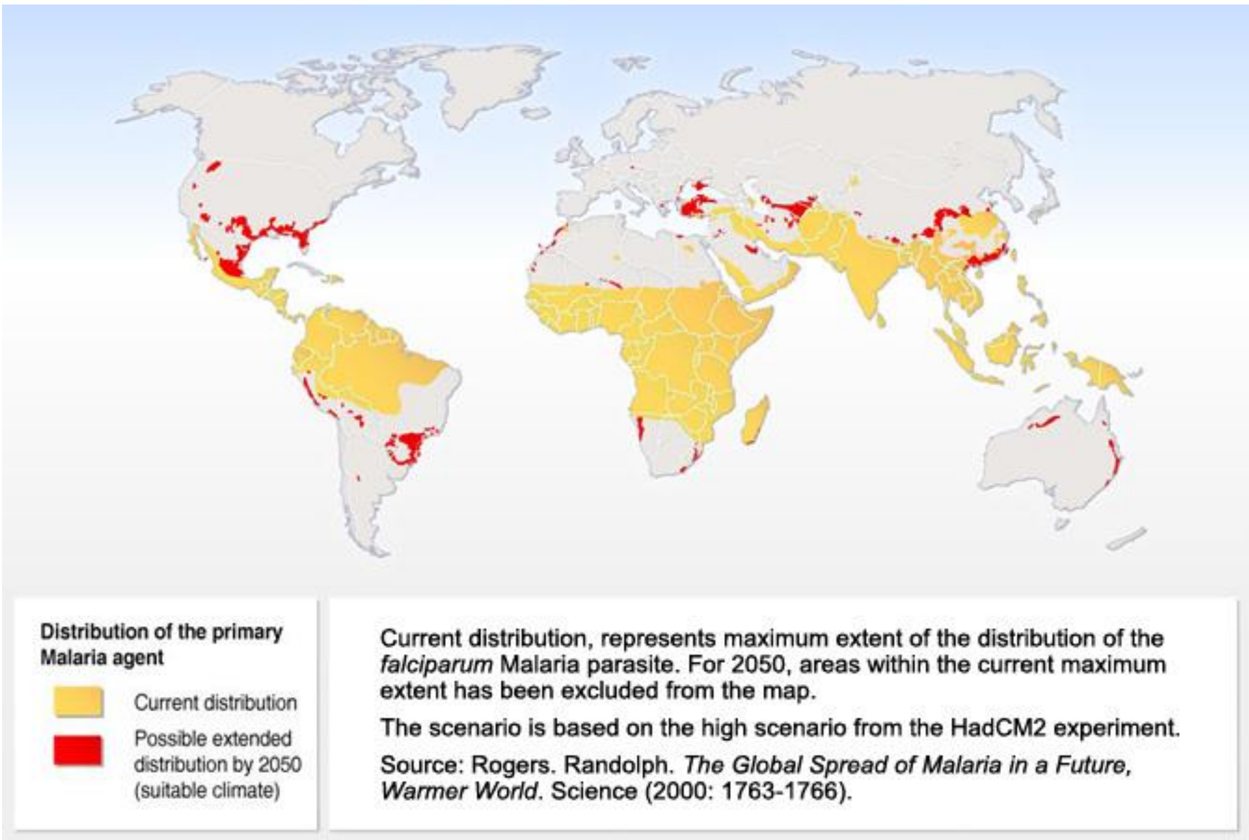


Fig. 4.2.2 Projected spread of malaria by 2050 due to global warming

Learning Outcome (i):

Discuss the effects of increased environmental stress (including increased temperatures and more extreme weather conditions) as a result of global climate change, on habitats, organisms, food chains and niche occupation.

5 EFFECTS OF ENVIRONMENTAL STRESS

5.1 Impact on Distribution of Animals and Plants

As temperatures increase, the habitat ranges of **many species are moving towards northern latitudes and to higher elevations** where temperatures are cooler, so as to stay within their ideal range of environmental conditions. In recent decades, in both land and aquatic environments, plants and animals have moved to higher elevations at a median rate of 0.011 kilometres per decade, and to higher latitudes at a median rate of 16.9 kilometres per decade.

