



**DEPARTMENT OF AQUACULTURE
FACULTY OF AGRICULTURE**

**AKU4301
FISH ENDOCRINOLOGY**

**PRACTICAL 4
OSMOREGULATION**

**LECTURER:
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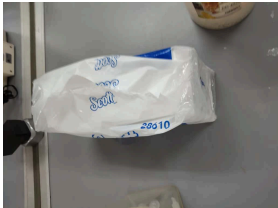
INTRODUCTION

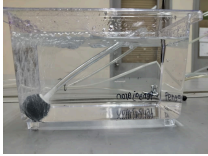
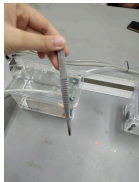
Osmoregulation is an active regulation of the osmotic pressure of an organism's fluid to maintain the homeostasis of the organism's water content. It keeps the organism's fluid from becoming too diluted or too concentrated. The process involved excretion of metabolic waste and others (i.e. hormones) through the skin surface and the kidneys. A sudden change in osmotic pressure can put great stress on the osmoregulatory system in fish. Many vertebrates show changes in pigmentation or pigmentary patterns that are correlated with environmental factors or particular events in life, e.g. warning, stress, attraction. The pigmentary responses of most fishes are under the control of melanophores and other chromatophores, and they involve the stimulation of melanotropin (α -MSH) hormone.

OBJECTIVE

1. To determine the effect of different salinity levels on the behaviour of fish.
2. To determine the salinity tolerance of freshwater fish.
3. To determine the effect of salinity on the melanophores on the fish scales.

MATERIALS

Material	Function
<i>Carassius auratus</i> (Gold fish)	To allow the collection of behavioral and scale data of fish in different salinity levels. 
Tank	To allow the preparation of different salinity levels. 
Air pump	To maintain oxygen level and air flows. 
Tissue	Hand wiping, absorbing liquid, and surface protection to prevent spills and contamination. 

Hand Glove	To protect hand from harmful substances and reduce the risk of contamination. 
Air stone	To increase the level of dissolved oxygen in the tank. 
Refractometer	To identify the salinity of the prepared tank so that it reaches the ideal salinity level. 
Compound microscope	To allow the observation of fish scales in different time duration. 
Salt	To be used in preparation of different salinity levels. 
Microscope slide and cover slip	To put the scale in different duration to be observed under a microscope.  
Forceps	To get the scale from the sample fish in different salinity and time duration. 

METHODOLOGY

1. 5 different salinities (0 ppt, 5 ppt, 10 ppt, 15 ppt, and 20 ppt) were prepared.
2. Two fish were placed in each tank with different salinities.
3. Each fish's behavior was observed for 20 minutes with 5-minute intervals.
4. For every 5 minutes, scales from the fish were removed and observed under the compound microscope.
5. The fish's behavior and color changes in scale were recorded in the table below.

RESULT

SCALES OBSERVATION UNDER A COMPOUND MICROSCOPE

Table 1

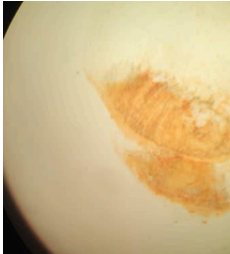
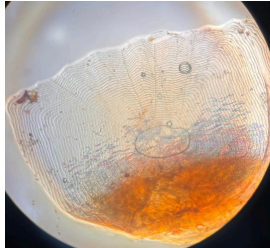
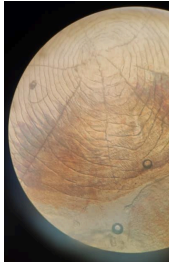
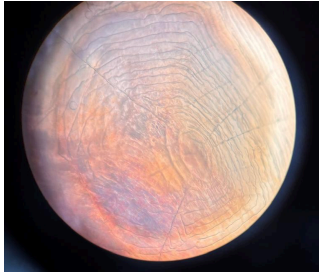
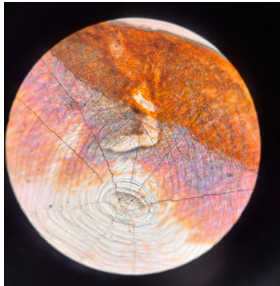
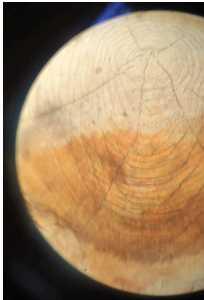
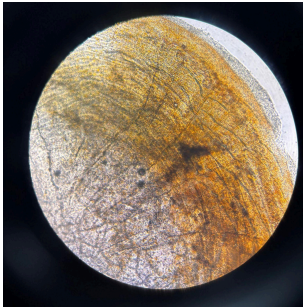
TIME DURATION	SALINITY LEVEL	
	0 ppt (CONTROL)	5 ppt
15 MIN		
30 MIN		
45 MIN		
60 MIN		

