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Reading Report

Reporter: Li Shuai

PART 1

Impact Factor: 6.494

MOLECULAR ECOLOGY

Molecular Ecology (2012) 21, 3100–3102

NEWS AND VIEWS

PERSPECTIVE

Intestinal microbiota composition in fishes is influenced by host ecology and environment

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Approximately 28 000 fish species comprise nearly half of all vertebrate diversity and represent a broad range of physiologies, ecologies and natural histories (Nelson 2006). Fishes therefore represent an important vertebrate group for understanding the evolution and ecology of host–micro-

Vertebrates

digestive tracts

Gut microbiota

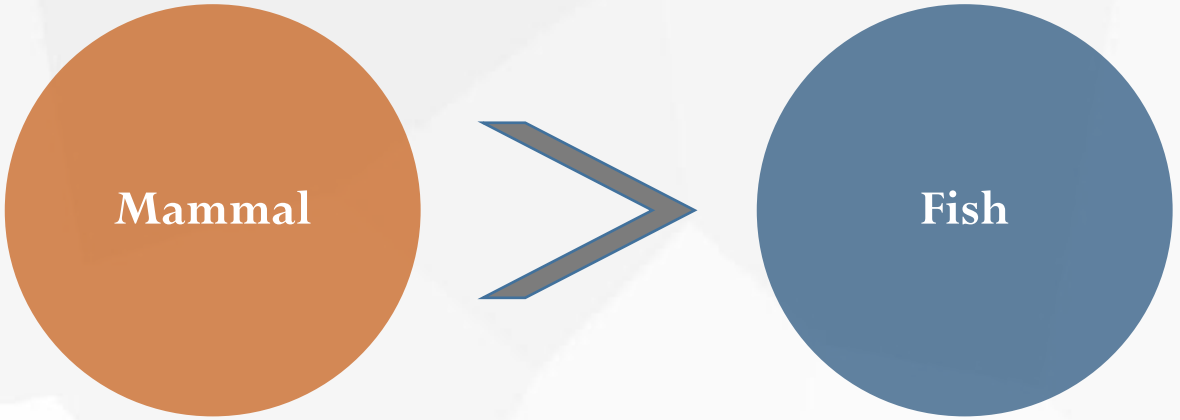
Be colonized by Complex assemblages of micro-organisms

Intense interest point：

1. How gut microbial communities are assembled ?

2. How they impact host fitness ?(Sekirov et al. 2010).

The order of research：



MOLECULAR ECOLOGY

Molecular Ecology (2012) 21, 3363–3378 doi: 10.1111/j.1365-294X.2012.05552.x

Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis

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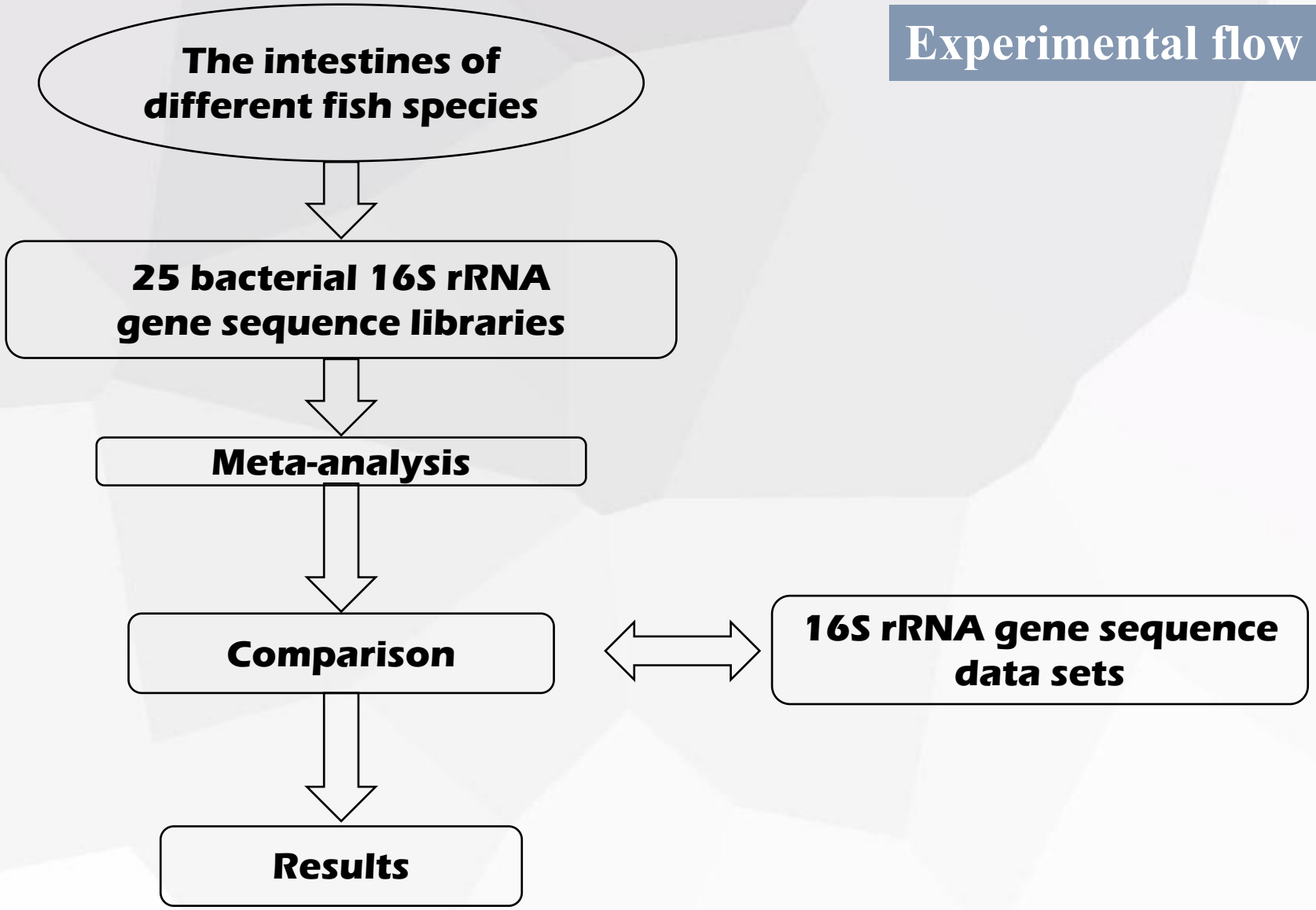
Experimental flow graph



