

Comparative vulnerability of riverine benthic and aquifer stoneflies in a large alluvial river floodplain

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10 October 2025

Montana AWRA



**FLATHEAD LAKE
BIO STATION**
UNIVERSITY OF MONTANA

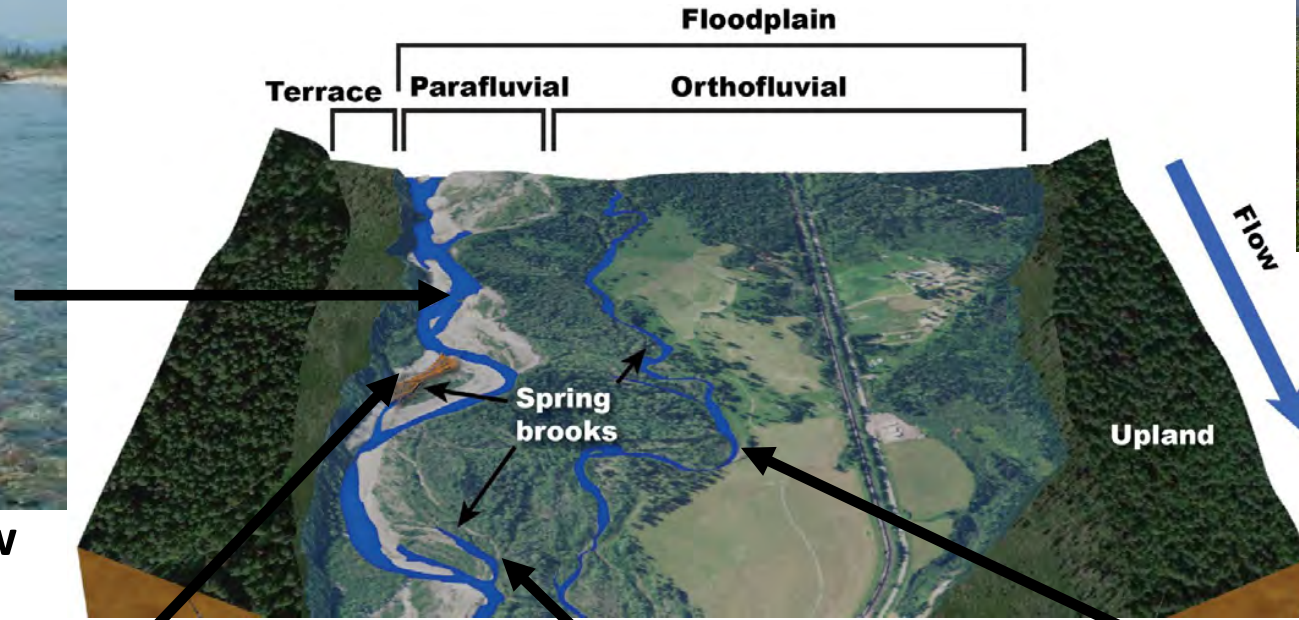




Habitat Complexity



Main channel shallow shoreline



Beaver ponds



Parafluvial ponds



Parafluvial spring brook



Orthofluvial spring brook

The Discovery - 1973



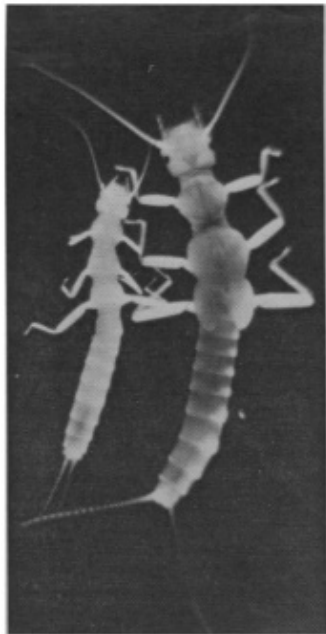
Hyporheic Communities of Two Montana Rivers

Author(s): Jack A. Stanford and Arden R. Gaufin

Source: *Science*, Aug. 23, 1974, New Series, Vol. 185, No. 4152 (Aug. 23, 1974), pp. 700-702

Published by: American Association for the Advancement of Science

Stable URL: <http://www.jstor.com/stable/1738736>



Tobacco River. The galleries are approximately 10 m long and were constructed much like a sand-gravel filter; perforated pipes surrounded by graded gravels (0.6 to 2.5 cm) were embedded horizontally at a depth of 6 m (20 feet) in floodplain gravel. The three galleries deliver water to two cylindrical concrete reservoirs, each of which resembles a well 2.5 m in diameter and 6.2 m deep. The reservoirs are connected to allow simultaneous operation of two large pumps. Water stands static in the pump reservoirs at 1.8 m below ground level, corresponding to the water table in the immediate area. After chlorination, water is pumped from the reservoirs to a large holding tank for gravity dispersal to domestic users. Very little stress is placed on the galleries when the system is pumped at capacity [5700 liters (1500 gallons) per minute], and the static level does not change. Only during periods of high use in late summer is the system pumped at capacity. The water delivered is of high quality and remains

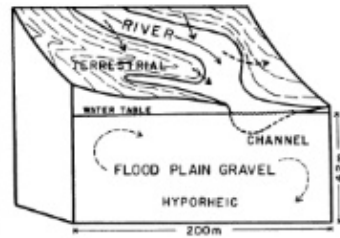
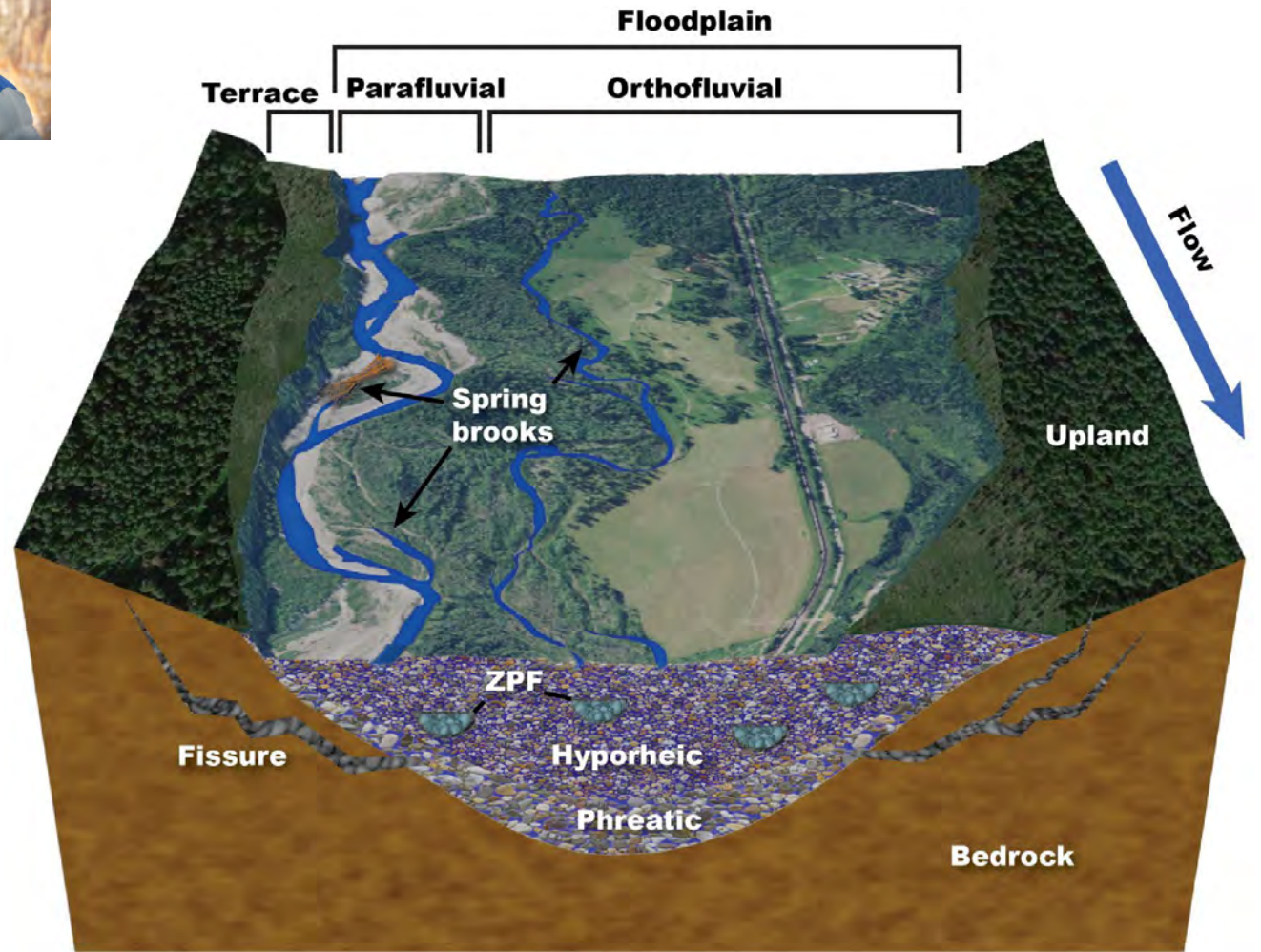


Fig. 2. Habitats utilized by stream organisms of the Flathead River. Dashed arrows indicate circulation of water from channel to hyporheic areas and vice versa. Dimensions refer to minimum floodplain width (200 m) and probable minimum vertical distribution (4.2 m) of hyporheic organisms.

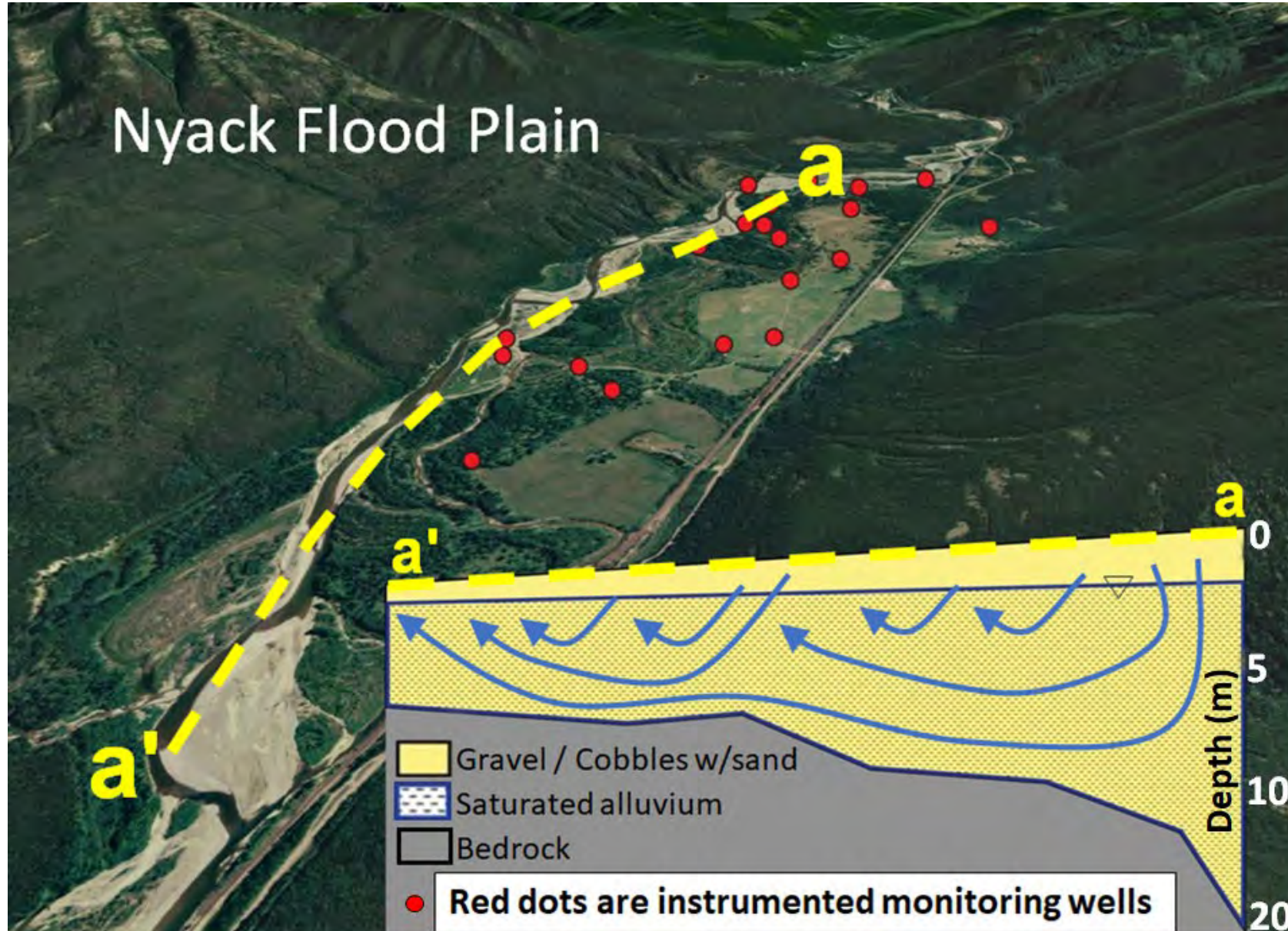
gimes, substrata, and biota are remarkably similar in both rivers. Specifically, plecopteran species compositions are quite comparable.

