

Supplementary Data

Table S1. Environmental variables for three freshwater sampling locations in the Han River.

Sampling location	Geographic coordinate		Sampling date	Water temperature (°C)	Salt (ppm)
	Latitude	Longitude			
Paldang Lake (PL)	37°31'58"	127°18'58"	Apr. 25, 2022	20.3	142.3
Jamsil Bridge (JB)	37°31'23"	127°05'54"	May. 4, 2022	18.7	100.7
Haengju Bridge (HB)	37°35'45"	126°48'29"	May. 4, 2022	18.7	158.3

Table S2. Read count information of eDNA and eRNA sequences.

Molecule	Location	Raw reads	Filtered reads	Denoised reads	Merged reads	Non-chimeric reads	Total number of ASVs
eDNA	PL	364,409	329,063	319,251	165,796	75,978	258
	JB	434,073	401,458	386,001	270,586	137,110	219
	HB	360,111	348,175	335,606	266,315	112,980	212
eRNA	PL	270,082	256,879	244,456	201,474	98,810	346
	JB	421,892	413,422	388,294	312,914	151,276	331
	HB	231,926	213,373	205,792	185,582	53,292	455

Table S3. Comparison of species identified by eDNA and eRNA metabarcoding and previous specimen-capture survey (PSCS) data

Order	Family	Genus	Species	PL			JB			HB		
				PSCS	eDNA	eRNA	PSCS	eDNA	eRNA	PSCS	eDNA	eRNA
Anabantiformes	Channidae	<i>Channa</i>	<i>Channa argus</i>		•			•			•	
Anguilliformes	Anguillidae	<i>Anguilla</i>	<i>Anguilla japonica</i>					•				
Beloniformes	Hemiramphidae	<i>Hyporhamphus</i>	<i>Hyporhamphus sajori</i>							•		
Centrarchiformes	Centrarchidae	<i>Lepomis</i>	<i>Lepomis macrochirus</i>		•	•		•	•		•	•
		<i>Micropterus</i>	<i>Micropterus salmoides</i>	•	•	•	•	•	•	•	•	•
		<i>Siniperca</i>	<i>Siniperca scherzeri</i>		•			•			•	
Clupeiformes	Engraulidae	<i>Coilia</i>	<i>Coilia nasus</i>			•			•	•	•	•
Cypriniformes	Acheilognathidae	<i>Acheilognathus</i>	<i>Acheilognathus chankaensis</i>		•			•			•	
			<i>Acheilognathus rhombeus</i>					•	•			
			<i>Acheilognathus yamatsutae</i>		•			•				
		<i>Rhodeus</i>	<i>Rhodeus ocellatus</i>					•				
			<i>Rhodeus uyekii</i>				•					
		<i>Tanakia</i>	<i>Tanakia lanceolata</i>				•	•				
			<i>Tanakia limbata</i>			•						
	Cobitidae	<i>Iksookimia</i>	<i>Iksookimia koreensis</i>		•			•			•	
		<i>Misgurnus</i>	<i>Misgurnus anguillicaudatus</i>	•	•	•		•	•		•	
			<i>Misgurnus mohoity</i>		•			•			•	
	Cyprinidae	<i>Carassius</i>	<i>Carassius auratus</i>	•	•	•		•	•	•	•	•
		<i>Cyprinus</i>	<i>Cyprinus carpio</i>	•			•			•		
		<i>Pseudogobio</i>	<i>Pseudogobio esocinus</i>	•			•					
		<i>Sarcocheilichthys</i>					•					
			<i>Sarcocheilichthys variegatus</i>				•					
	Gobionidae	<i>Abbottina</i>	<i>Abbottina rivularis</i>		•	•		•				
		<i>Gobio</i>	<i>Gobio gobio</i>		•							
		<i>Hemibarbus</i>	<i>Hemibarbus labeo</i>	•	•		•			•		