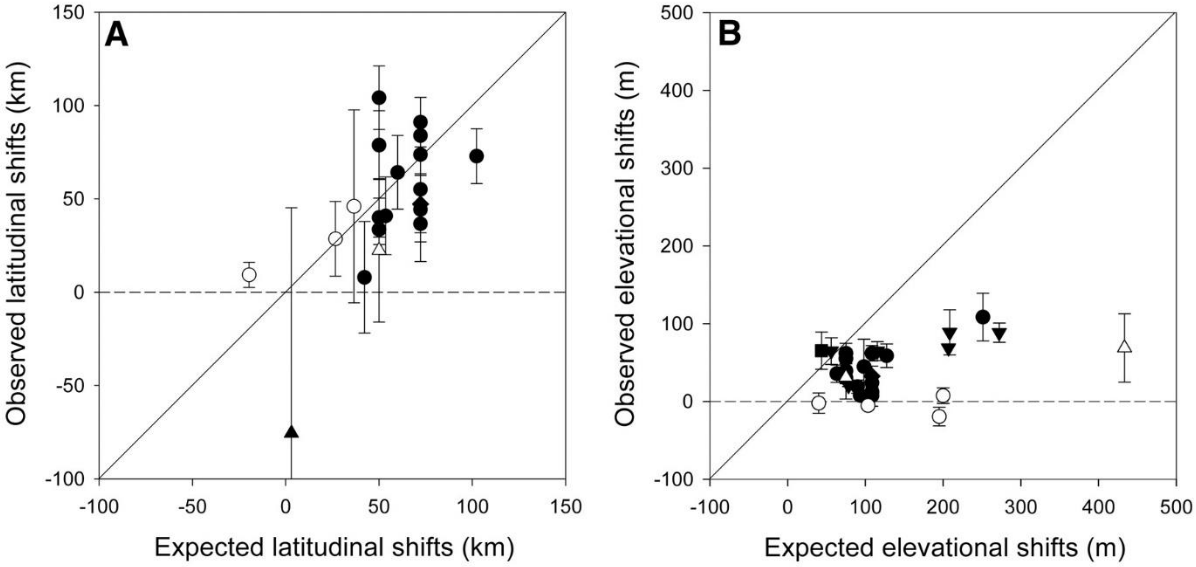


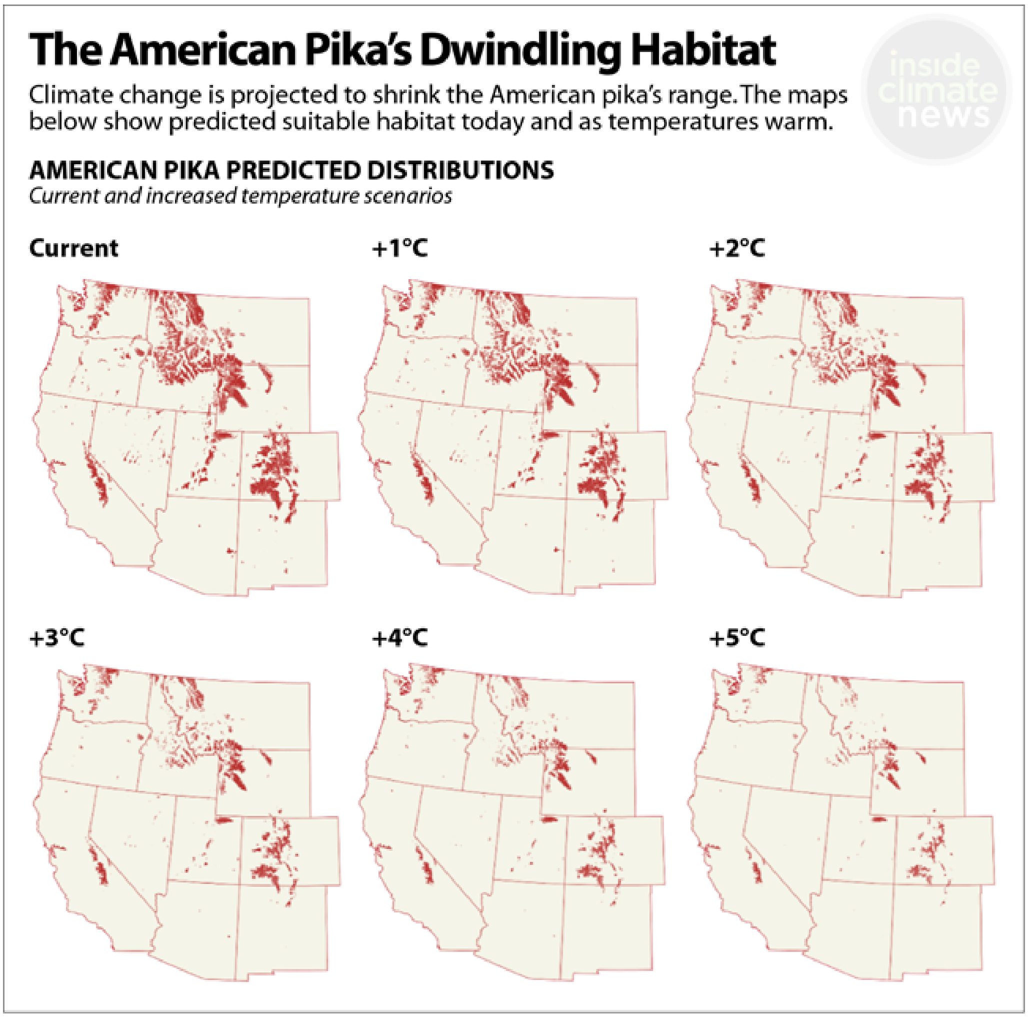
Fig. 5.1.1 Range shifts of species as a result of high levels of climate warming

5.1.1 Shift to higher elevations

While some species may experience an expansion in range as a result of migration, for others it may mean movement into less hospitable habitat, increased competition, or reduction in range, with **some species having nowhere to go** because they are already at the top of a mountain or at the northern limit of land suitable for their habitat.

- An example would be the American pika, a small mammal that lives on Western mountaintops. It has adapted to life in mountainous areas that rarely get above freezing and can die when exposed to temperatures as mild as 26°C. As a result of global warming, the pika is forced to move to higher altitudes to find tolerable alpine temperatures. Trapped at the top, alpine wildlife such as the pika are vulnerable to the damaging effects of global warmings, including vegetation changes, invasion of new predators and pests, reduced winter snowpack and increases in extreme weather conditions.

Fig 5.1.2 The American pika



SOURCE: Michael T. Calkins, et al. PAUL HORN / InsideClimate News

Fig 5.1.3 The dwindling habitat of the American pika as a result of climate change

5.1.2 Shift towards northern latitudes

Forests can be classified into three different types according to latitude – **tropical, temperate and boreal forests**.

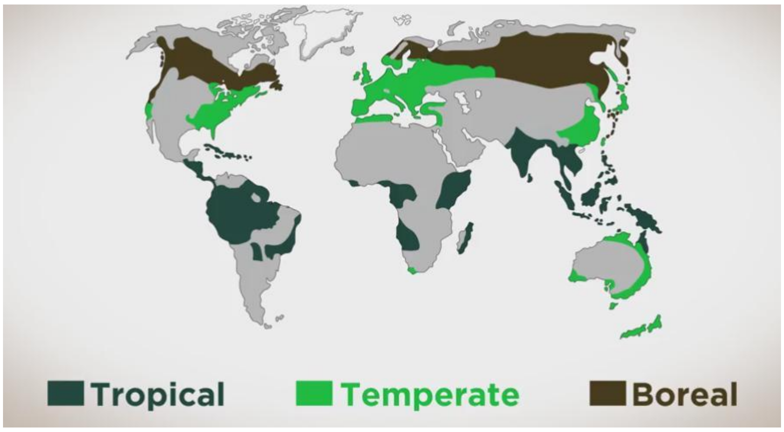


Fig 5.1.4 Classification of forests according to latitude

Whether forests will gain or lose ground depends on the regional characteristics of their environment, including water availability, temperature and more importantly, how fast the species can migrate. **Compared to animals, plants are more sedentary and hence may be in greater danger of being impacted by climatic changes**. The rate of climate change is likely to exceed the migration rates of most plant species. For plant species which cannot migrate fast enough to escape the consequences of climate change, they could become extinct.