Table 2

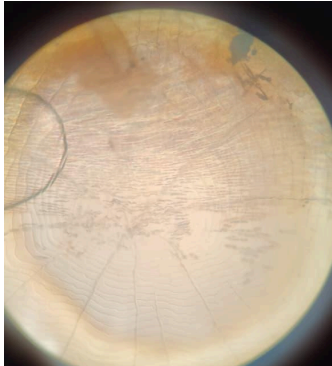
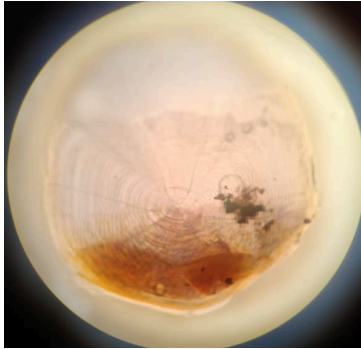
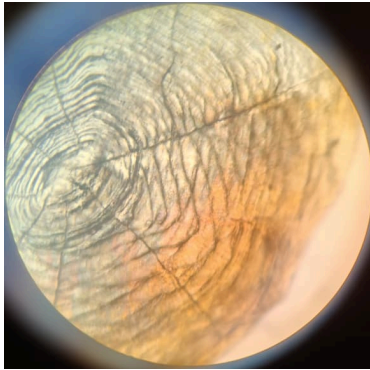
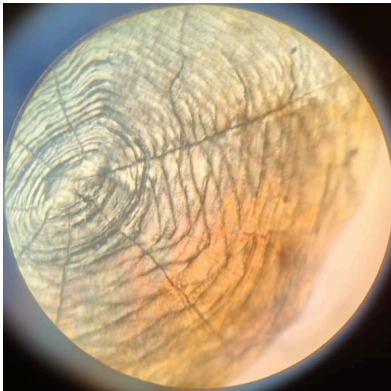
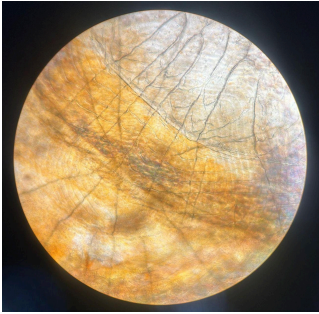
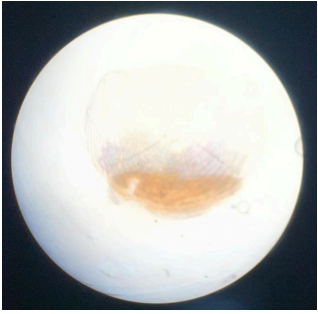
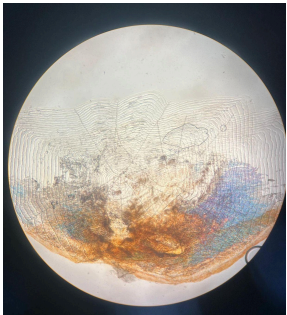
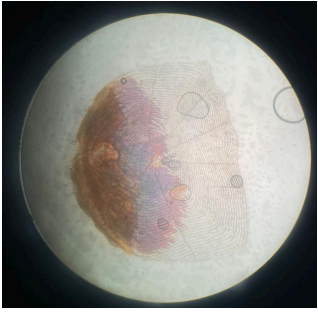
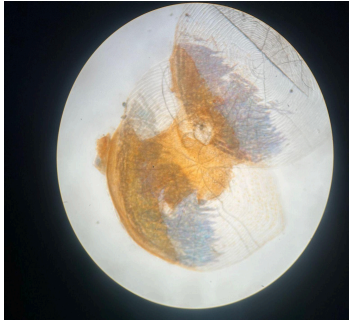
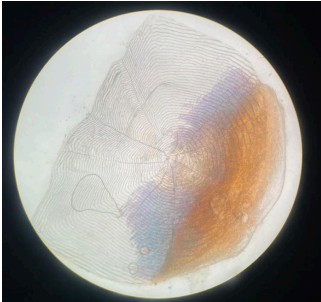
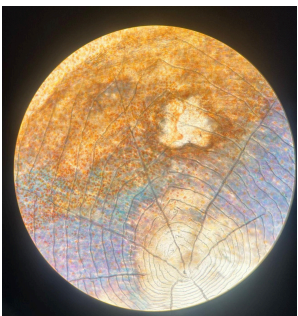
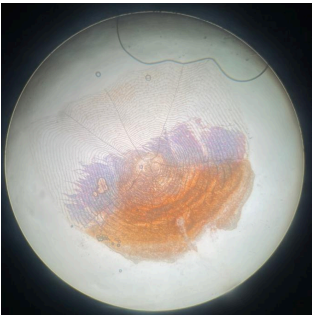
TIME DURATION	SALINITY LEVEL	
	10 ppt	
15 MIN		
30 MIN		
45 MIN		
60 MIN		