		<i>Pseudopungtungia</i>	<i>Pseudopungtungia nigra</i>	•		•		•		
		<i>Pseudorasbora</i>	<i>Pseudorasbora parva</i>			•				
		<i>Pungtungia</i>	<i>Pungtungia herzi</i>	•		•	•		•	•
	Leuciscidae	<i>Alburnus</i>	<i>Alburnus alburnus</i>						•	
		<i>Phoxinus</i>	<i>Phoxinus keumkang</i>	•		•			•	
		<i>Rhynchocypris</i>	<i>Rhynchocypris oxycephala</i>	•						
	Xenocypridae	<i>Chanodichthys</i>	<i>Chanodichthys erythropterus</i>			•			•	
		<i>Culter</i>	<i>Culter alburnus</i>	•	•	•	•		•	•
		<i>Hemiculter</i>	<i>Hemiculter eigenmanni</i>						•	
			<i>Hemiculter leucisculus</i>						•	
		<i>Nipponocypris</i>	<i>Nipponocypris temminckii</i>	•		•			•	
		<i>Opsariichthys</i>	<i>Opsariichthys uncirostris</i>	•		•			•	
		<i>Zacco</i>	<i>Zacco platypus</i>	•	•	•	•		•	
Gobiiformes	Gobiidae	<i>Acanthogobius</i>	<i>Acanthogobius hasta</i>			•		•	•	•
			<i>Acanthogobius lactipes</i>						•	
		<i>Gymnogobius</i>	<i>Gymnogobius urotaenia</i>						•	
		<i>Rhinogobius</i>	<i>Rhinogobius brunneus</i>	•		•				
			<i>Rhinogobius similis</i>		•	•	•	•	•	•
		<i>Tridentiger</i>	<i>Tridentiger bifasciatus</i>						•	•
			<i>Tridentiger brevispinis</i>	•	•	•	•	•	•	•
	Odontobutidae	<i>Odontobutis</i>	<i>Odontobutis interruptus</i>	•	•		•		•	
			<i>Odontobutis platycephala</i>	•	•		•		•	•
Mugiliformes	Mugilidae	<i>Mugil</i>	<i>Mugil cephalus</i>	•		•	•		•	•
		<i>Planiliza</i>	<i>Planiliza haematocheilus</i>	•			•	•	•	•
Osmeriformes	Plecoglossidae	<i>Plecoglossus</i>	<i>Plecoglossus altivelis</i>	•		•			•	
Pempheriformes	Lateolabracidae	<i>Lateolabrax</i>	<i>Lateolabrax japonicus</i>						•	
			<i>Lateolabrax maculatus</i>						•	
Perciformes	Cottidae	<i>Trachidermus</i>	<i>Trachidermus fasciatus</i>						•	
Salmoniformes	Salmonidae	<i>Oncorhynchus</i>	<i>Oncorhynchus masou</i>	•						

Table S4. List of saltwater and brackish water fishes detected at each location by metabarcoding. PL: Paldang Lake; JB: Jamsil Bridge; HB: Haengju Bridge.

Order	Family	Genus	Species	PL		JB		HB	
				eDNA	eRNA	eDNA	eRNA	eDNA	eRNA
Clupeiformes	Clupeidae	<i>Clupea</i>	<i>Clupea harengus</i>					•	
Gadiformes	Gadidae	<i>Gadus</i>	<i>Gadus</i> sp.			•			
Scombriformes	Scombridae	<i>Scomberomorus</i>	<i>Scomberomorus niphonius</i>			•			
Pleuronectiformes	Paralichthyidae	<i>Paralichthys</i>	<i>Paralichthys olivaceus</i>					•	
Chaetodontiformes	Leiognathidae	<i>Nuchequula</i>	<i>Nuchequula</i> sp.	•					
Scombriformes	Scombridae	<i>Scomber</i>	<i>Scomber</i> sp.			•			
Lophiiformes	Lophiidae	<i>Lophius</i>	<i>Lophius litulon</i>	•		•		•	
Clupeiformes	Engraulidae	<i>Engraulis</i>	<i>Engraulis japonicus</i>			•			