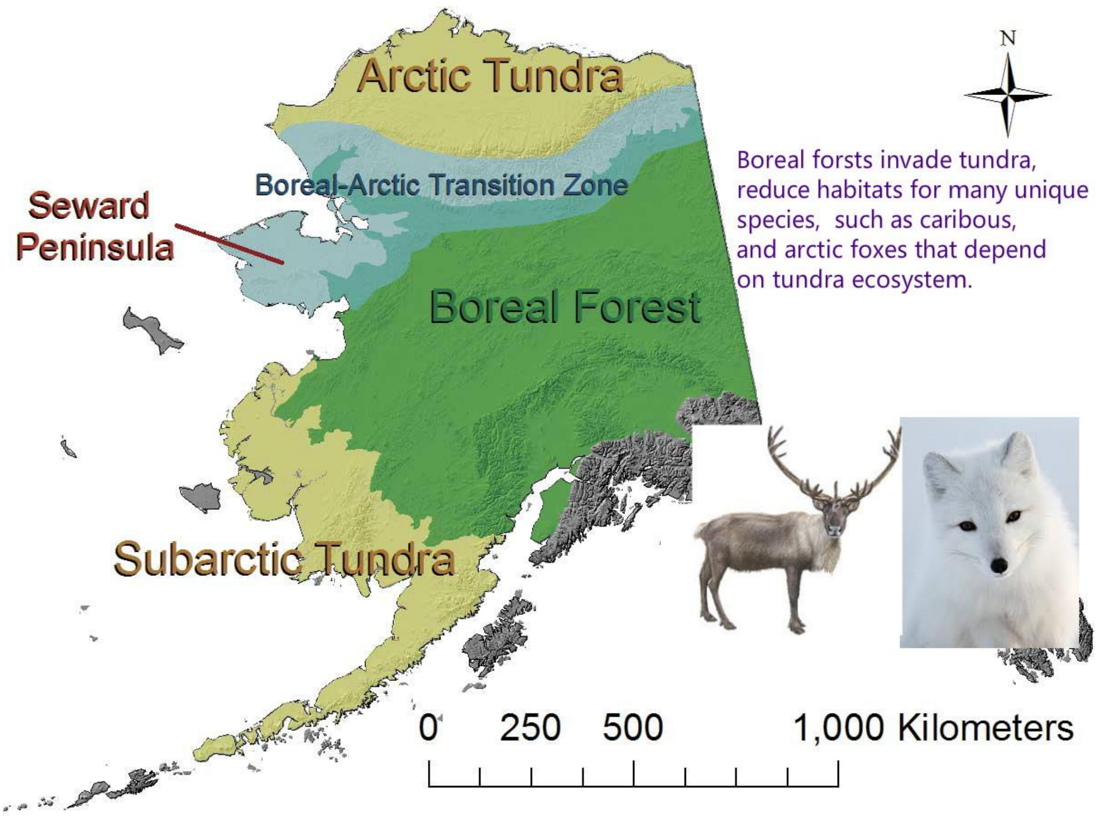


Fig. 5.1.5 Projected shifts in forest types

5.1.3 Impact on Feeding and Breeding grounds for Animals

Climate change can also impose environmental stress which adversely impacts many animal species. For example:

- Increased water levels threaten coastal habitats, resulting in the **reduction of breeding grounds** for sea turtles and **feeding grounds** for waterbirds.
- In the Arctic and Antarctic, substantial contraction in sea-ice cover is predicted to **reduce the breeding and feeding habitat** of species such as seals and polar bear.
- In marine ecosystems, **large scale shifts in phytoplankton abundance** in response to temperature changes affected the demography of secondary consumers in the food chain such as squid, fishes, etc.
- As rivers and streams warm, **warm water fish are expanding into areas previously inhabited by cold-water species**. As waters warm, cold-water fish, including many highly valued trout and salmon species, are losing their habitat.