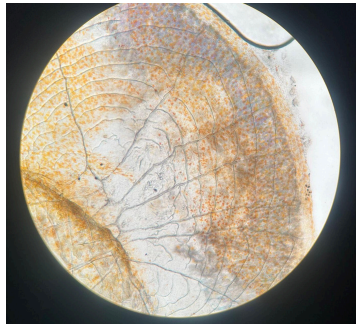
Table 3

TIME DURATION	SALINITY LEVEL	
	15 ppt (TANK 1)	15 ppt (TANK 2)
5 MIN		
	Fish 1	Fish 1
		
	Fish 2	Fish 2
10 MIN		
	Fish 1	Fish 1
		
	Fish 2	Fish 2

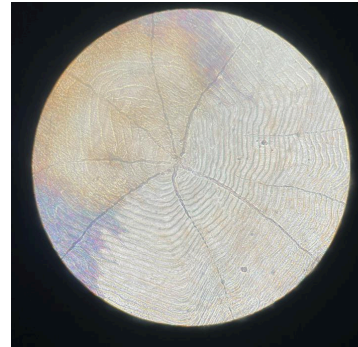
15 MIN



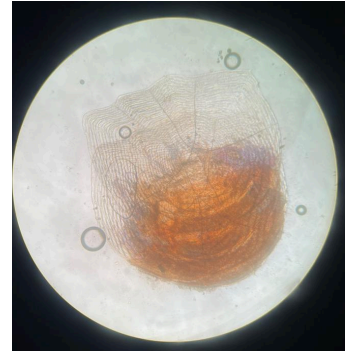
Fish 1



Fish 2

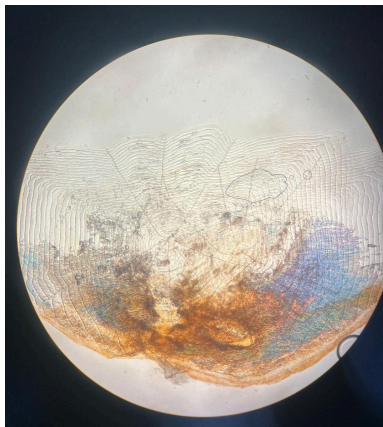


Fish 1

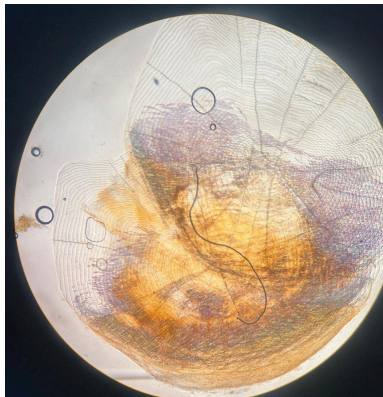


Fish 2

20 MIN



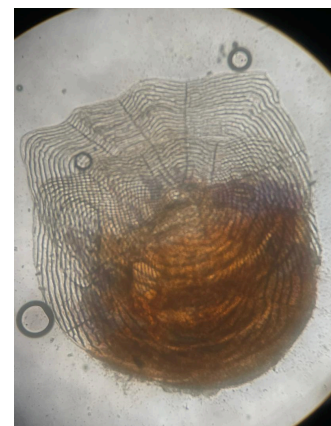
Fish 1



Fish 2

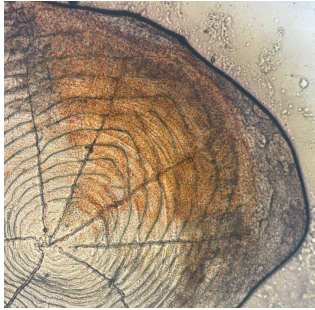
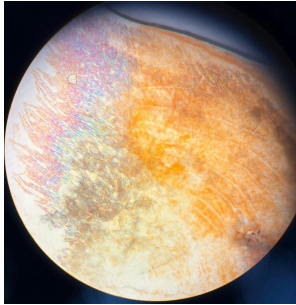
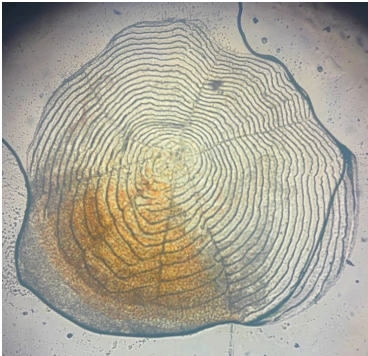
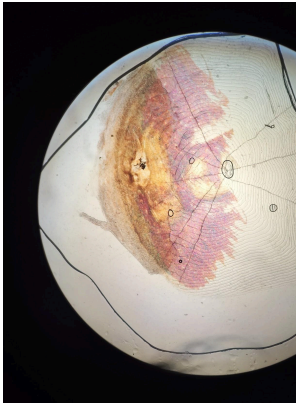
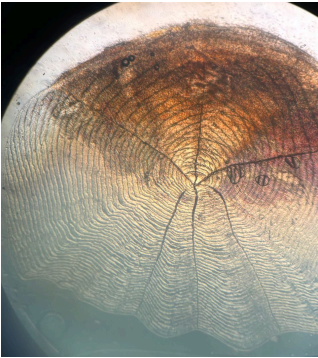
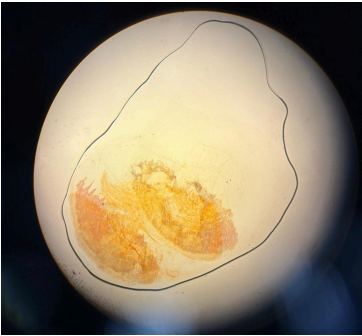
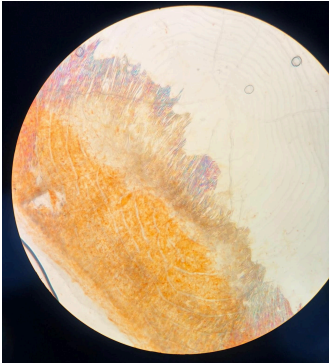


Fish 1



Fish 2

Table 4

TIME DURATION	SALINITY LEVEL	
	20 ppt (TANK 1)	20 ppt (TANK 2)
5 MIN		
10 MIN		
15 MIN		
20 MIN	-	

SWIMMING BEHAVIOR

SALINITY	SWIMMING BEHAVIOR
0 ppt	15 MIN: Normal behaviour
	30 MIN: Normal behaviour
	45 MIN: Normal behaviour
	60 MIN: Normal behaviour
5 ppt	15 MIN: Normal swimming and good coloration
	30 MIN: Remained still, then swam back
	45 MIN: Gasping for air
	60 MIN: Slightly Weakened