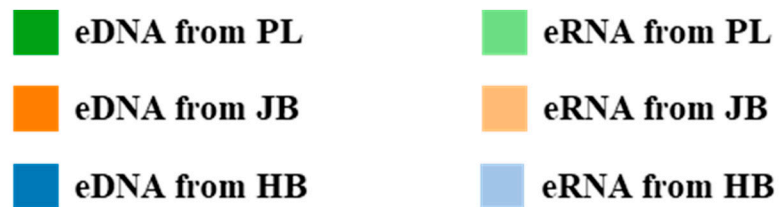
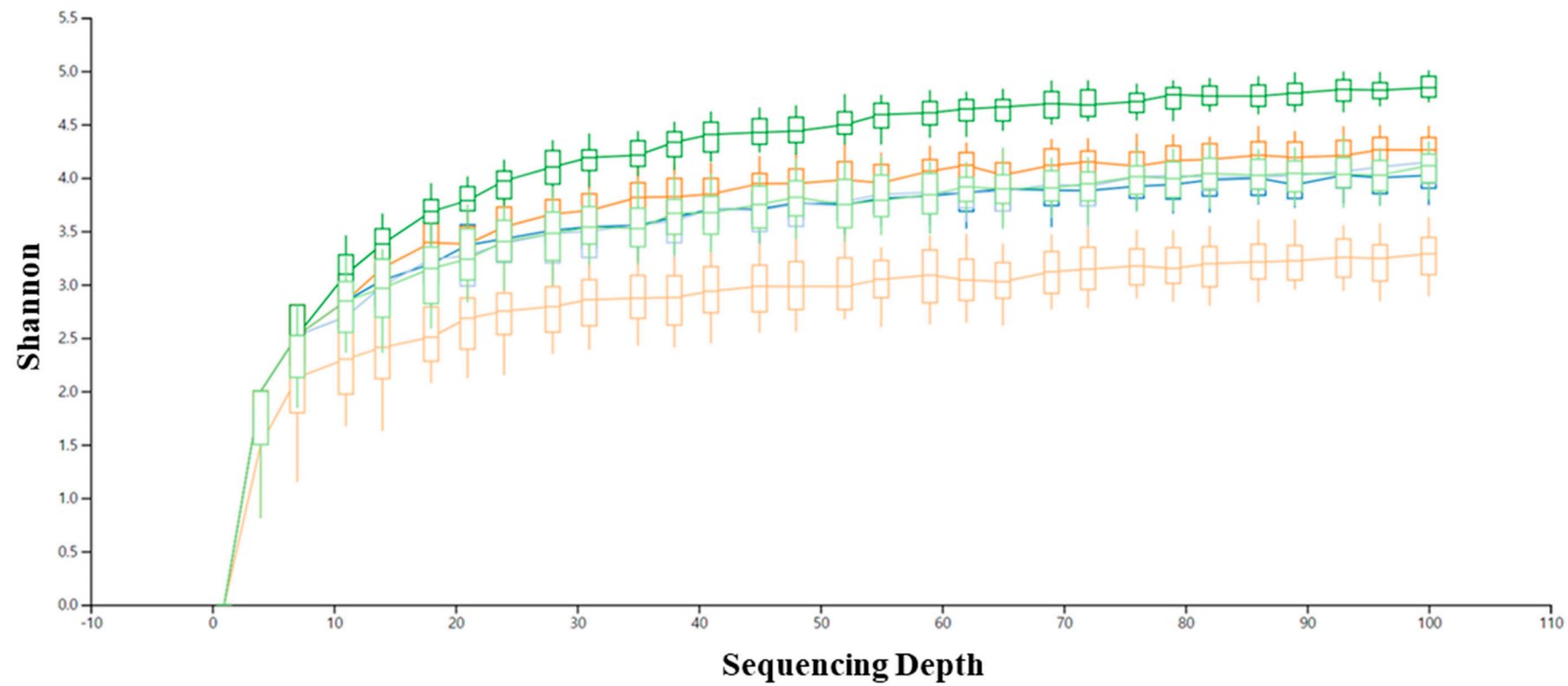


Figure S1. Rarefaction curves of eDNA and eRNA metabarcoding using 12S rRNA. Curves show predicted total diversity by sequencing depth. The X axis represents sample sequencing depth and Y axis represents species richness, as estimated by the Shannon index. The locations and molecules corresponding to each curve are shown on the bottom of the figure. PL: Paldang Lake; JB: Jamsil Bridge; HB: Haengju Bridge.