The galleries yielded *Paraperla frontalis* (Banks) most abundantly with as many as 100 nymphs accumulating in the nets each week (see Fig. 1). Several hundred fresh specimens could be



Discovery greatly expanded the scale of the hyporheic zone concept

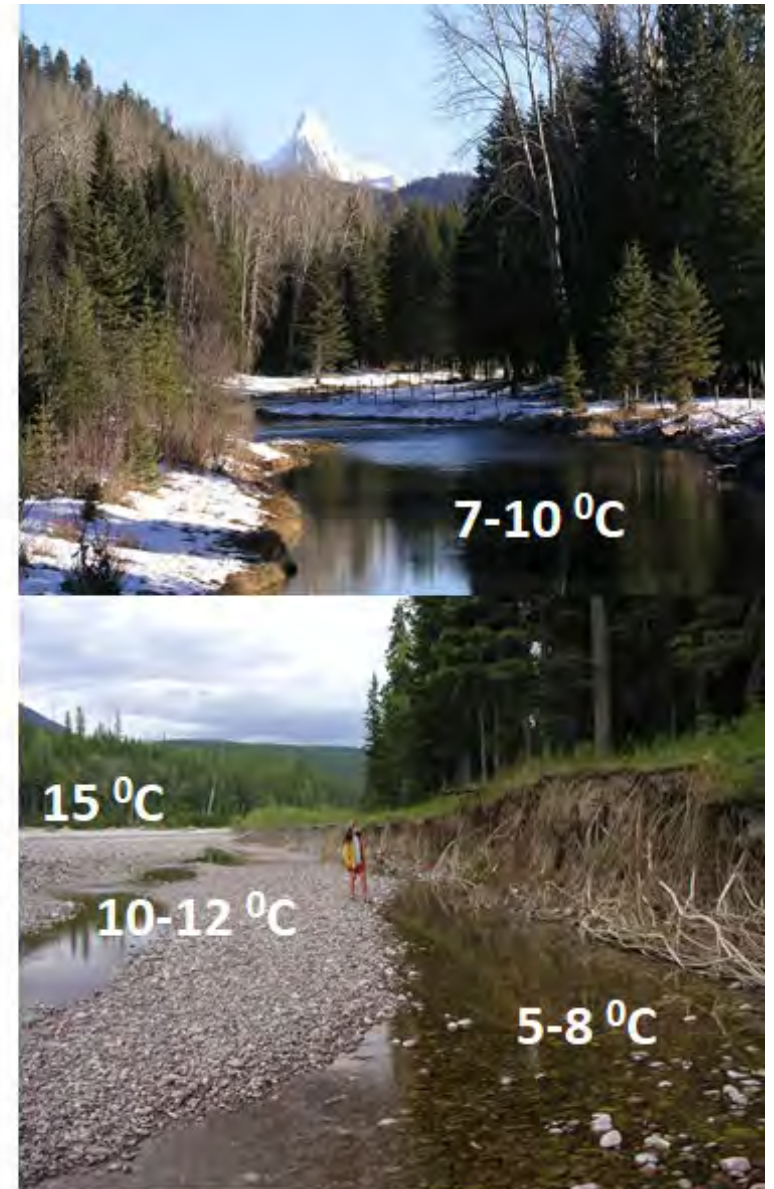
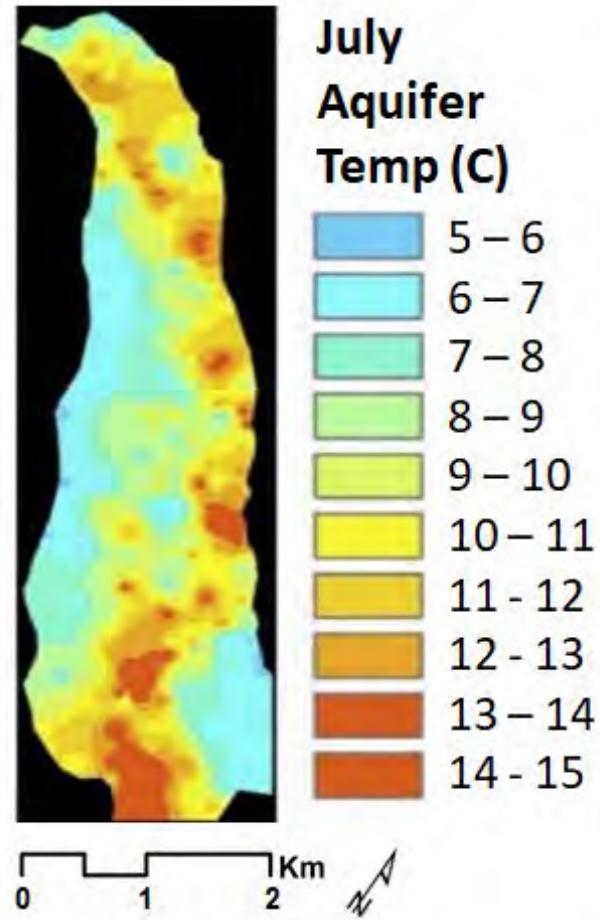
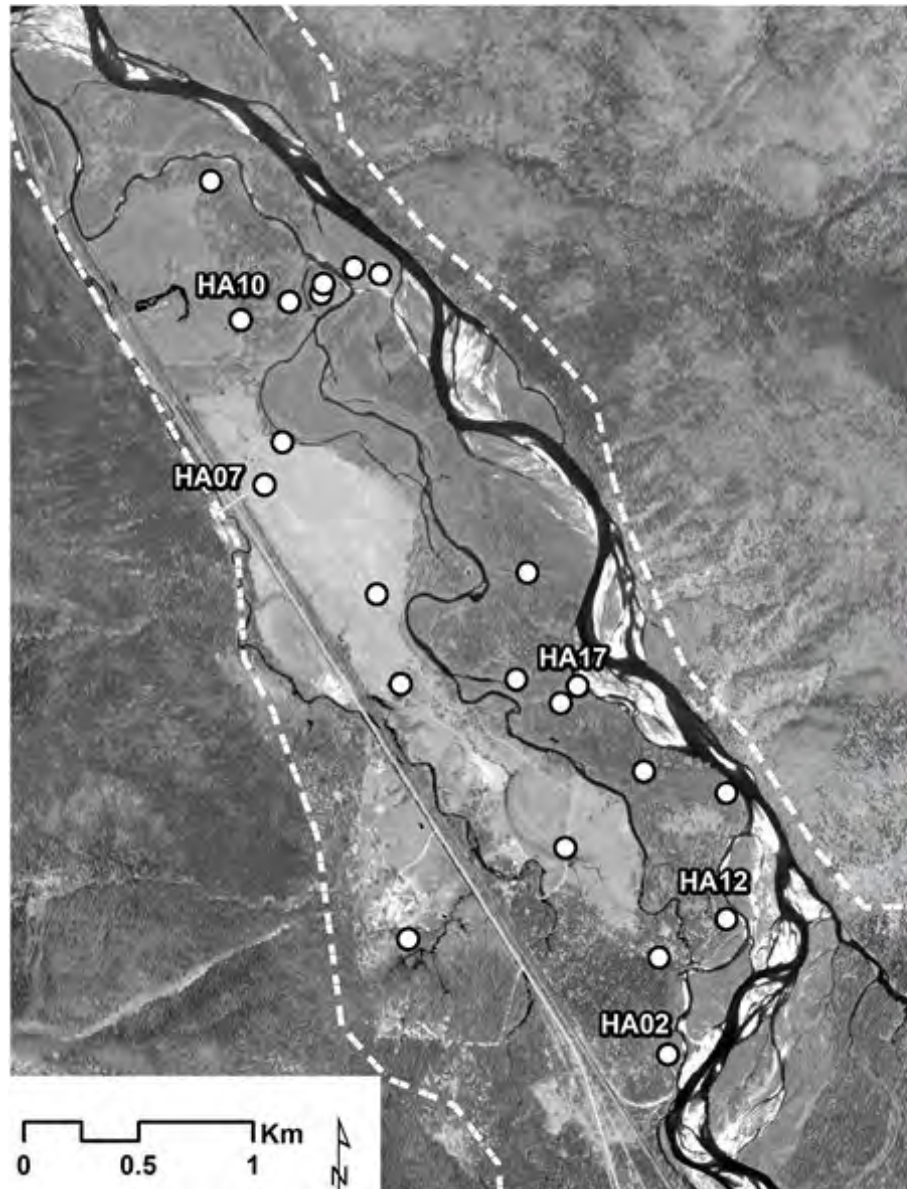
Geomorphology & Hydrology



- ~10 km long by 2 km wide
- 30% of the river water enters the aquifer
- Flow paths are highly variable

Stanford et al. 2005; Whited et al. 2007, Helton et al. 2012; Poole et al. 2006;

Temperature Patterns



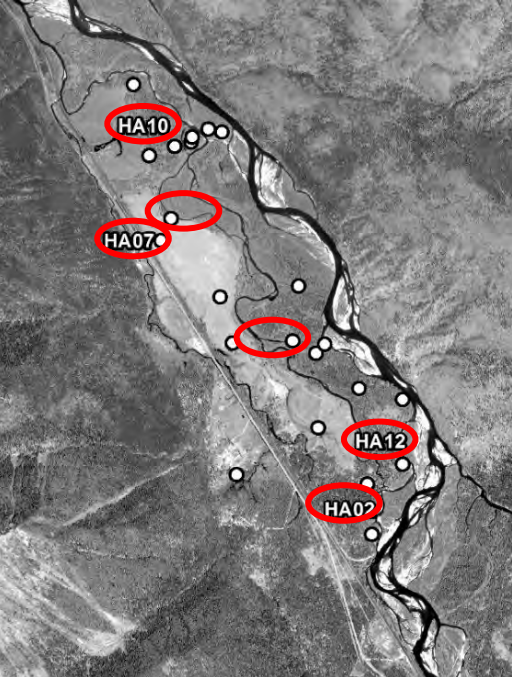
Life Cycle



- Amphibiotic: aquifer-river-terrestrial life cycle
- Nymphs must navigate the aquifer to return to the river
- Emerge from the river channel as winged adults



Field Collection Methods





- Species:
Paraperla frontalis
Kathroperla perdita
Isocapnia grandis
Isocapnia integra
Isocapnia integra
- Sampling vertical profiles can be done using baffles
- This floodplain swale is 1.2km from the river channel
- Aquifer water outwells in the spring

Over 3 Decades of Studies

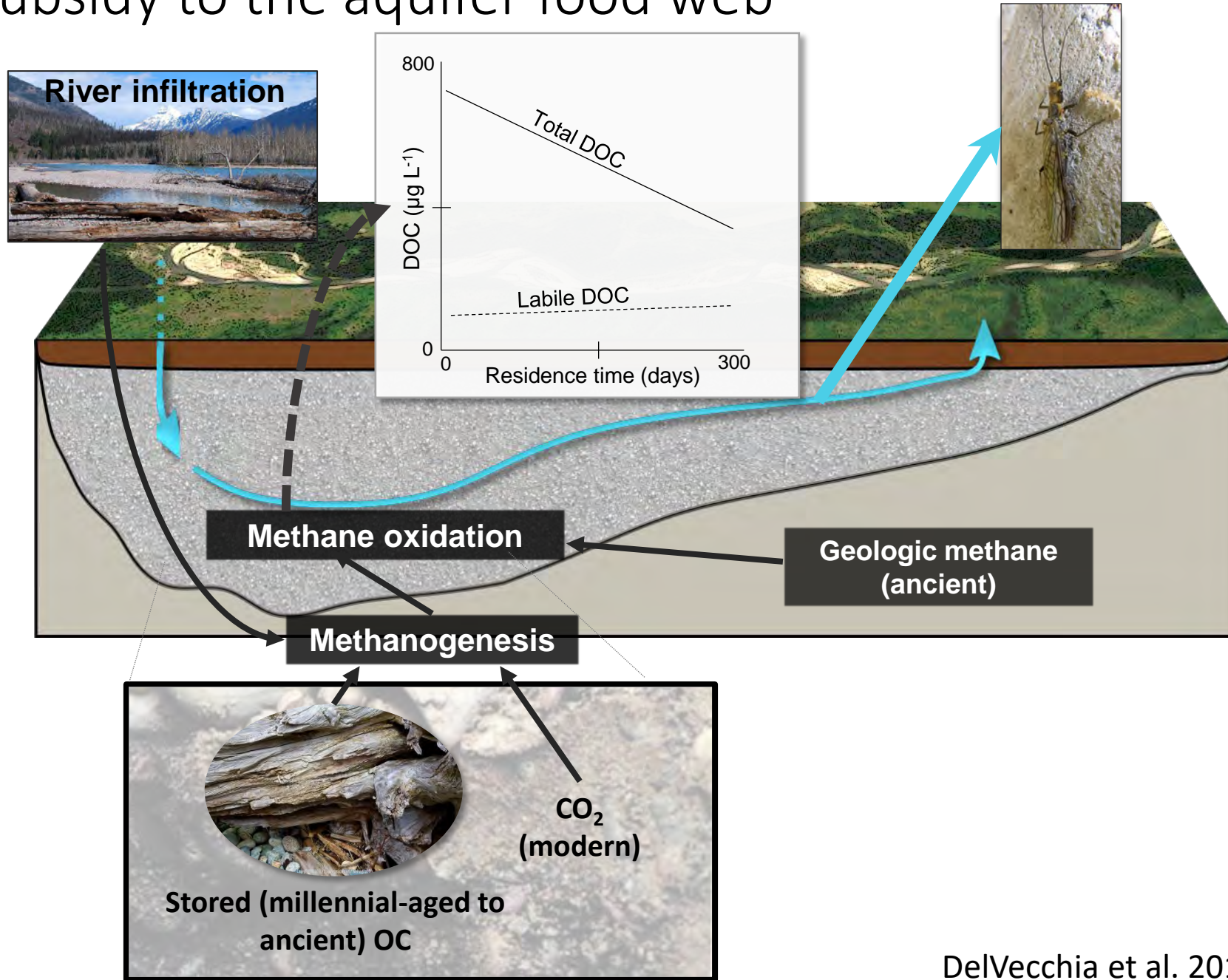
- Hydrology, temperature patterns, aquifer biogeochemistry
- Distribution and abundance of aquifer stoneflies
- Adaptations of aquifer stoneflies

How can alluvial aquifers provide enough energy to support huge numbers of aquifer stoneflies?