Results：

1. Variation in gut microbiota composition in fishes is strongly correlated with species habitat salinity, trophic level and possibly taxonomy.（comparison within groups）
2. Fish gut microbiota compositions are often similar to those of other animals and contain relatively few free-living environmental bacteria.（comparison among groups）

Suggestion：

The gut microbiota composition of fishes is not a simple reflection of the micro-organisms in their local habitat but may result from host-specific selective pressures within the gut(Bevins & Salzman 2011).

Approximately 28 000 fish species comprise nearly half of all vertebrate diversity and represent a broad range of physiologies, ecologies and natural histories (Nelson 2006).

Important vertebrate group

The research of the gut microbiota of fishes ：

Culture-based approaches

Culture-independent DNA sequence-based approaches

Few studies have compared the intestinal bacterial diversity of multiple species .

(Roeselers et al. 2011)

Ecological and environmental factors

More fish species?

This article by Sullam et al. (2012) is significant because it presents phylogenetic and statistical meta-analyses of intestinal microbiotas from the largest number of fish species to date.

24 published culture-dependent and culture-independent libraries

+

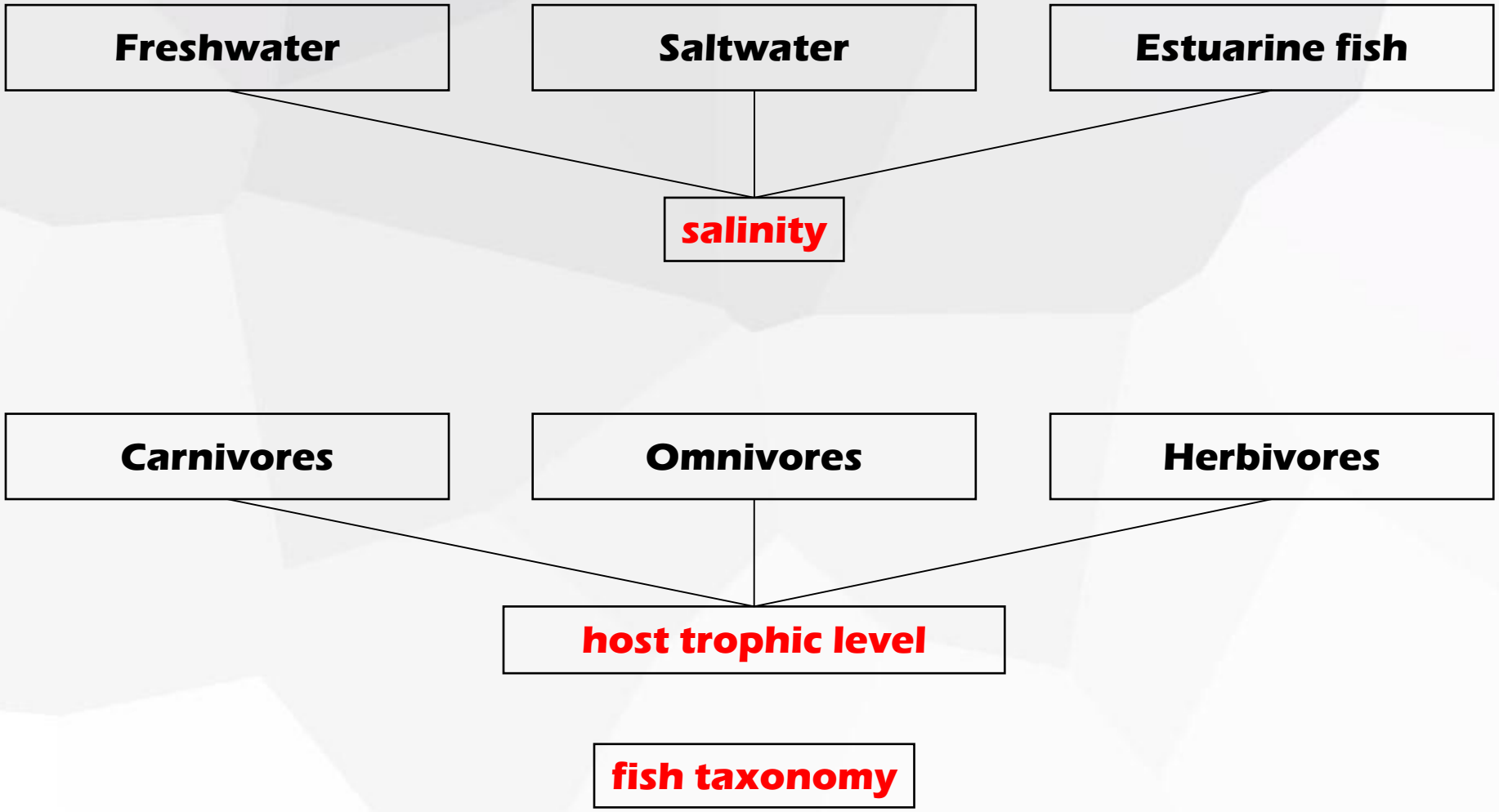
A new library from Trinidadian guppy, Poecilia reticulata