10 ppt	15 MIN: Erratic swimming
	30 MIN: Gasping for air
	45 MIN: Stayed near aeration
	60 MIN: Slow Movement
15 ppt (TANK 1)	5 MIN: Dorsal-normal, regular opercular movement; x dorsal-normal, regular.
	10 MIN: Slight increase for both in movement activity.
	15 MIN: Fish 2 is erratic & gasping for air, Fish 1 has minimal change in activity and is slightly gasping for air.
	20 MIN: Fish 2 is erratic & gasping for air & has an increased opercular ventilation rate; Fish 1 is starting to adapt & tolerating.
15 ppt (TANK 2)	5 MIN: Dorsal-normal, regular opercular movement; x dorsal-normal, regular.
	10 MIN: Slight increase for both in movement activity.
	15 MIN: Fish 2 is erratic & gasping for air; Fish 1 has minimal change in activity and is slightly gasping for air.
	20 MIN: Fish 2 is erratic & gasping for air & has an increased opercular ventilation rate, Fish 1 is starting to adapt & tolerating.
20 ppt (TANK 1)	5 MIN: Fish movement is normal, regular opercular movement
	10 MIN: Both fish are gasping for air slightly quicker, and the fish movement is starting to be erratic
	15 MIN: Both fish is gasping for air much quicker and the fish movement is more erratic