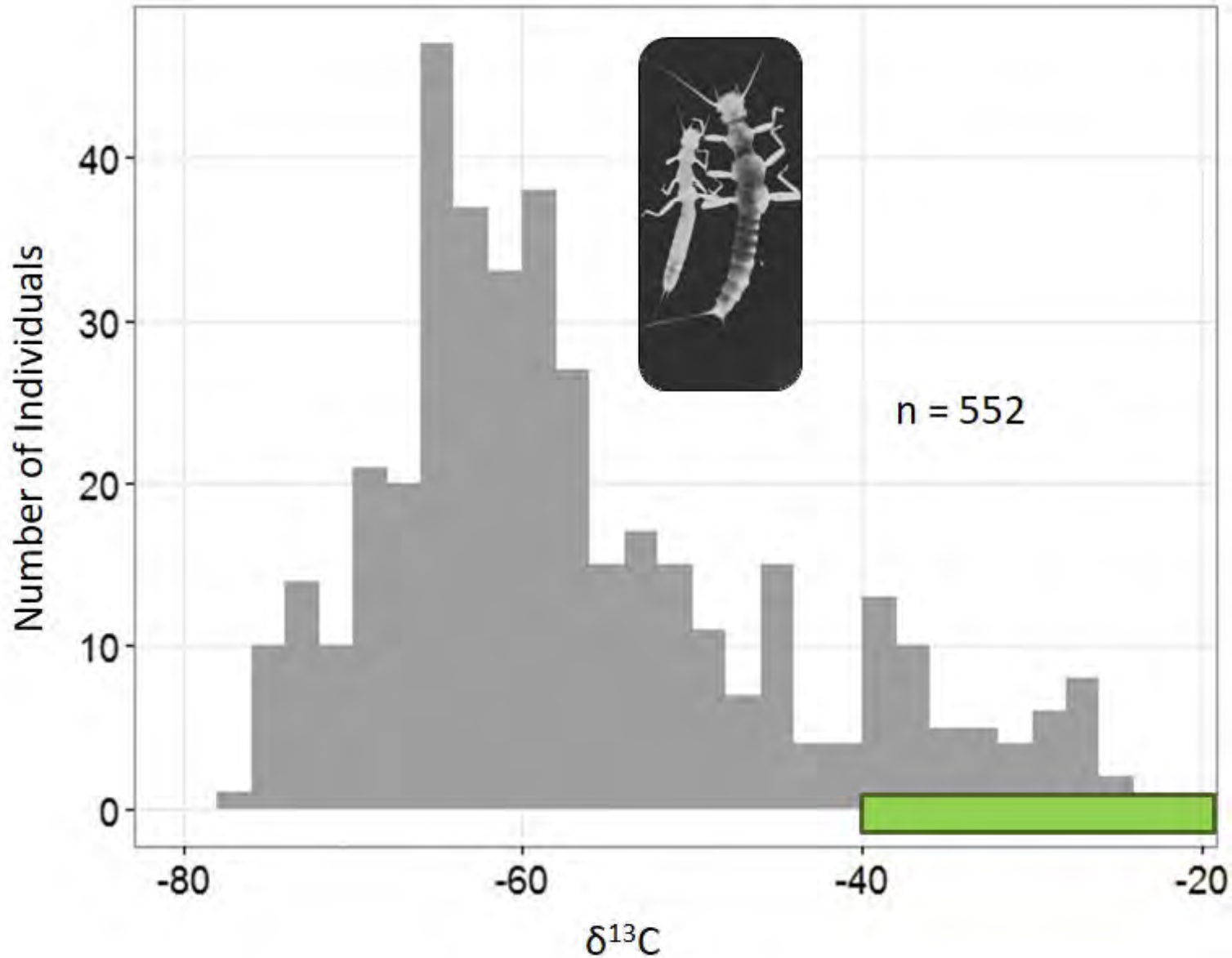
- The paradox of the hyporheos – low microbial production and DOC



Methane subsidy to the aquifer food web



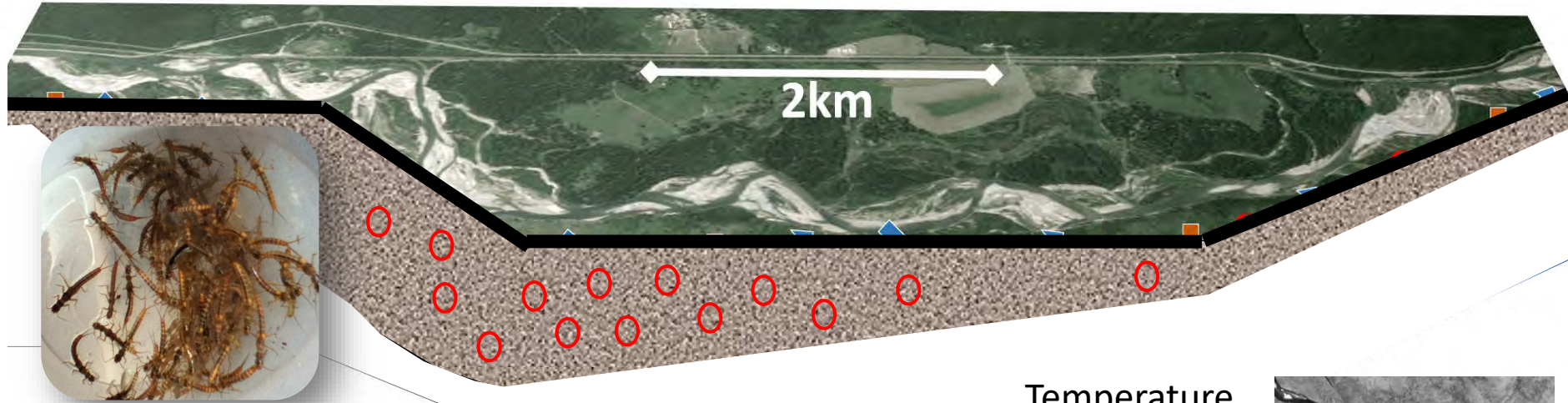
Biomass incorporated into the aquifer food web



- Aquifer stonefly tissues substantially depleted

Overarching Questions

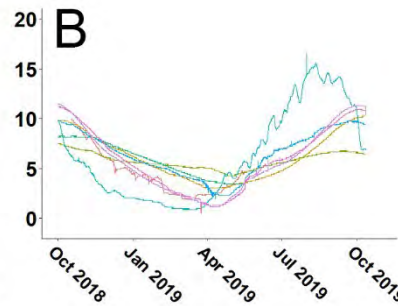
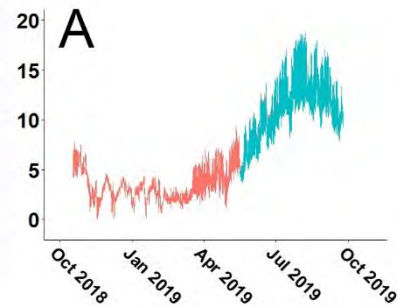
How are aquifer stoneflies adapted to live in this unique environment and how vulnerable are they to climate change?



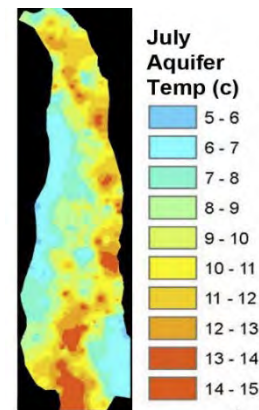
Low Oxygen Tolerance?

Mechanisms underlying tolerance?

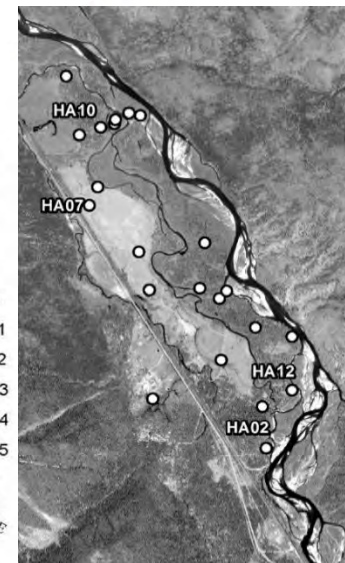
Ability to Forage?