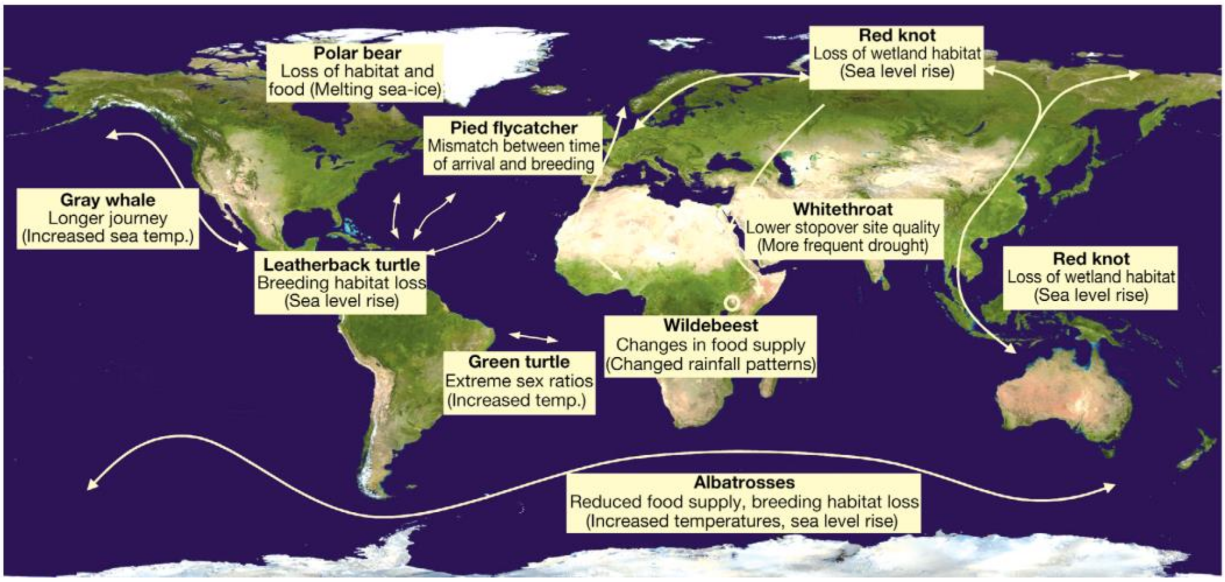


Fig. 5.1.6 Climate change impacts feeding, breeding and migration of animals

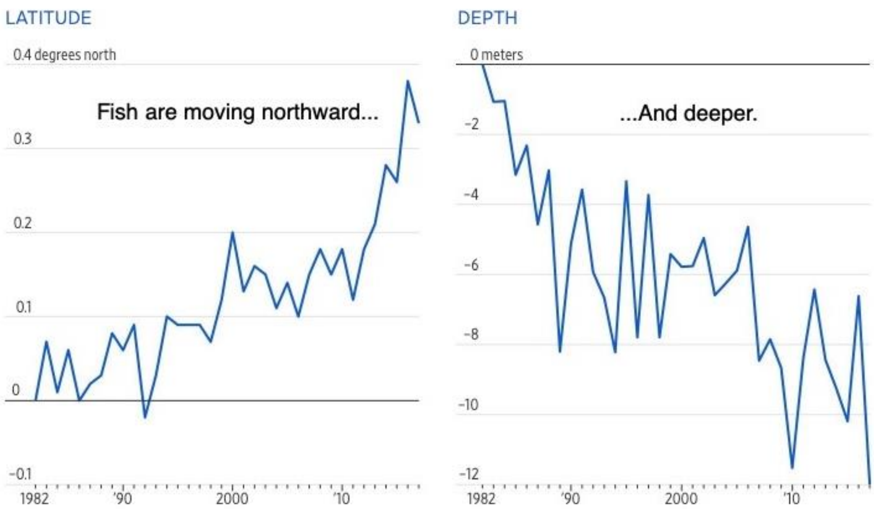


Fig. 5.1.7 Average change in latitude and depth for marine fish in US

Climate change disturbs the current state of ecosystems, resulting in changes to animals' physiological responses, various predator-prey interactions, and range shifts for some animal species. **The impact of climate change on one species can ripple through a food web and affect a wide range of other organisms**.

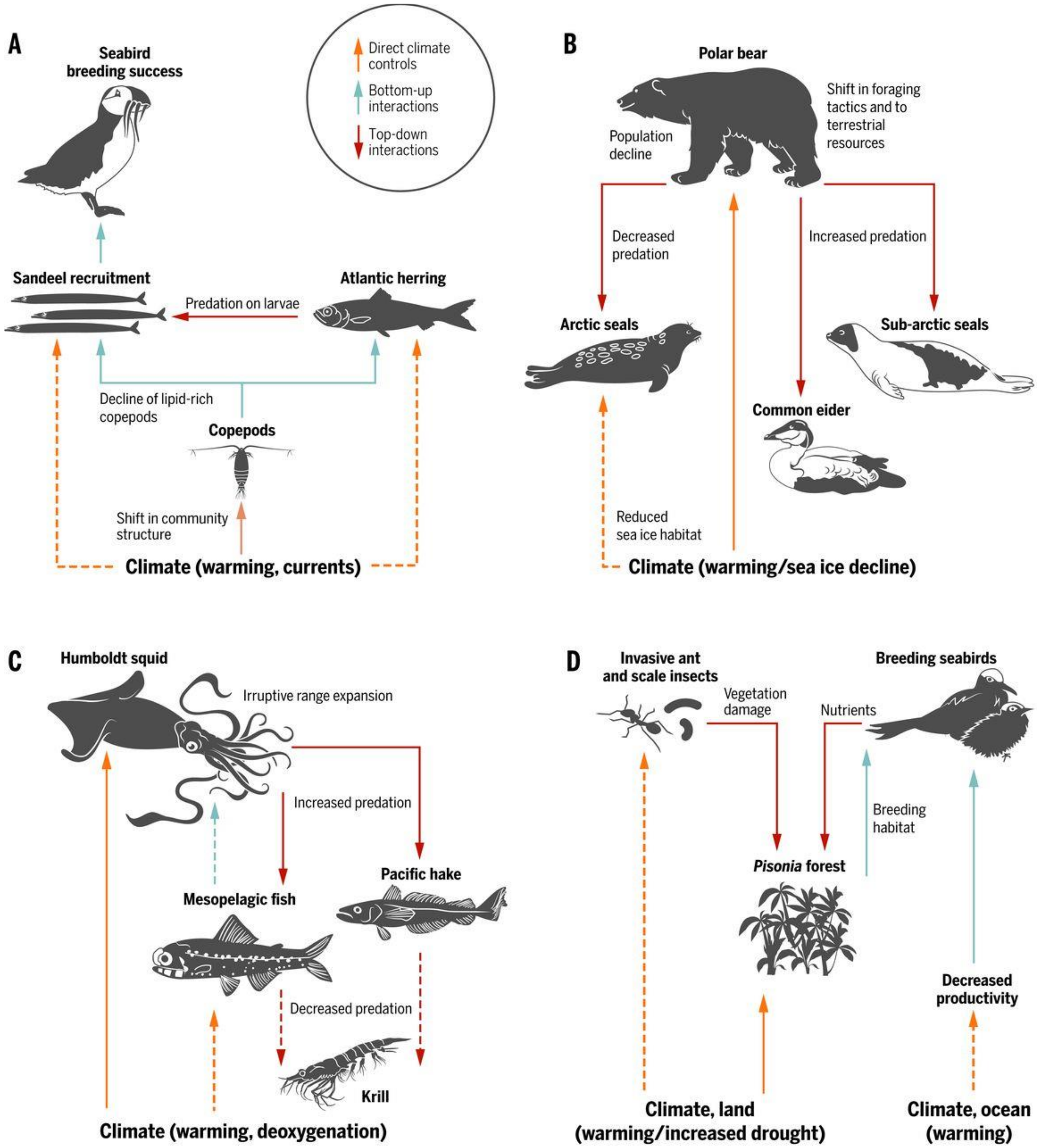


Fig. 5.1.8 Changes to predator-prey interactions due to climate change

5.2 Impact on Breeding Success of Animals and Plants

Phenology refers to the timings of cyclical or seasonal biological events, such as migrations, egg laying, flowering, mating and hibernation. Many species take their cues about when to embark on these events from environmental cues such as changes in temperature, precipitation and daylight**. Climate change is confusing these signals, disrupting the life cycle and seasonal events of wildlife.**

For example:

- American flowering plants like columbines and wild geraniums are blooming earlier than before.
- North American Fowler's toads are breeding six days earlier than they did a decade ago.
- Mamots are ending their hibernations about three weeks earlier than they did 30 years ago.
- Tree swallows are nesting and laying their eggs earlier.
- Birds are migrating to their nesting grounds earlier. In North America, one species of hummingbird has ceased to migrate.