	20 MIN:-
20 pp (TANK 2, GROUP 3)	5 MIN: Abnormal swimming
	10 MIN: Slow swimming, go near aeration
	15 MIN: Slow swimming, sometimes stops and floats, presence of faeces
	20 MIN: Swim vertically, swim near aeration, drunk

Discussion

When a fish is placed in a stressful environment or faced with a sudden change of salinity level, it can respond with pale colour of scales, clamped fins, erratic swimming, and reduced appetite (Agarwal et al., 2024). Refer to our results after the experiment has been done in the freshwater species; two symptoms out of four listed symptoms have been clearly shown in the experiment, which are pale colour and erratic swimming. The chromatophores aggregation may be caused by a higher salinity water level because in higher salinity, the stress response axis may be activated, and pigment granules will be aggregated to the cell center (Nivethitha & Pragasan, 2024).

According to the result observed from the experiment, we can ensure that a high salinity level can cause chromatophore aggregation in fish scales, and with the increase of the salinity level, the chromatophore aggregations become more obvious and can be formed in a shorter duration. This idea is supported by the fish scales image for 15 ppt, fish 1, and fish 2 at 10 minutes, which shows the presence of chromatophores, and it becomes apparent at the fourth observation. Same as for the result of 20 ppt salinity level, the result for tank 2 fish scales at 10 min shows the symptom of chromatophore aggregation, and the chromatophores form at the next time duration of observation. Hence, the colour of the fish becomes pale or lighter, and a few dark spots can be identified under a microscope.

As the fish is set to be in a different salinity environment, the swimming behavior shows that it is experiencing acute osmotic shock. Refer to the table of swimming behavior above; the fish in 0 to 10 ppt have mild to moderate stress and show erratic swimming and get near to aeration to get more air, which is considered a light symptom, while the fish exposed to 15 to 20 ppt have strong stress response, and some even show vertical swimming and “drunk-like” movement which means that it is indicating serious physiological disturbance. Based on the article written by Nivethitha & Pragasan in 2024, they also mention that a sudden change in salinity can cause fish to experience osmotic stress and stimulate the production of stress hormones such as cortisol, which may cause aggregation of pigment granules.

While the colour changed on scales of 0 ppt fish, this might happen because of a sudden shock caused by human error. As we need to pick up scales from the fish to identify them under a microscope, human error may occur as we frighten the sample fish. This explains why fish with 0 ppt, which stay in the control treatment, also have scales that have a slightly different colour over time.

Conclusion

In conclusion, the experiment demonstrated that rising salinity levels significantly affected freshwater fish's swimming habit and scale colour. Fish scales became whiter and dark pigment patches were evident under a microscope due to chromatophore aggregation, which was accelerated at salinities between 15 and 20 ppt. Additionally, the fish showed symptoms of osmotic stress, including irregular swimming, vertical movement, and "drunk-like" behaviour, which are indicative of physiological disruption brought on by abrupt changes in salinity. Even though the control group also showed minor colour changes (0 ppt), they were probably caused by handling stress or human error during sampling. Overall, the results demonstrate that fish physiology and behaviour can be adversely affected by high salinity through stress-related reactions.

References

Journal

1. Agarwal, D., Shanmugam, S. A., Kathirvelpandian, A., Eswaran, S., Rather, M. A., & Rakkannan, G. (2024). Unraveling the impact of climate change on fish physiology: A focus on temperature and salinity dynamics. *Journal of Applied Ichthyology*, 2024(1). <https://doi.org/10.1155/2024/5782274>
2. Nivethitha, P., & Pragasan, L. A. (2024). Assessing Colouration in Ichthyology: Insights from the Melanophore Index Perspective. *Asian Journal of Fisheries and Aquatic Research*, 26(5), 32–52. <https://doi.org/10.9734/ajfar/2024/v26i5764>
3. Spaeth, R. A. (1913). The physiology of the chromatophores of fishes. *Journal of Experimental Zoology*, 15(4), 527–585. <https://doi.org/10.1002/jez.1400150405>