Temperature



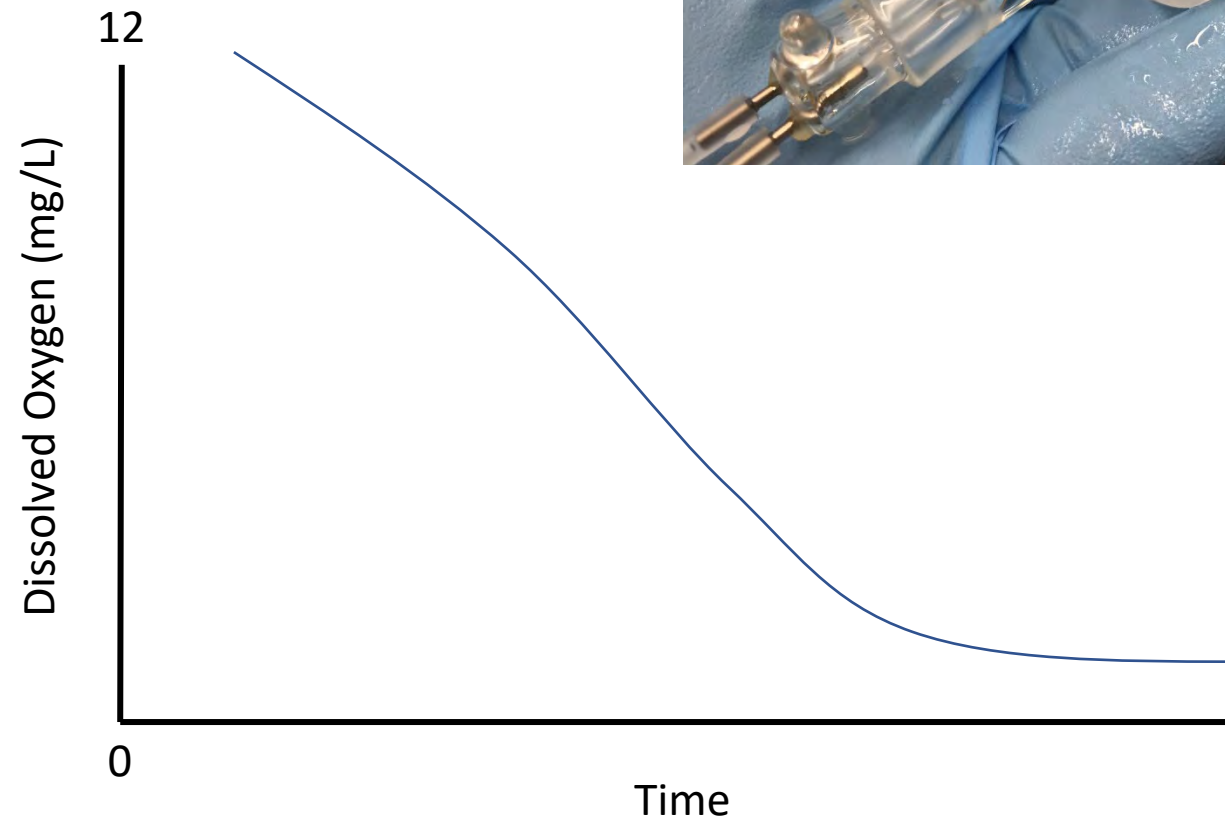
0 0.5 1 2 Km



Metabolic Rate Methods



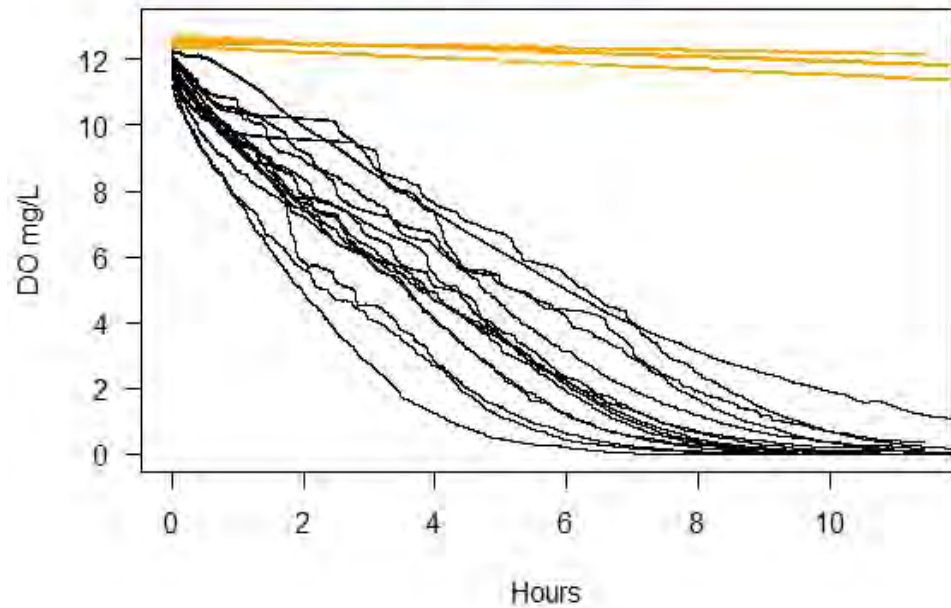
Metabolic Rate Methods



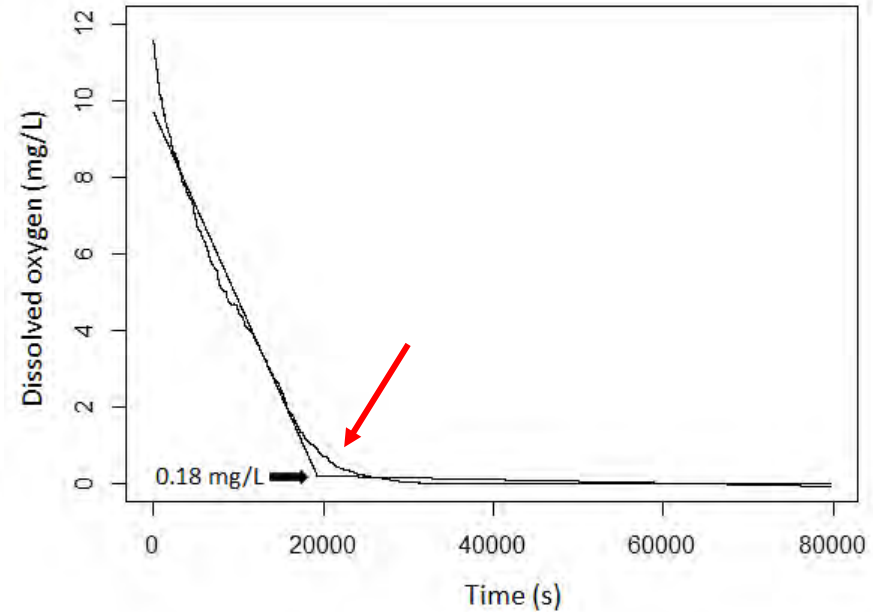
Metabolic Rate Methods

Aquifer – *P. frontalis*

Oxygen depletion curves



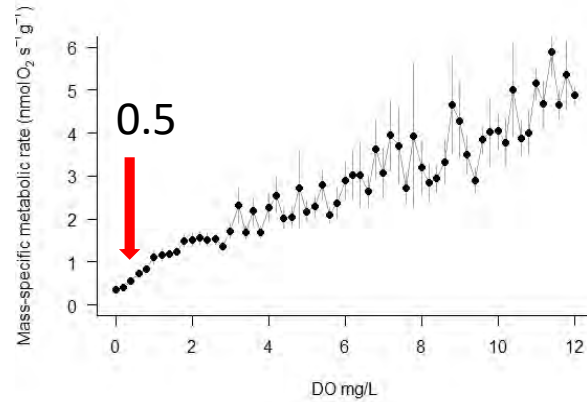
- Mass specific metabolic rates



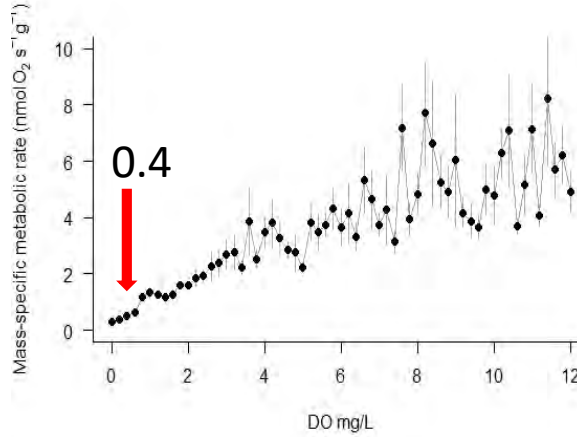
- Break points

Metabolic Rate Results

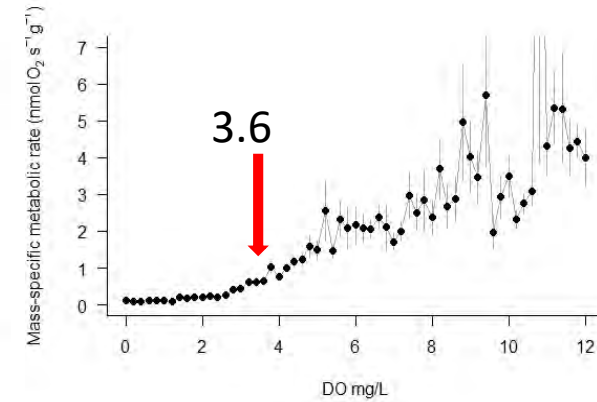
Aquifer – *P. frontalis*



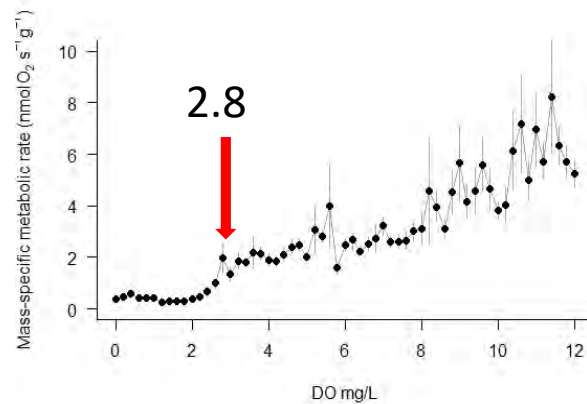
Aquifer – *Isocapnia*



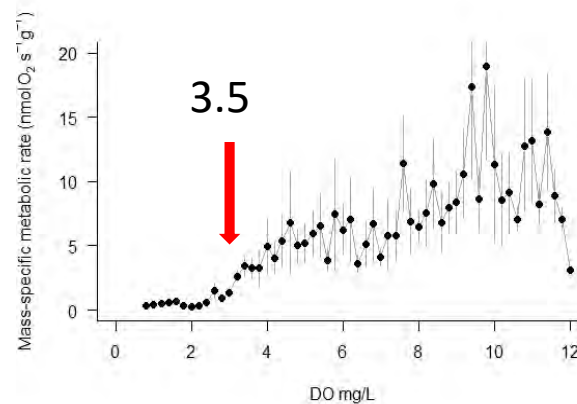
Benthic – *P. californica*



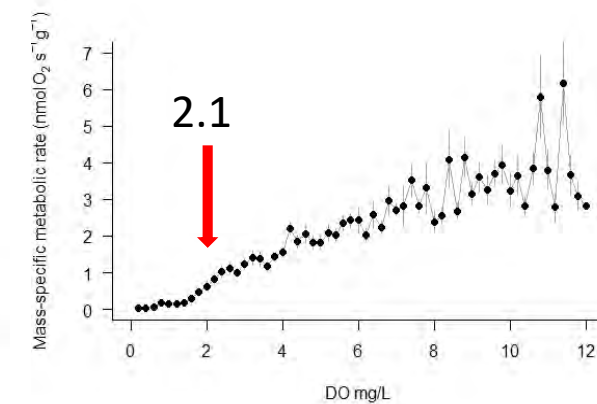
Benthic – *P. badia*



Benthic – *C. sabulosa*

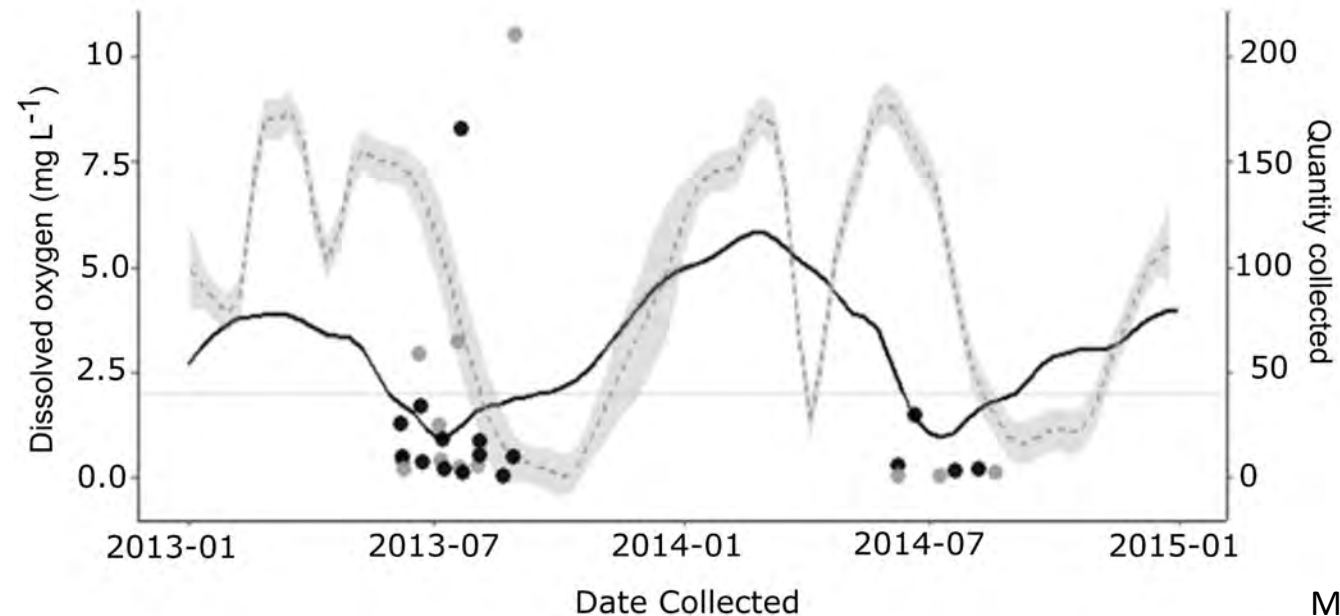
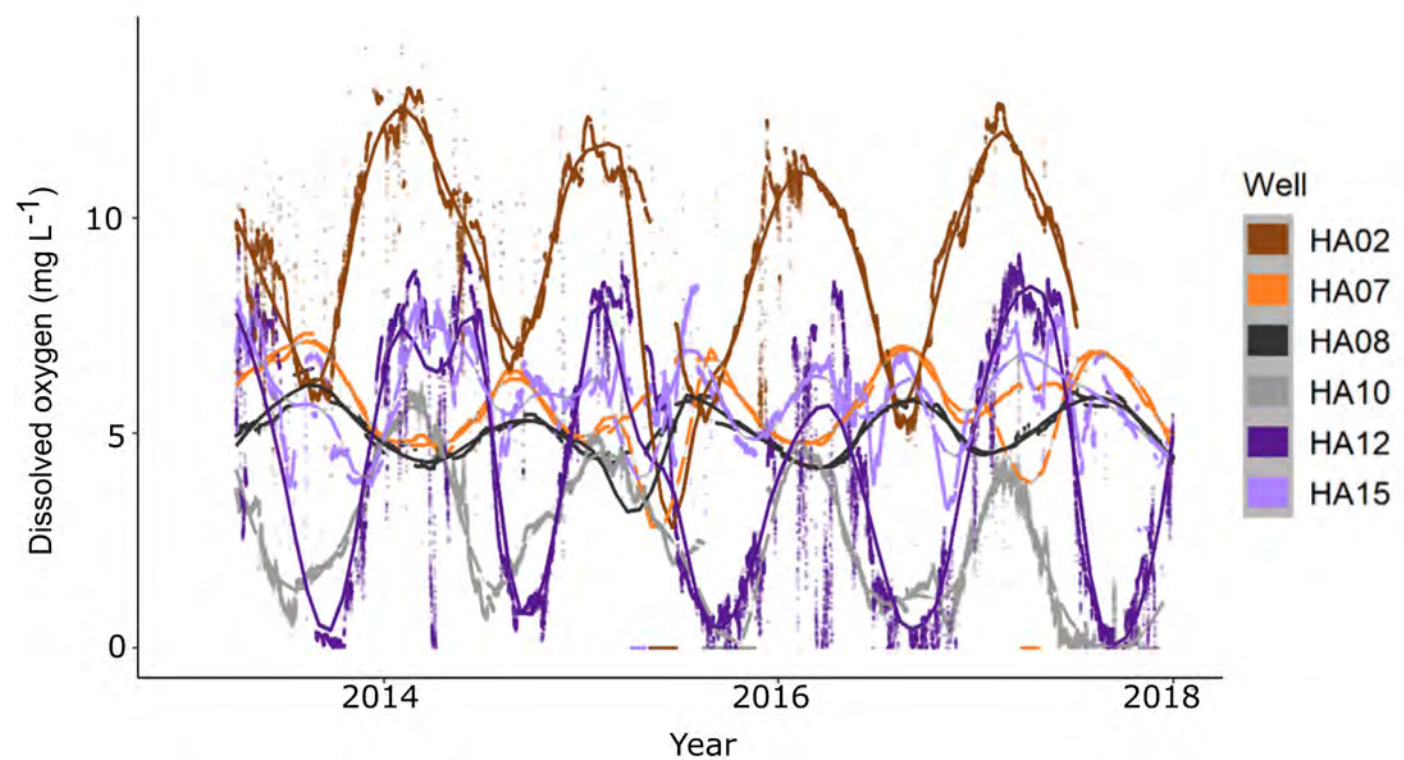