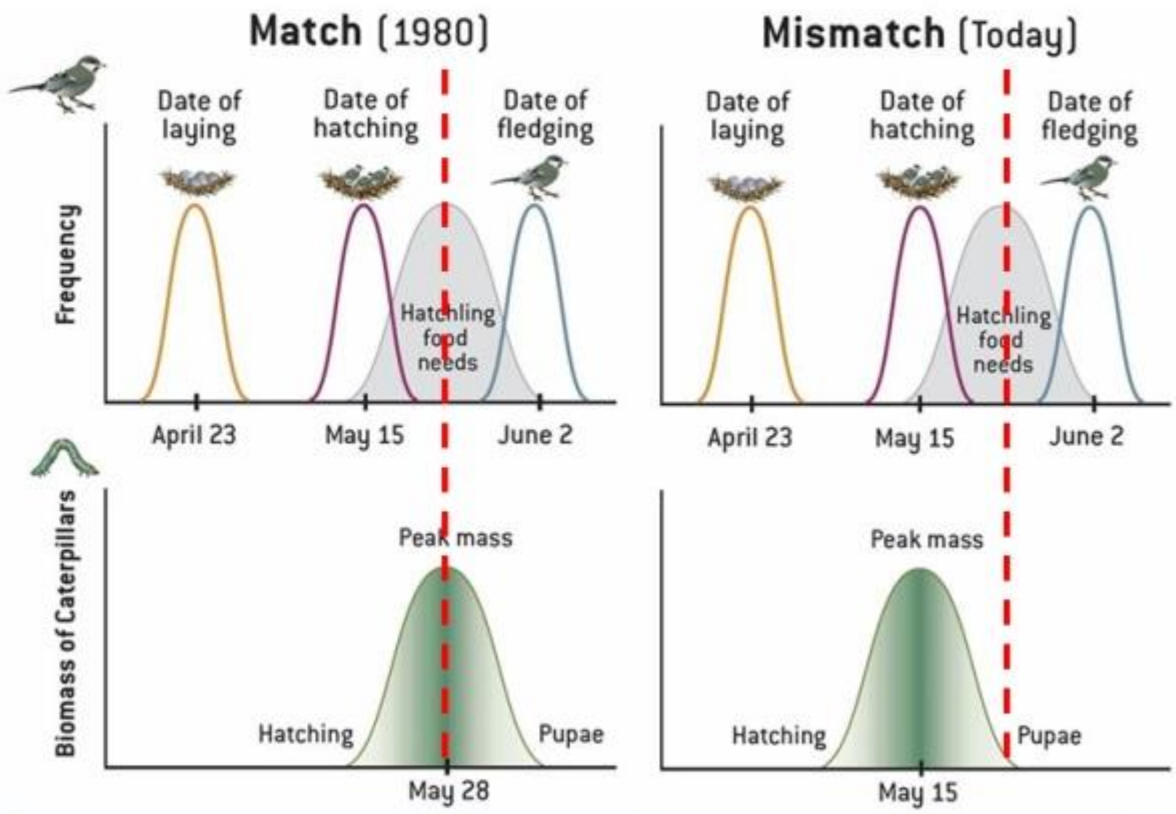


Fig. 5.2.1 Impacts of climate change on birds

Changes such as these can lead to **mismatches in the timing** of migration, breeding and food availability. Species may **get out of sync with other species in their ecosystem or with other natural events**, which in turn **impact their survivability and reproductive success**.

Learning Outcome (j):

Discuss how climate change affects the rich biodiversity of the tropics including the potential loss of this rich reservoir for biomedicines and genetic diversity for food.

6 EFFECT ON BIODIVERSITY

6.1 Loss of Biomedicines

Increase in global temperature increases the risk of extinction of animals and plants. **Over a quarter of all pharmaceutical products come from rainforests**; they have been used in treatment for diseases such as leukemia, malaria, snake bites, as well as cervical, breast and testicular cancers. However, it is estimated that 140 species of plants, animals and insects are lost every day as they become extinct from the loss of rainforest land through fires, logging, urbanisation and intensive mono-cultivation.

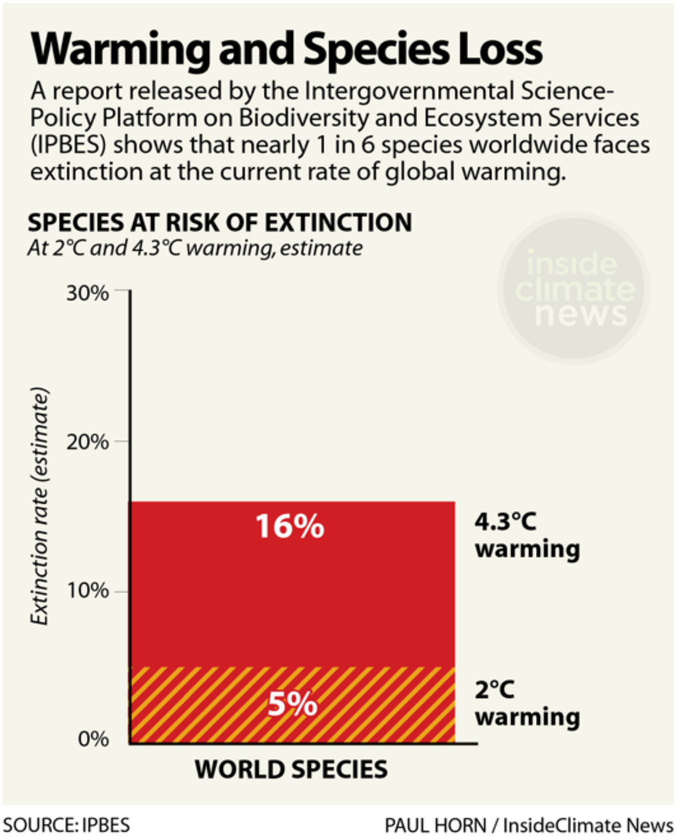


Fig. 6.1.1 Species lost due to climate change

Examples of plants with medicinal value include:

a) Cocoa • The cocoa tree is a tropical tree native to South America from which chocolate is made. Cocoa is also often used as medicine. Cocoa seeds are used for intestinal diseases, diarrhoea, asthma, bronchitis and as an expectorant for lung congestion. The seed coat is also used for liver, bladder, and kidney ailments like diabetes, as a tonic and as a general remedy. Cocoa butter is used for high cholesterol.	
b) *Calophyllum lanigerum* • In 1987, an Illinois team obtained samples from many trees in Sarawak, one of which was *Calophyllum lanigerum*. It was later discovered that Calanolide A, a compound obtained from the bark and latex of the tree was very effective against activity of HIV-1. However, the team could no longer locate the plant in the original site upon their return.	

6.2 Loss of Genetic Diversity of Foods

- **Approximately 80% of the foods we eat come from the tropical rainforest directly or indirectly**. These include avocados, coconuts, squash, yams, bananas, plantains, mangoes, pineapple, citrus fruits, chocolate, vanilla, ginger, peppercorns, and cinnamon. Some other foods grown in the rainforest also include nuts, coffee, tea, rice, and corn.

- **Genetic diversity in crop production is vital to the sustainability of a food source**. Without genetic variability among the genetic strains, there is little chance that the world's food supply will be able to thrive in adversity (e.g. pestilence or disease). Crops grown from seeds with narrow genetic base are more susceptible to diseases.

- **Gene flow from wild plants can be used to fortify modern varieties** against this vulnerability. Without rainforests, this opportunity is lost, as is the chance to develop entirely new food plants.

- The loss of local species and varieties, especially in the rich tropic regions, usually results in **irreversible loss of the genetic diversity** they contain. This has dangerously shrunk the gene pool that is available for natural selection, and for selection by farmers and plant breeders, and has consequently **increased the vulnerability of agricultural crops** to sudden changes in climate, and to the appearance of new pests and diseases.

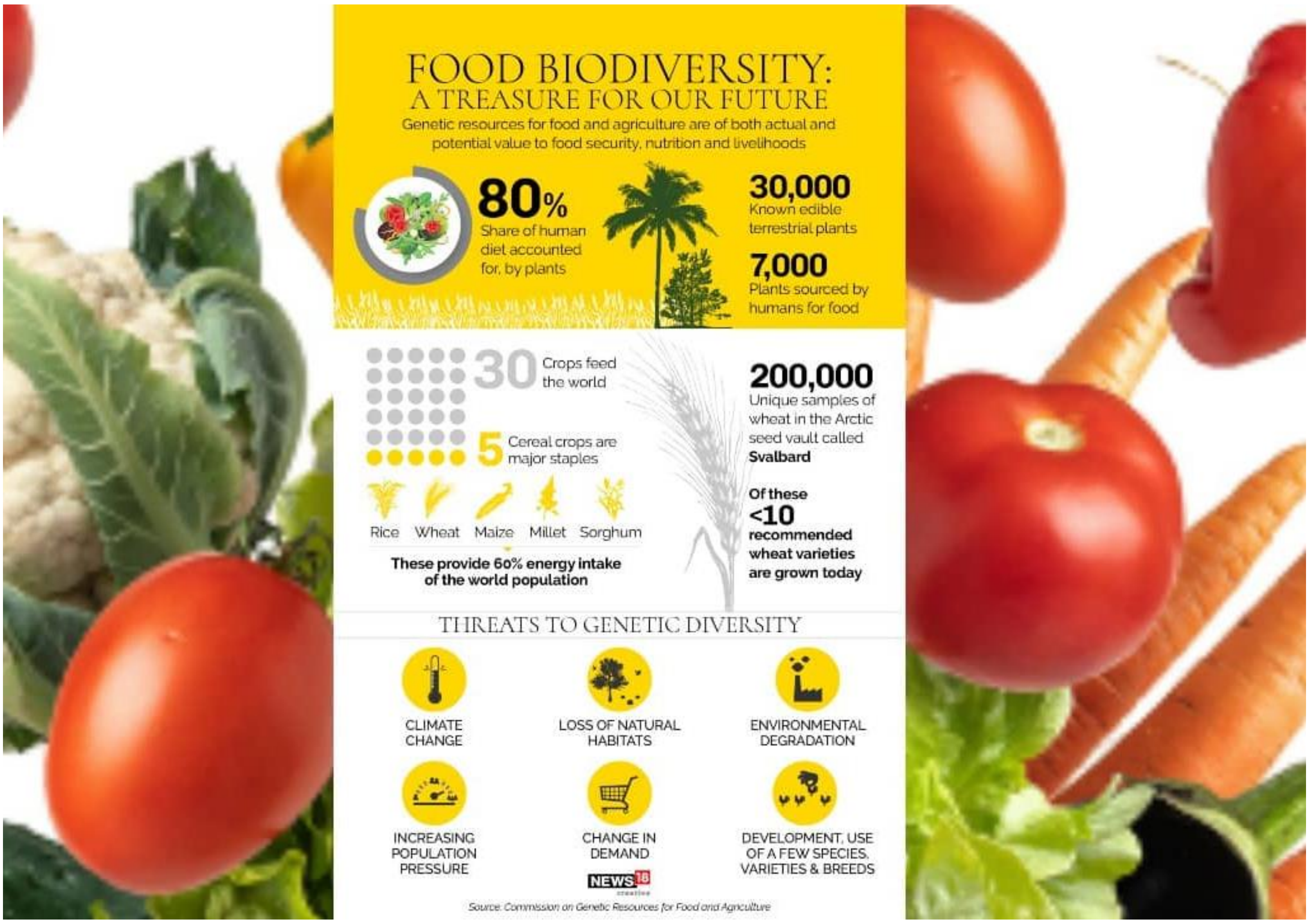


Fig. 6.2.1 Threats to genetic diversity in foods

Biostatistics: Student t-test
(Self-Directed Learning)

Learning outcome:

1. Use the t-test to test the significance of differences between two sets of given data.

Resources:

1. T Test (Student's T-Test): Definition and Examples. (2020, May 29). Retrieved June 07, 2020, from https://www.statisticshowto.com/probability-and-statistics/t-test/

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1 ORIGIN OF THE T-TEST

The T-Distribution, also known as Student's T Distribution, gets its name from William Sealy Gosset who first published it in English literature in his 1908 paper titled Biometrika using his pseudonym "Student" because his employer preferred staff to use pen names when publishing scientific papers instead of their real name, so he used the name "Student" to hide his identity.
Gosset worked at the Guinness Brewery in Dublin, Ireland, and was interested in the problems of small samples – for example, the chemical properties of barley with small sample sizes. Gosset devised the t-test as an economical way to monitor the quality of stout. The t-test work was submitted to and accepted in the journal Biometrika and published in 1908. Company policy at Guinness forbade its chemists from publishing their findings, so Gosset published his statistical work under the pseudonym "Student".

> **NOTE:** You are not expected to remember the equation or what the symbols stand for.
> **You are expected to be able to use the equations to calculate standard deviations, to test for significant differences between the means of two small unpaired samples.**
>
> You will be given access to the equations, the meaning of the symbols, a t-table distribution table.

A ***t* value** can be calculated based on the difference between the two means, standard deviation of the samples and degrees of freedom.

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{{s_1}^2}{n_1} + \frac{{s_2}^2}{n_2}}} \qquad s = \sqrt{((\sum (x - \bar{x})^2)/(n-1))}$$

where s = standard deviation; $\bar{x}$ = mean

$\sum$ = sum of; n = sample size

x = observation

Standard deviation (s) is the dispersion of data in a normal distribution. It indicates how accurately the mean represents sample data. Low standard deviation means data are clustered around the mean, and high standard deviation indicates data are more spread out.

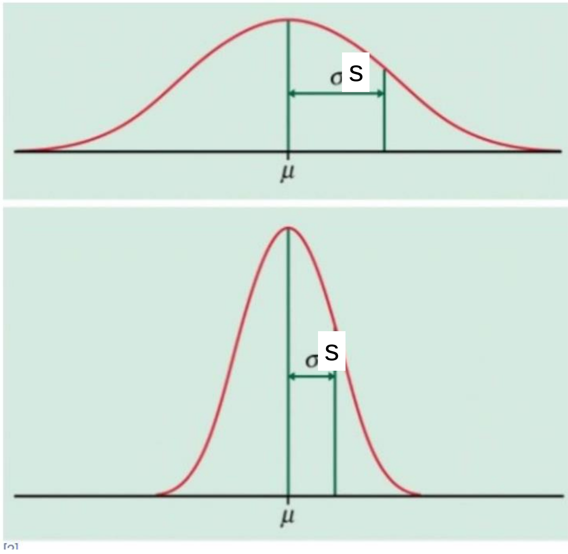

Fig. 1.1 High and low standard deviation curves

A data is said to follow a **normal distribution** when the values of the data are dispersed evenly around one representative value.

2 T-VALUE

The **t-value** is a ratio between the difference between two groups and the difference within the groups. The larger the t-value, the more difference there is between groups. The smaller the t score, the more similarity there is between groups.
Similiar to chi-square values and their associated p-values, every t-value has a p-value to go with it.

A **p-value** is the probability that the results from your sample data occurred by chance.

- The P stands for probability and measures how likely it is that any observed difference between groups is due to chance. Being a probability, P can take any value between 0 and 1.
- Values close to 0 indicate that the observed difference is unlikely to be due to chance, whereas a P value close to 1 suggests no difference between the groups other than due to chance.

3 HYPOTHESIS

Before you carry out the t-test, you need to formulate both the null and alternative hypothesis.

- The **null hypothesis** states that the **difference between the means** of two small unpaired samples **is not significant** and **any difference is due to chance** alone.
- The **alternative hypothesis** states that the **difference between the means** of two small unpaired samples **is significant** and **any difference is NOT due to chance**.

4 CALCULATION OF T-VALUE

Month	Monthly mean precipitation in year X /mm	
	1961	2016
May	50.35	100.52
June	180.55	168.33
July	200.40	280.34
August	180.22	300.45
September	230.18	192.33
October	35.22	92.76

Precipitation in 1961 /mm		Precipitation in 2016 /mm	
x	**$(x - \bar{x})^2$**	**x**	**$(x - \bar{x})^2$**
50.35	9178.278678	100.52	7850.255336
180.55	1183.130678	168.33	432.2934028
200.40	2942.700844	280.34	8320.784336
180.22	1160.537778	300.45	12393.9978
230.18	7060.480711	192.33	10.29340278
35.22	12306.20444	92.76	9285.570803
$\bar{x}_1$ = 146.1533333	**$\sum(x - \bar{x})^2$ = 33831.33313**	**$\bar{x}_2$ = 189.1216667**	**$\sum(x - \bar{x})^2$ = 38293.19508**
s_1 = 82.25731959		**s_2 = 87.51365046**	
n_1 = 6		**n_2 = 6**	

Step 2: Calculation of experimental t-value ($t_{calculated}$)

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{{s_1}^2}{n_1} + \frac{{s_2}^2}{n_2}}}$$

$$\boldsymbol{t} = |\mathbf{146.153} - \mathbf{189.122}|/\sqrt{(\mathbf{1127.711} + \mathbf{1276.440})}$$

$$\boldsymbol{t} = \mathbf{42.969/49.032}$$

$$\boldsymbol{t} = \mathbf{0.876}\ \textbf{(3.s.f.)}$$

5 CALCULATION OF DEGREES OF FREEDOM

Calculation of Degree of Freedom (Df/ v)

Degree of freedom is calculated as the sum of number of samples for both groups minus 2 (due to 2 tailed-test used).

$$v = n_1 + n_2 - 2$$

6 IDENTIFY THE CRITICAL T-VALUE (2 TAILED T-TEST)

Degrees of freedom *(df)*	.2	.15	.1	.05	.025	.01	.005	.001
1	3.078	4.165	6.314	12.706	25.452	63.657	127.321	636.619
2	1.886	2.282	2.920	4.303	6.205	9.925	14.089	31.599
3	1.638	1.924	2.353	3.182	4.177	5.841	7.453	12.924
4	1.533	1.778	2.132	2.776	3.495	4.604	5.598	8.610
5	1.476	1.699	2.015	2.571	3.163	4.032	4.773	6.869
6	1.440	1.650	1.943	2.447	2.969	3.707	4.317	5.959
7	1.415	1.617	1.895	2.365	2.841	3.499	4.029	5.408
8	1.397	1.592	1.860	2.306	2.752	3.355	3.833	5.041
9	1.383	1.574	1.833	2.262	2.685	3.250	3.690	4.781
10	1.372	1.559	1.812	2.228	2.634	3.169	3.581	4.587

Table 1

A **statistically significant** test result ($P \leqslant 0.05$) means that the test hypothesis should be rejected. A P value greater than 0.05 means that no effect was observed.

