

HS2 Learning Legacy supporting document

Table 1. GCN status of ponds surveyed from April to October using ethanol precipitation (EP) kits and filtration (F) kits. Cells are shaded according to kit result (navy = negative, turquoise = positive, grey = inconclusive), and white cells represent months where a pond was not surveyed due to drying out and/or health and safety concerns. Numbers in turquoise cells indicate eDNA scores for positive kits.

Pond	Month and eDNA capture method														Notes	
	Apr		May		Jun		Jul		Aug		Sep		Oct			
	EP	F	EP	F	EP	F	EP	F	EP	F	EP	F	EP	F		
1																No disagreement between eDNA capture methods.
2																No disagreement between eDNA capture methods.
3																Filtration kit tested positive for inhibition in September, thus result was inconclusive as inhibitors could be responsible for non-detection of GCN DNA.
4						1										Positive for GCN (low eDNA score) using filtration kit in June only. Filtration kit tested positive for inhibition in September, thus result was inconclusive as inhibitors could be responsible for non-detection of GCN DNA.
5	1	12		12	1			12		10						Positive for GCN (low eDNA scores) with ethanol precipitation kits in April and June only. GCN detection with filtration kits also inconsistent albeit high eDNA scores observed in-season and early out-of-season.
6	1	11		12		12		12		4						Positive for GCN (low eDNA scores) with ethanol precipitation kit in April only. GCN detection with filtration kits consistent until August.
7		12	5	12		12	1	12		10						Positive for GCN with ethanol precipitation kits in May and July only, with low eDNA score in July. GCN detection with filtration kits consistent until August.
8		1	3	7			7	12		12		1				Positive for GCN with ethanol precipitation kits in May and July only, with low eDNA score in May. GCN detection with filtration kits consistent until September, excluding negative result in June.
9		7	10	1	4	12	12	12								Negative for GCN with ethanol precipitation kit in April only, otherwise GCN detection with both kit types consistent from April to July.
10	9	10	3	12	1	12	4		1	1	1	1				Negative for GCN with filtration kit when ethanol precipitation kit positive in July only. GCN detection

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	Apr		May		Jun		Jul		Aug		Sep		Oct								
	EP	F	EP	F	EP	F	EP	F	EP	F	EP	F	EP	F							
															typically stronger for in-season months compared to out-of-season months.						
11	2	12	12	12	12	1	12	11	12	5	5					Negative for GCN with filtration kit when ethanol precipitation kit positive in September only. Strong GCN detection in all months, except October.					
12	6	12	10	2	12	12	12	12	12	12	1	12					Perfect agreement between kit types from April to September. Strong GCN detection in all months, except October.				
13	12	12	12	7	6	12	11	11	12	5	2					Negative for GCN with filtration kit when ethanol precipitation kit positive in September only. Strong GCN detection in all months, except October.					
14	2	8	12	12	12	2	11			11	11	1	3			Negative for GCN with filtration kit when ethanol precipitation kit positive in July only. Strong GCN detection from April to August.					
15			6	5	12			11	4	12	3	12	10	4			Negative for GCN with ethanol precipitation kits in April and June, otherwise GCN detection with both kit types consistent from April to September.				
16	12			12	12	12	11	12			7							Negative for GCN with filtration kits in April, July and August. Strong GCN detection from April to August.			
17			12														Negative for GCN with ethanol precipitation kit in April.				
18						1														Negative for GCN with ethanol precipitation kit in May.	
19	2	9														Positive with both kit types in April.					
20	1	12	1	7			12	1	12	3	1			5	1	1	Negative for GCN with ethanol precipitation kits in June and September, otherwise GCN detection with both kit types consistent from April to October.				
21	3	12			12	12	12														Negative for GCN with ethanol precipitation kit in May.
22	1	12	8	12	11	12	12	2	4	2							Perfect agreement between kit types from April to August. GCN detection typically stronger for in-season months compared to out-of-season months.				
23			9	10	12														Negative for GCN with ethanol precipitation kit in April.		

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	Apr		May		Jun		Jul		Aug		Sep		Oct		
	EP	F	EP	F	EP	F	EP	F	EP	F	EP	F	EP	F	
24														2	Positive for GCN with filtration kit in October.
25							12	12	12	12	3	6		2	Negative for GCN with ethanol precipitation kit in October.