Benthic – *H. pacifica*



Aquifer Oxygen Dynamics and Stonefly Tolerance to Hypoxia

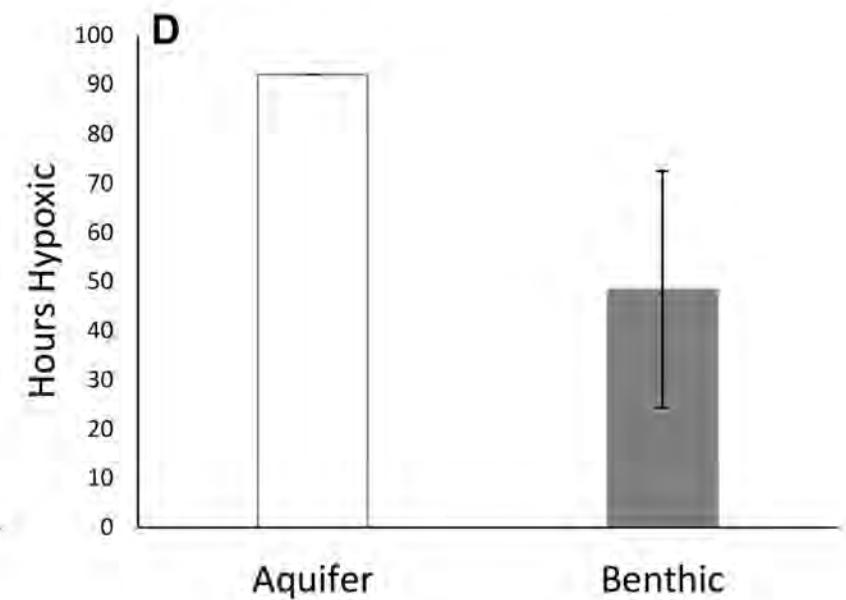
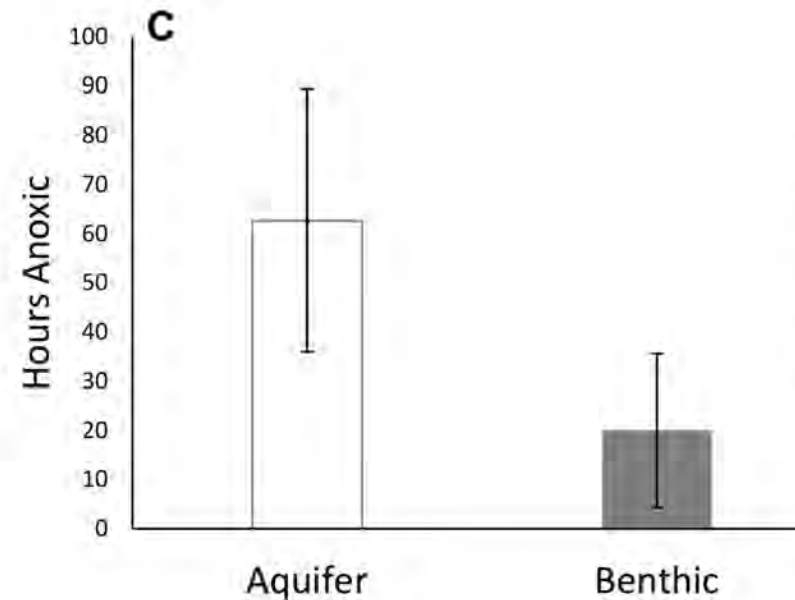
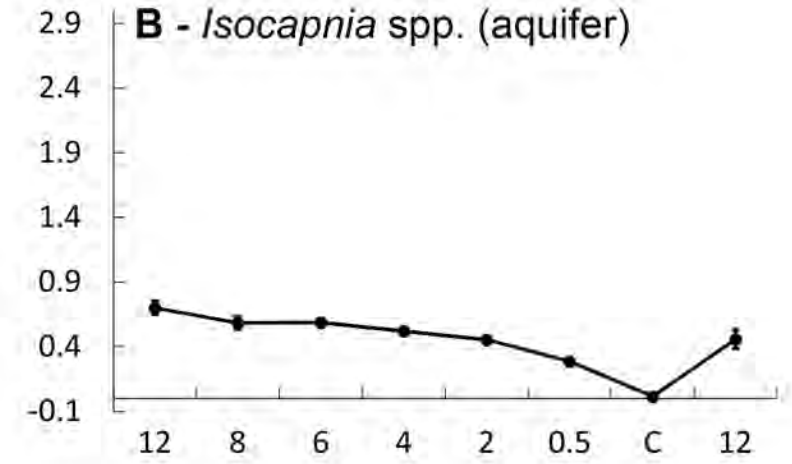
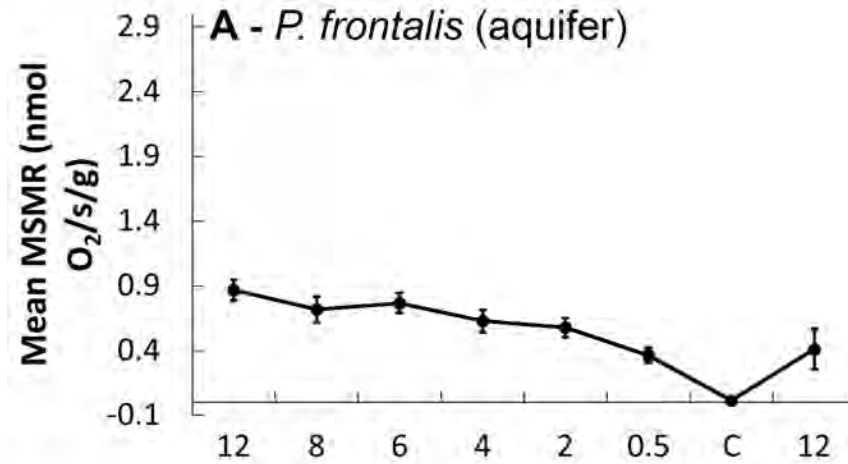
- Aquifer is “mostly” well oxygenated
- Stoneflies routinely collected from low oxygen wells



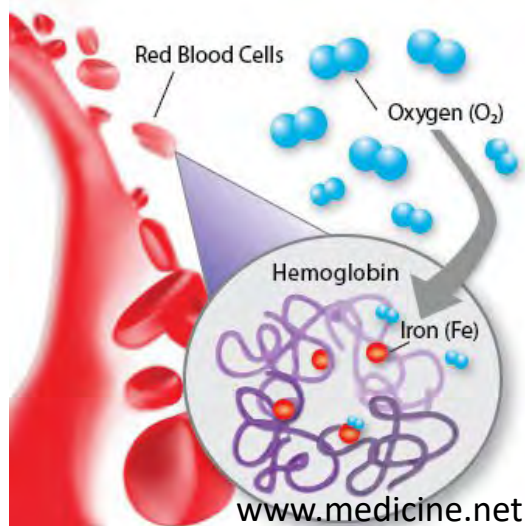
Aquifer stoneflies are adapted to anoxia & hypoxia



- Survival and metabolic rates studies
- Stoneflies can survive and continue moving for days in hypoxia & anoxia
- Stoneflies are able to tolerate repeated exposure to hypoxia and anoxia



Respiratory Protein?



Blood's Rainbow

Blood's color depends on the protein it uses to carry oxygen

Hemoglobin	Hemerythrin	Hemocyanin	Chlorocruorin
<p>Most mammals, birds, reptiles, amphibians and fish have red blood. The color comes from an iron ion in heme within hemoglobin. Fair-skinned people appear to have blue blood in their veins, but it's an optical illusion based on the properties of light.</p>	<p>Leeches, earthworms, and flatworms have violet blood. Hemerythrin needs two iron ions to capture an oxygen molecule (hemoglobin uses one). Also, despite its name, hemerythrin does not contain heme.</p>	<p>Most spiders, crustaceans, snails, slugs, octopuses and squid have blue blood. It relies on copper, rather than iron, to carry oxygen. The blood of horseshoe crabs is used to test for bacterial contamination in injected medicines.</p>	<p>Marine worms shaped like Christmas trees, feather dusters or lipstick tubes have green blood. They use chlorocruorin, which is similar to hemoglobin but with less oxygen-binding power. It floats freely in the bloodstream rather than existing within blood cells.</p>

www.nigms.nih.gov

Brittle Star



www.nespmarine.edu.au

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A respiratory hemocyanin from an insect

Silke Hagner-Holler, Axel Schoen, Wolfgang Erker, James H. Marden, Rainer Rupprecht, Heinz Decker, and Thorsten Burmester

PNAS January 20, 2004 101 (3) 871-874. <https://doi.org/10.1073/pnas.0305872101>

Edited by John H. Law, University of Arizona, Tucson, AZ, and approved November 14, 2003 (received for review September 12, 2003)

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Perla marginata - Italy



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Hemocyanin genes detected!

Paraperla frontalis
Isocapnia grandis
Kathroperla perdita

Black highlights =
 Oxygen binding sites

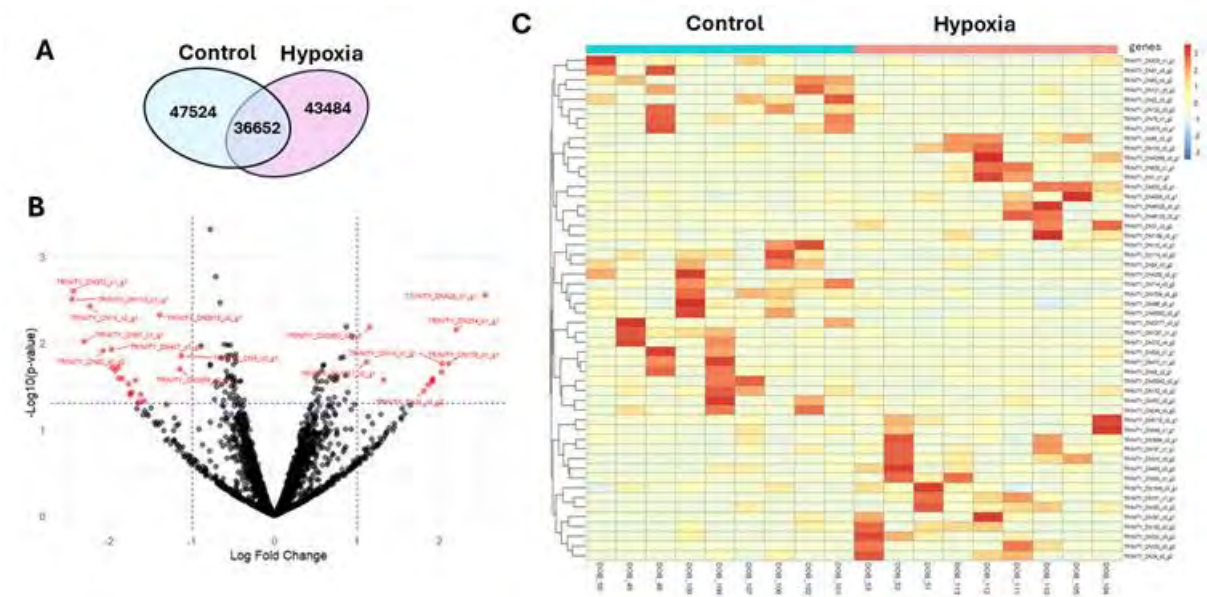
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Per.hc1	VAYLGEDVGLNS	HHSHWHMDF	PFWWKAAEY	GIEKDRKG	ELFFYMHHQ	MIARYDFER	LSNW
Per.hc2	VAYLGEDIGVMS	HHAHWHMDF	FFFWWKRT.	YDVTKDRR	ELFFYMHHQ	MVSRFDAER	LSNN
KP-3	CRCAGEDGRKNS	HHAHWHMDF	PSISKRALR	QTVFHYLR	LFFYMHHQ	PIRWRESY	PEPPN
KP-11	CRCAGEDKYENS	HHAHWHMDF	PSISSRRA	LQTVKDYRL	LSFFYMHHQ	SASFNIMIC	WWTYW
KP-13	CRCAGEDKYENS	HHAHWHMDF	PSISSRRA	LQYLDVYRL	LSFFYMHHQ	YGGNRTQ	NLRIR
IG-650	FLEYHSDNELSS	HHAHWHMDF	PLLRRGG	NKNTMDG	PTELFFYMHHQ	VIKPFQDR	ID
IG-427	FLETHSPNELSS	HHAHWHMDF	PLLRRGD	GVNYDIL	AKVLELFFYMHHQ	SYSNENCS	VKEKLL
IG-467	FLEYGSDNEVSS	HHAHWHMDF	PLLRRGK	GYGWPPH	ITGELFFYMHHQ	SYSNENCC	YCENH
PF-1981	VCRS.ETSGANI	HHAHWHMDF	PVSSSFG	FGVKSFS	SPSFI	ELFFYMHHQ	LOSVRW
PF-1995	VCRS.GTSLATI	HHAHWHMDF	PVENMFK	FGHRRF	PELGE	ELFFYMHHQ	FTATTVE
PF-2227	VCRL.GTSLATI	HHAHWHMDF	PVGNKMF	GGHRRF	PELGE	ELFFYMHHQ	FTARTVE
PF-2230	VARL.ETSLATI	HHAHWHMDF	PVGTVCR	SEISLAI	TDF	ELFFYMHHQ	NYDLML