Worked E.g.

At p = 0.05 and degree of freedom = ***10***, the calculated t-value (***0.876***) is ***less*** than the critical value (***2.228***). The difference in precipitation in the year 1961 and 2016 is ***not significant***.

7 CHECKPOINT

PRACTICE QUESTION 1

A group of naturalists studying Darwin's finches on Galapagos Islands noticed an increase in beak length of the finches following a dry season on the Galapagos Islands. They formed a null hypothesis which states that a change in the season does not affect beak sizes. The naturalists measured the beak's length before and after a dry season. The table below shows the data collected.

Sample no.	Length of finch's beak / cm	
	Before dry season	After dry season
1	3.6	4.1
2	3.2	4.3
3	3.7	4.2
4	3.5	4.2
5	3.5	4.4
6	3.8	3.9
7	3.1	4.0
8	3.3	4.1

Conduct a t-test by completing the table below, to determine the probability that the difference in beak's length is due to chance and not due to change in season.

Before dry season		After dry season	
x	**$(x - \bar{x})^2$**	**x**	**$(x - \bar{x})^2$**
3.6	**0.01891**	4.1	**0.00250**
3.2	**0.06891**	4.3	**0.02250**
3.7	**0.05641**	4.2	**0.00250**
3.5	**0.00141**	4.2	**0.00250**
3.5	**0.00141**	4.4	**0.06250**
3.8	**0.11391**	3.9	**0.06250**
3.1	**0.13141**	4.0	**0.02250**
3.3	**0.02641**	4.1	**0.00250**
$\bar{x}_1 = 3.46250$	**$\sum(x - \bar{x})^2 = 0.41875$**	**$\bar{x}_2 = 4.15$**	**$\sum(x - \bar{x})^2 = 0.18$**
$s_1 = 0.24458$		**$s_2 = 0.16035$**	
$n_1 = 8$		**$n_2 = 8$**	

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{{s_1}^2}{n_1} + \frac{{s_2}^2}{n_2}}}$$

$\boldsymbol{t = |3.46250 - 4.15| / \sqrt{(0.007477 + 0.003214)}}$

$\boldsymbol{t = 0.6875/0.1034}$

$\boldsymbol{t = 6.65\ (3\ s.f.)}$

$v = n_1 + n_2 - 2 = 8 + 8 - 2 = 14$

The table below shows a portion of the t distribution table. Use the t distribution table to determine the critical value at *p = 0.05*.

Degree of freedom (*v*)	*Probability (p)*			
	0.10	0.05	0.025	0.01
11	1.796	2.201	2.593	3.106
12	1.782	2.179	2.560	3.055
13	1.771	2.160	2.533	3.012
14	1.761	2.145	2.510	2.977
15	1.753	2.131	2.490	2.947

1. At p = 0.05 and degree of freedom = ***14***, the calculated t-value (***6.65***) is ***more*** than the critical value (***2.145***).
2. The ***difference*** in beak's length of the Darwin's finches at Galapagos Island before and after a dry season is ***significant*** and is ***not due to chance*** alone.

PRACTICE QUESTION 2

A colorimeter passes a beam of light through a coloured filter into a solution and measures the light absorbance of that solution. A standard solution is used to set the colorimeter scale to zero (0) before taking any measurements.

Chlorophyll absorbs red light, so the chlorophyll concentration of a leaf extract can be measured using a colorimeter.

The table shows the students' results from the colorimeter measurements made on 10 samples of each of the two types of leaf.

type of leaf	ivy	geranium
mean absorbance ± *s*	0.28 ± 0.08	0.32 ± 0.1

1. $v = n_1 + n_2 - 2$ = ***10 + 10 – 2 = 18***

2. With the given scenario, formulate the:

   (i) null hypothesis

   There is no significant difference in the mean absorbance between the ivy and geranium leaf and any difference is due to chance.

   (ii) alternative hypothesis

   There is a significant difference in the mean absorbance between the ivy and geranium leaf and any difference is not due to chance.

3. Using the t-test equation and the information given, calculate the t-value.

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{{s_1}^2}{n_1} + \frac{{s_2}^2}{n_2}}}$$

$$\boldsymbol{t} = \frac{|\mathbf{0.28 - 0.32}|}{\sqrt{\mathbf{0.00064 + 0.001}}}$$

$$\boldsymbol{t} = \frac{|\mathbf{0.28 - 0.32}|}{\sqrt{\mathbf{0.00064 + 0.001}}}$$

$$\boldsymbol{t} = \frac{\mathbf{0.04}}{\sqrt{\mathbf{0.00064} + \mathbf{0.001}}}$$

$$\boldsymbol{t} = \frac{\mathbf{0.04}}{\mathbf{0.0405}}$$

$$\boldsymbol{t} = \mathbf{0.988}$$

4. With the t-calculated value, the degree of freedom and using part of the t-distribution table below, determine the probability of t-calculated value.

Degree of freedom (*v*)	*Probability (p)*			
	0.10	0.05	0.025	0.01
18	1.734	2.101	2.445	2.878
19	1.729	2.093	2.433	2.861
20	1.725	2.086	2.423	2.845

1. At p = 0.05 and degree of freedom = ***18***, the calculated t-value (***0.988***) is ***less*** than the critical value (***2.101***).
2. The ***difference*** in absorbance is ***not significant*** and is ***due to chance*** alone.