	70	80	90	100	110
Per.hc1	LHFVEPISFE..	DEIEHG	FYPQTTY	RVGGFE	FPARPDN
Per.hc2	LPQVEPLDWH..	HEIVEG	FAPGAMY	HNGQEF	PMRPDGM
KP-3	KKSG.QSAPW..	GKNHQ	NT.RYIIL	FLFLFLY	GIRMQQY
KP-11	KKSWLQSVRW..	RKKLQ	NITFH	ASTYMR	SSGKCSS
KP-13	KKSSLQPCGK..	ETPNT.	RMHYF	VERSWE	VQ.ECNI
IG-650	NFCGSYSWF	MGPCY	RMTEF	MLDIES	LRLLT
IG-427	NFCCSYRTE	KGFYFF	ASPVG	IPDKNA	QAKARP
IG-467	NFCCSILISE	KGFN	AGELH	MPRGFL	FLCFTR
PF-1981	RIRKVVSSQP..	RGERT	IRIHND	TLFCF	SCFMEE
PF-1995	EKWLA	VSPVG..	KEPSE	YITIH	YFVFD
PF-2227	KKSG.QSALW..	ERNTE	YITHA	LF	CF
PF-2230	MEIFAVSSVE..	KEITE	YNVSC	INVH	AILGEV

	120	130	140	150	160	170
Per.hc1	AILKHSVLT	KTGEH	IALDNE	HGIDIL	GLDMEP	SMETLH
Per.hc2	IIASGFVK	MTDG	HLVYLN	TTEGID	ILGLIV	ETLDH
KP-3	FHASTYMR	SSGK	CSSAL.	DL	SGYA	AMRRR
KP-11	FHRYIIL	RSFLY	GIRML.	DQ	QYMC	WLSL
KP-13	FHFYQIT	YRVGY	GFPMR.	PD	NYF	HWHDL
IG-650	TAHESQ	GFSIS	LLHPL	AYL	TRR	HKKQ
IG-427	TAIPGV	GFYIP	DKNA	QKYL	SKRP	VAQH
IG-467	TAQCSQ	FFLTS	YIHPL	AYP	DKNA	KKQ
PF-1981	CFSILG	SAVR	MQQY	CMWL	WLSL	TPFG
PF-1995	VHAILG	SEVQ	QRLV	GLVLR	VRCY	ASAG
PF-2227	FVFILG	SDP	VFIW	NKNA	ILYVA	LVPHA
PF-2230	FIKILG	SGGY	IIGFR	NHAG	LYSAL	LRHKS

Potential mechanisms

- Specialized abilities to extract oxygen from water given higher MR at low oxygen
 - Differential gene expression?

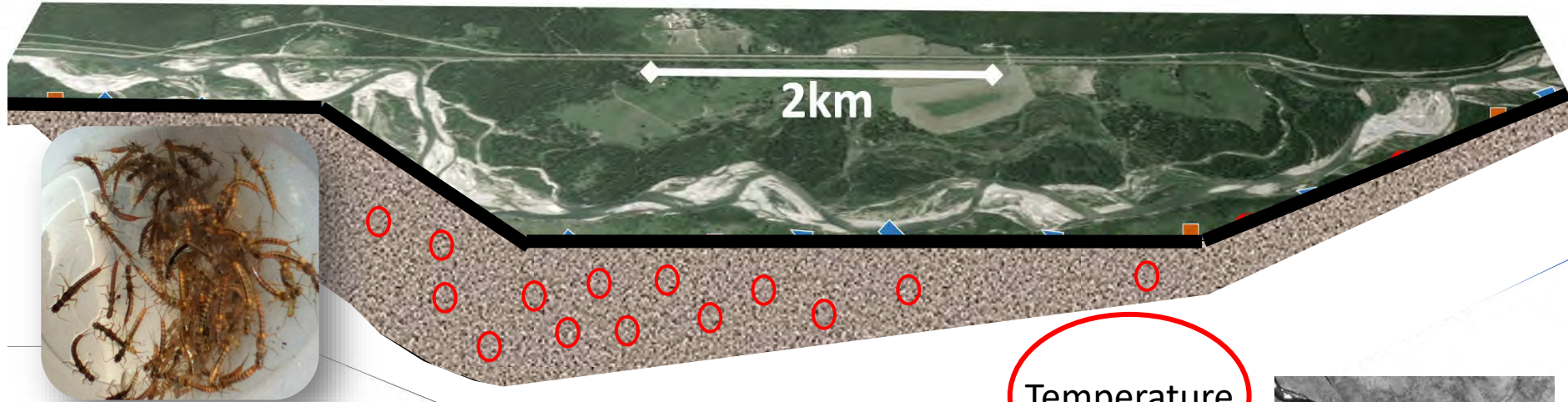


32 genes differentially expressed!

- Removed pairs: 0 min, 5 min, 1 hr

Overarching Questions

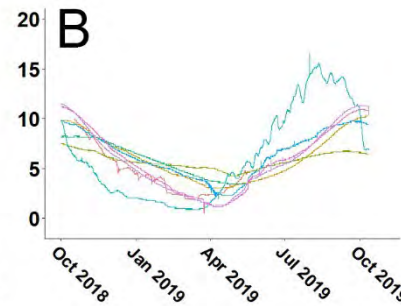
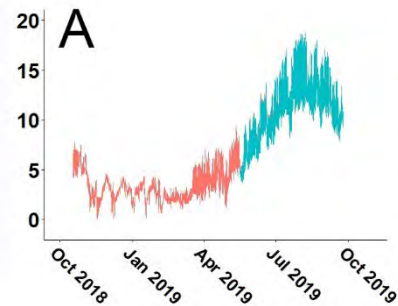
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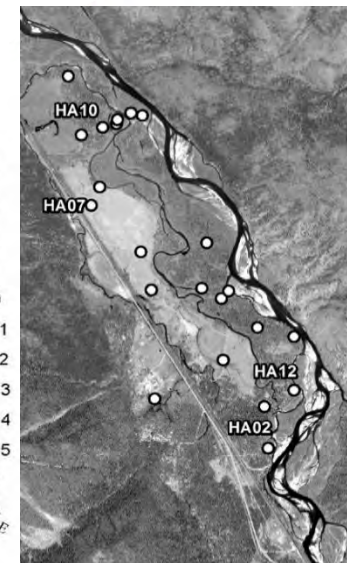
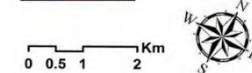
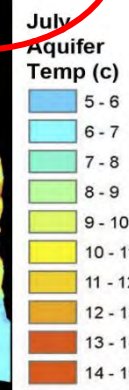
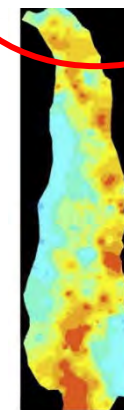
Low Oxygen Tolerance?

Mechanisms underlying tolerance?

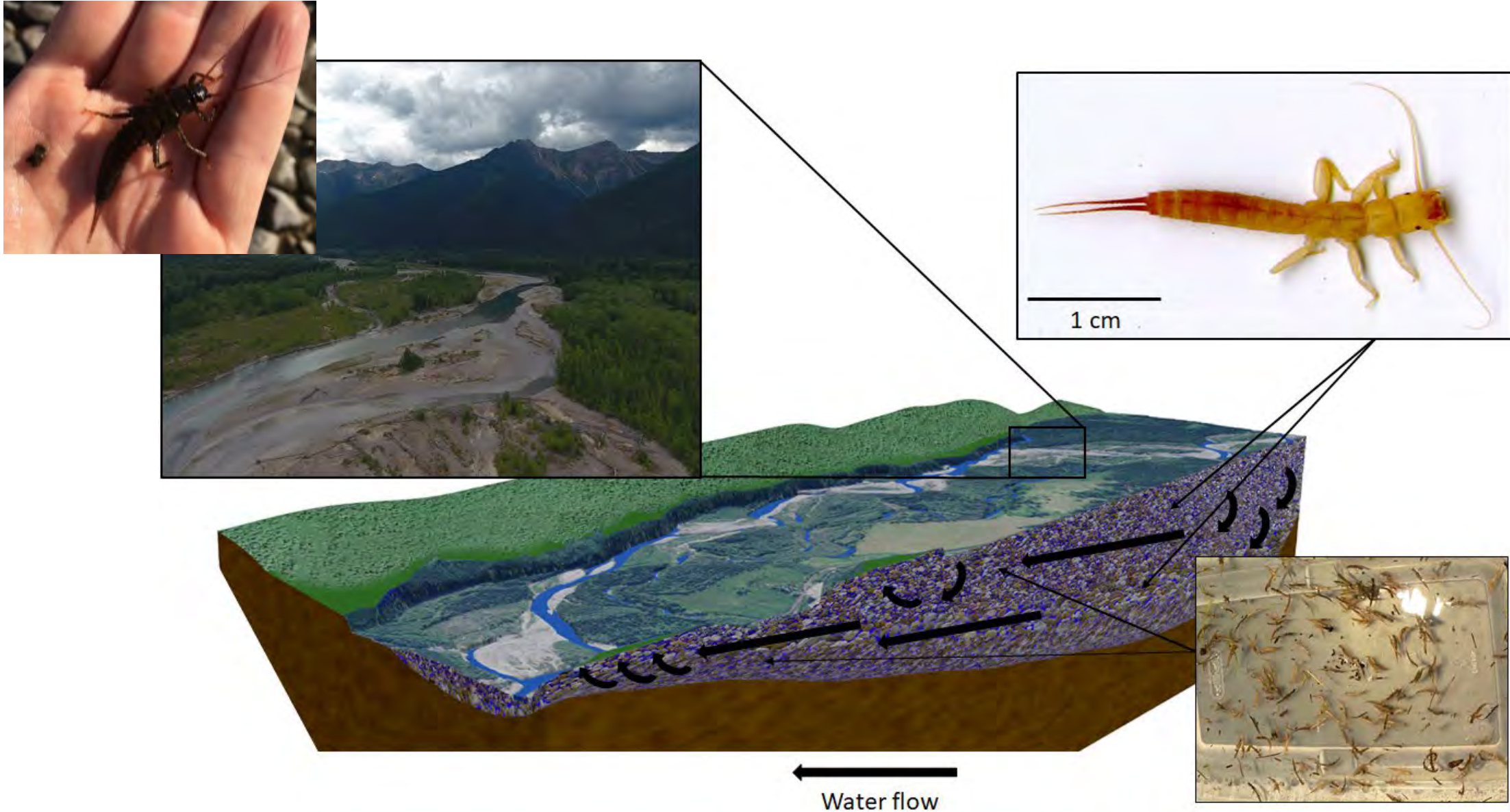
Ability to Forage?

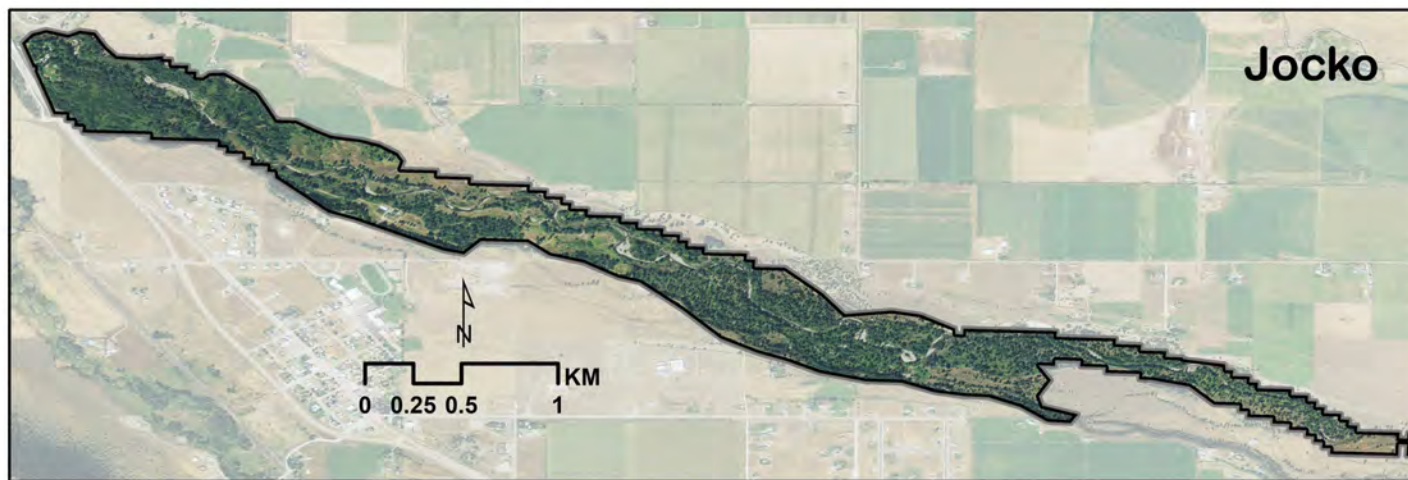
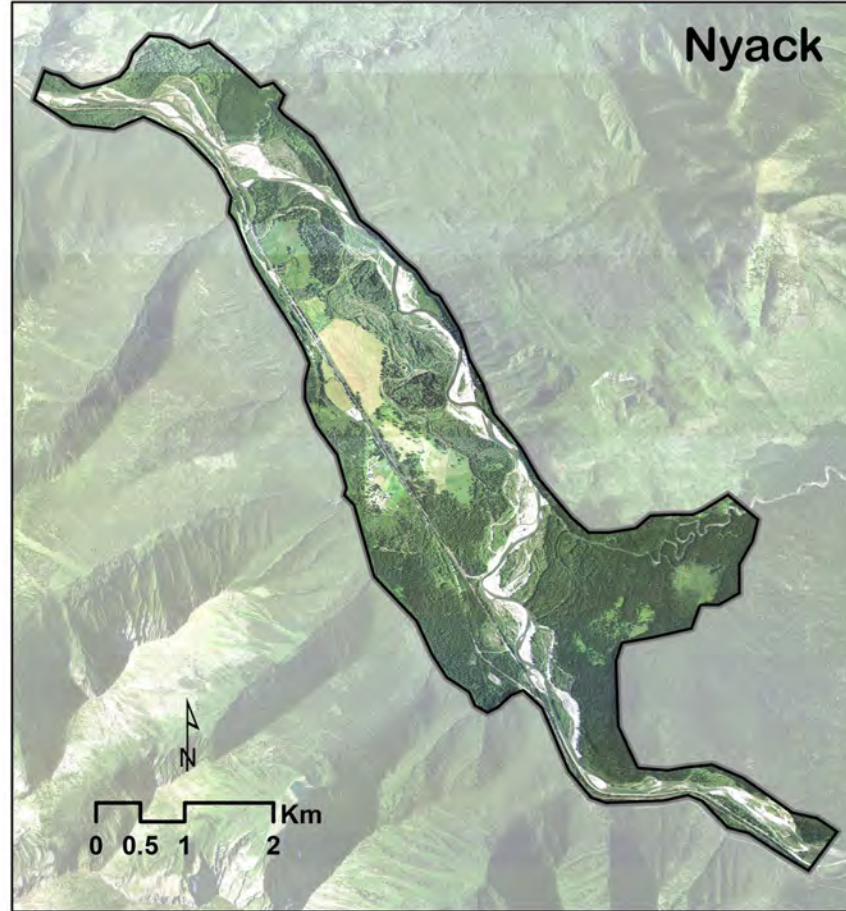
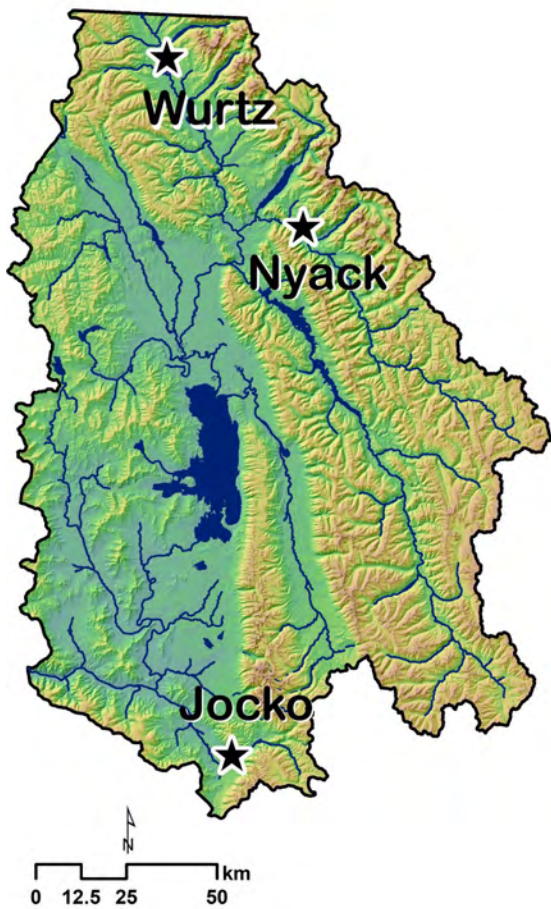


Temperature



Prediction: Benthic stoneflies would be less sensitive to warming temperatures compared to aquifer taxa





Methods:

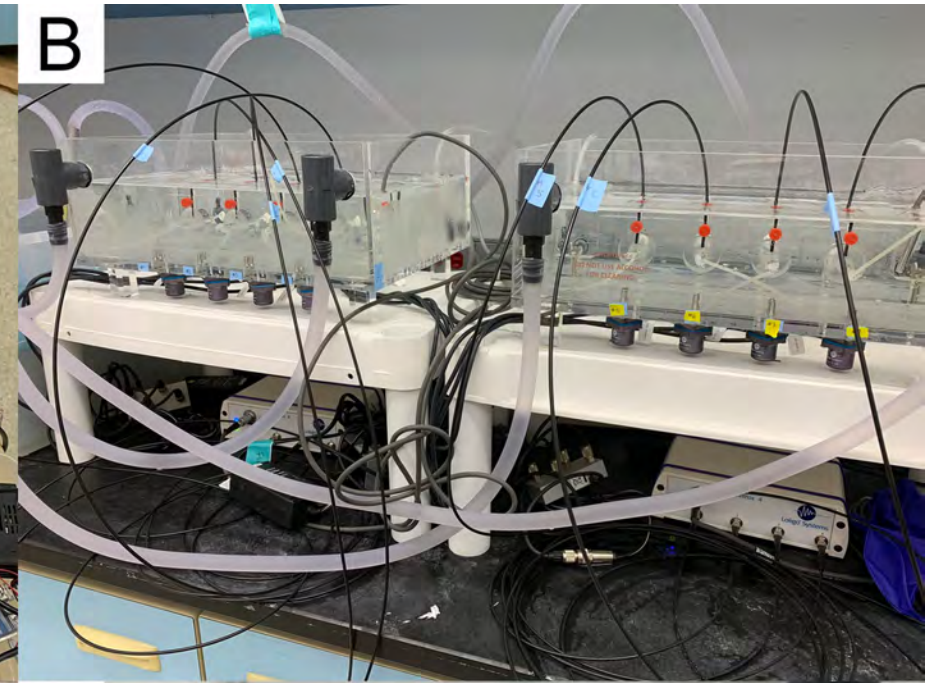
- Temperature data
- CT_{MAX}
- Metabolic rates

Benthic:

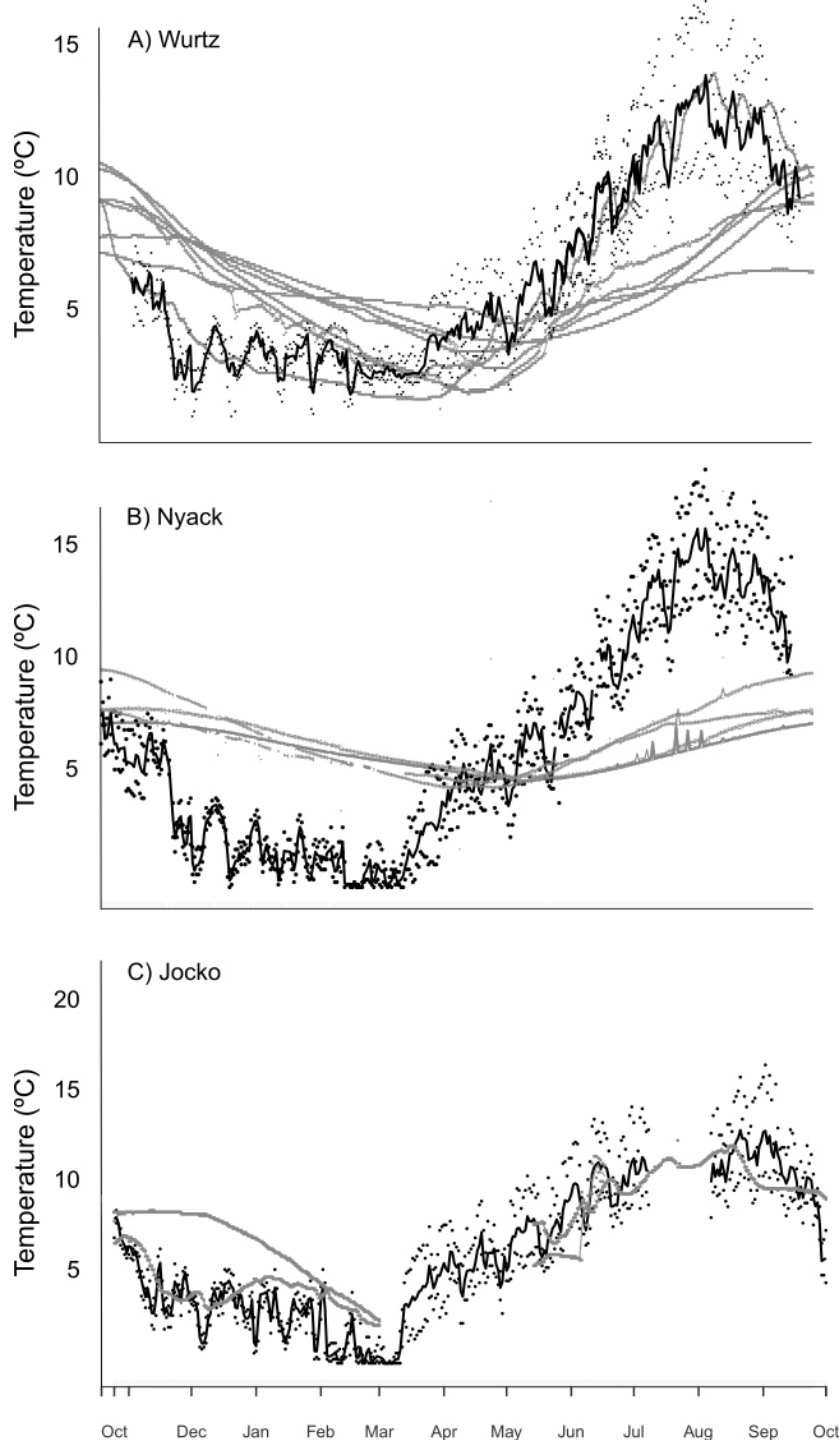
Claassenia sabulosa
Hesperoperla pacifica
Pteronarcys californica
Pteronarcella badia
Sweltsa coloradensis

Aquifer:

Isocapnia spp.
Paraperla frontalis
Kathroperla perdita



Results:

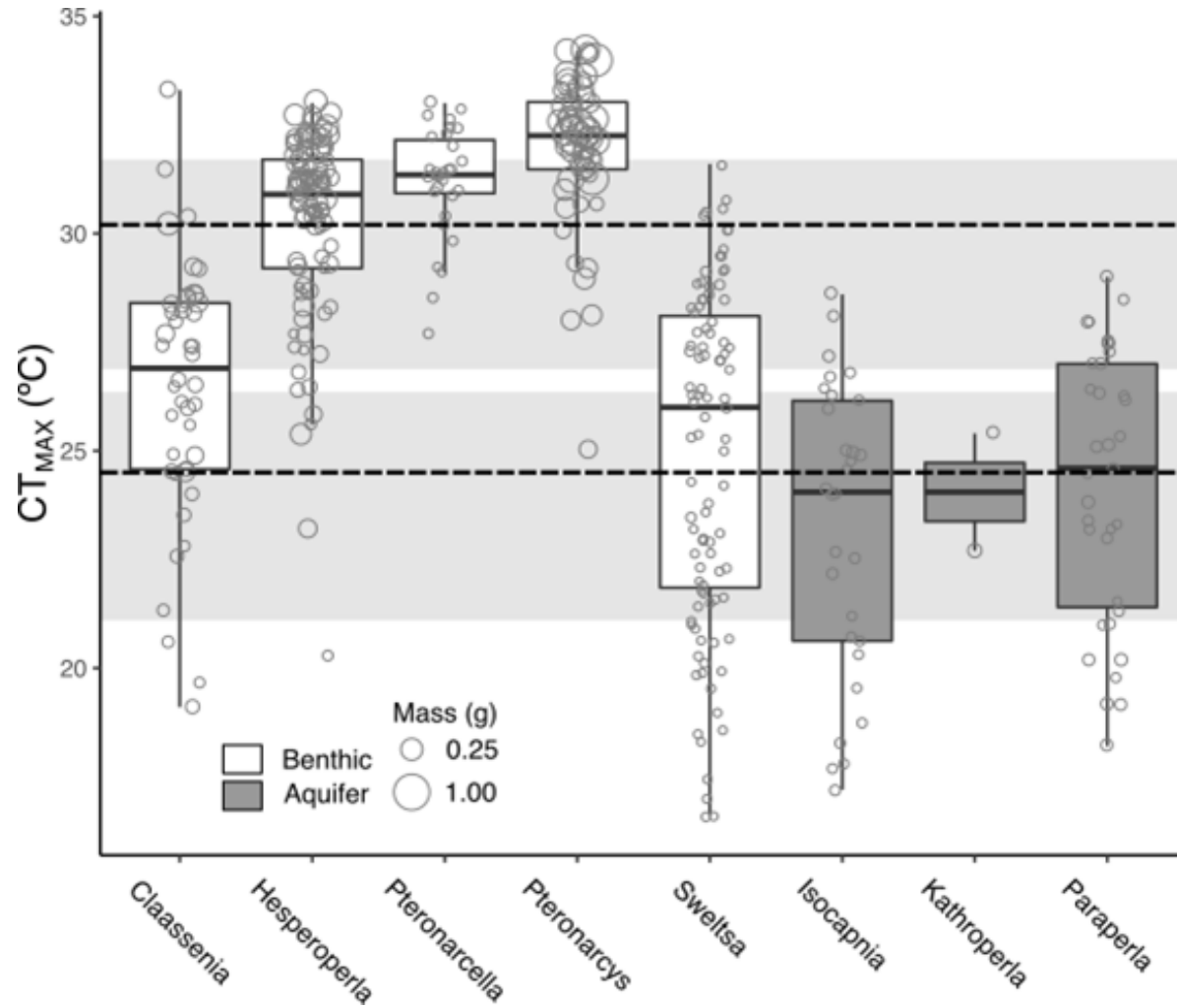


- Mean daily river temperature
- Mean daily aquifer temperature

Mean temperature was similar for aquifer and benthic habitats at the Nyack and Jocko, but was warmer in benthic vs. aquifer habitats at Wurtz

Benthic habitats were much more variable

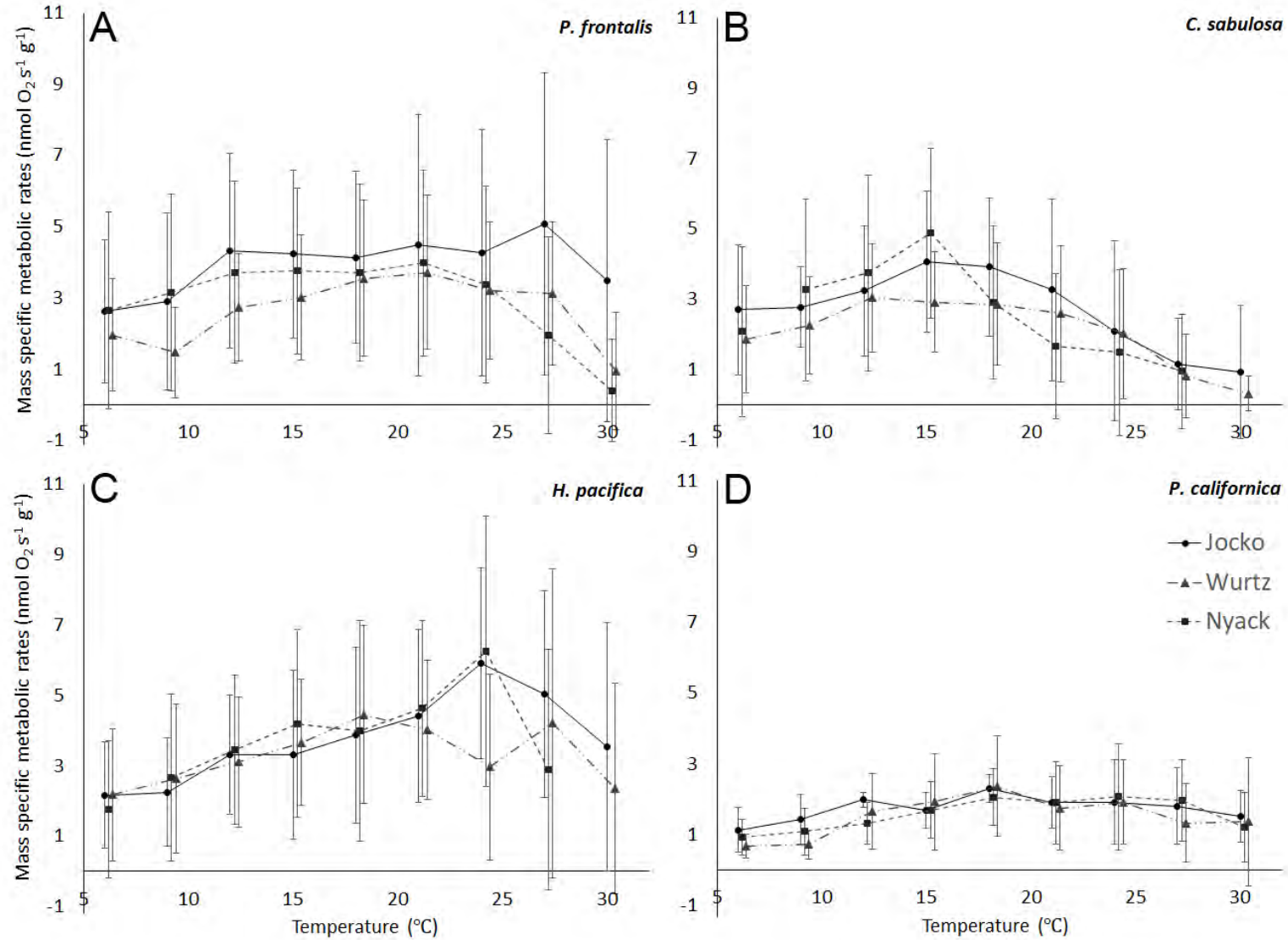
Results:



Benthic stoneflies had significantly higher CT_{MAX} values ($F = 6.68$, $P = 0.021$)

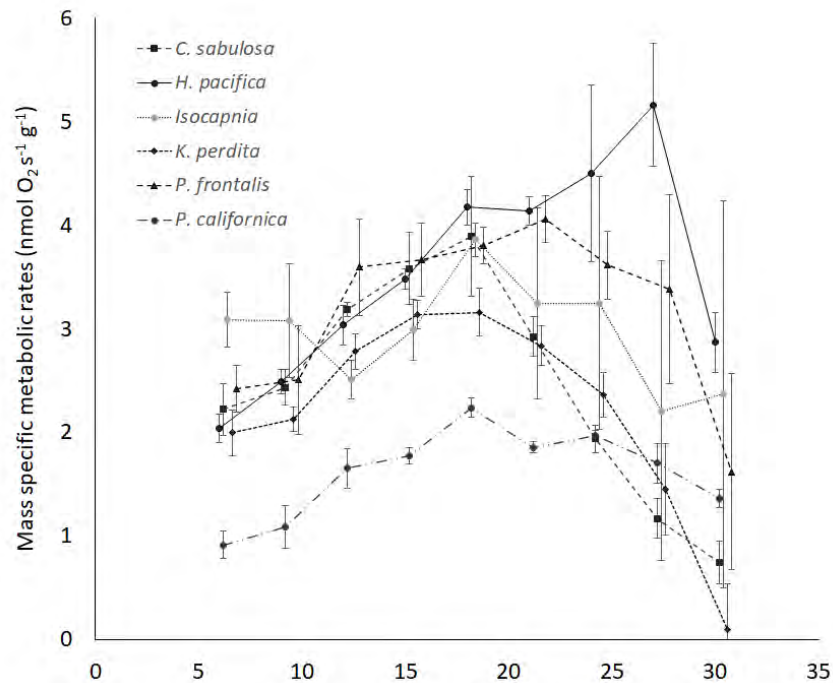
Also significant differences between genera and mass was significant

Results:



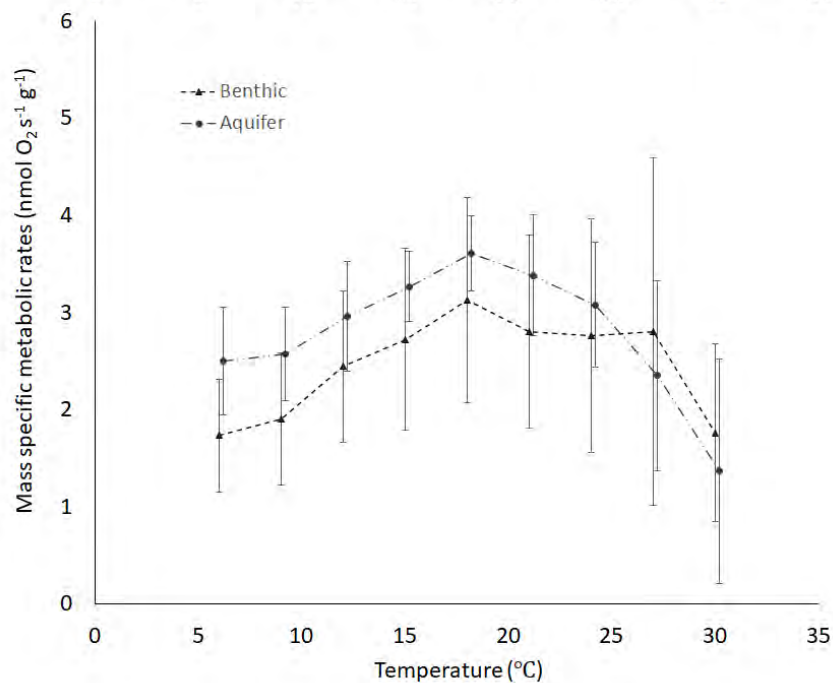
No difference in metabolic rates among floodplains ($F = 1.78$, $P = 0.171$)

Results:



No difference in metabolic rates between habitats ($F = 2.24$, $P = 0.194$)

But benthic stoneflies had a greater metabolic breath (difference between starting and max MR)



Aquifer stoneflies may be more vulnerable to warming temperatures because they had lower critical thermal maximum and lower metabolic breath

Take Home Messages:

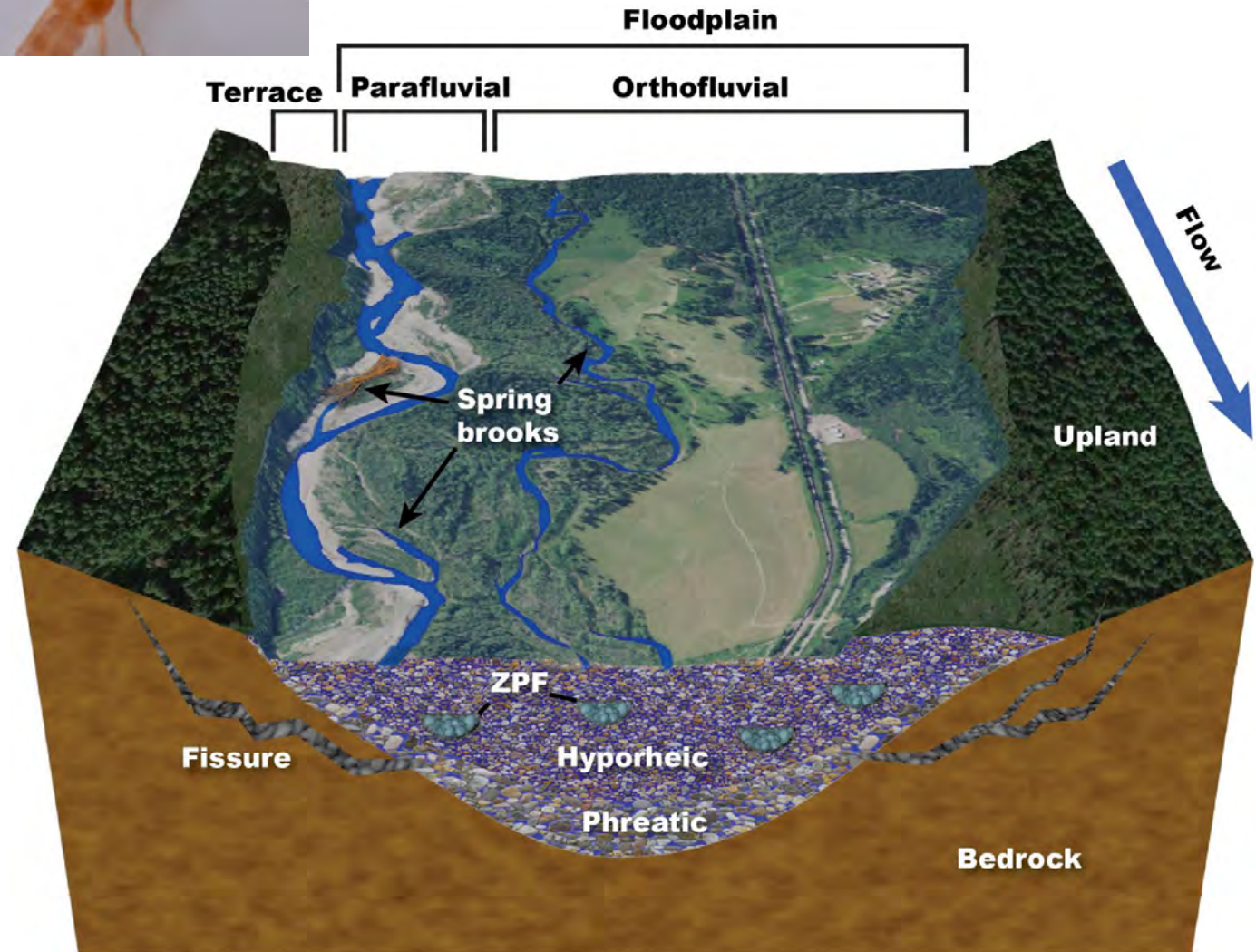
The discovery of aquifer stoneflies has helped shaped our understanding hyporheic zones and alluvial aquifers

Aquifer stoneflies are adapted to low oxygen conditions, surviving and moving much longer

Aquifer stoneflies may be less tolerant than benthic taxa to warming temperatures but also less vulnerable in alluvial aquifers

Investigation of temperature tolerance, plasticity, and mechanisms of adaptations is ongoing

There is still more to learn



Acknowledgments

Collaborators

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Field and Lab Crew Members

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OVERVIEW

Amphibitic stoneflies (Plecoptera) are integrators of ecosystem processes in alluvial aquifers of gravel-bed river floodplains

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