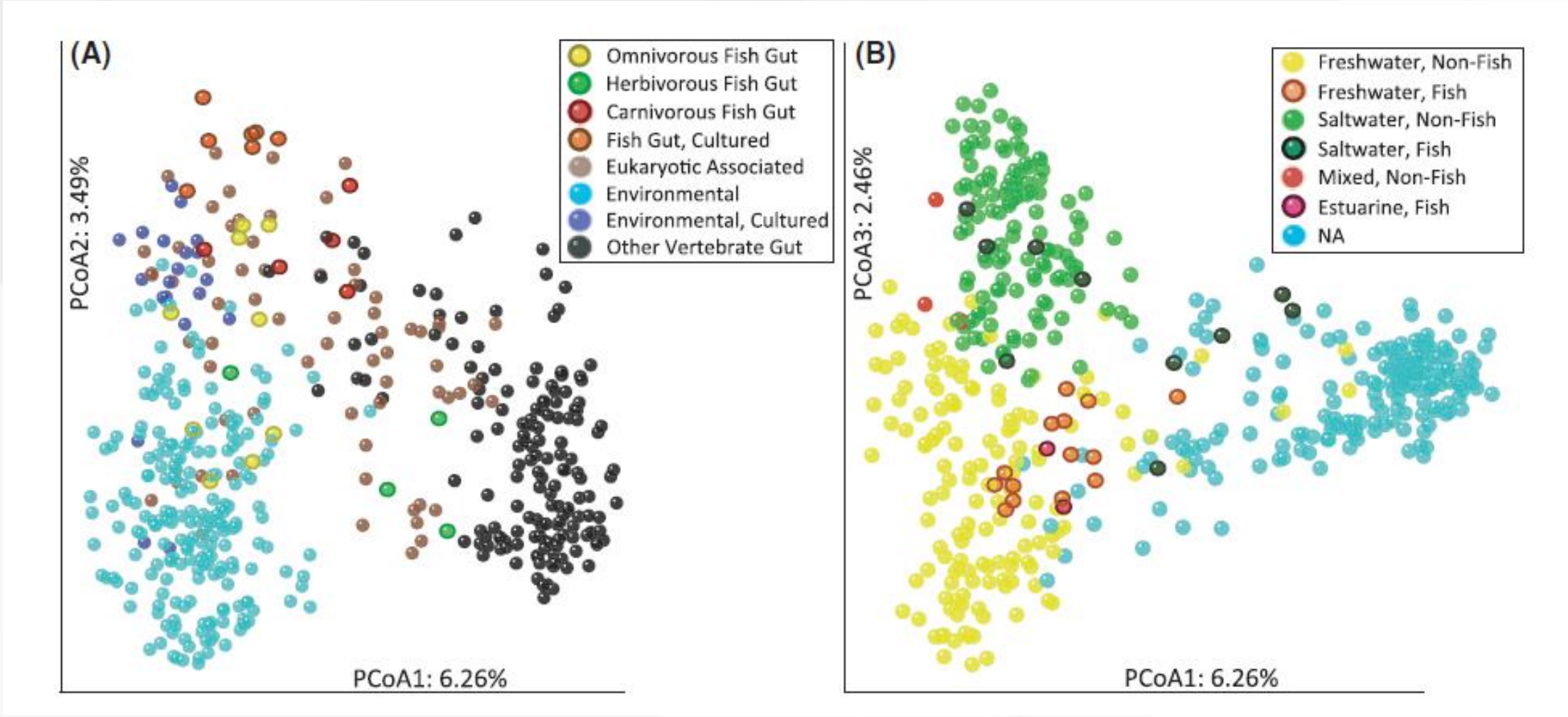
18 fish species

Water salinities

Trophic levels

Principal coordinate analysis (PCoA)





Co-evolution ?

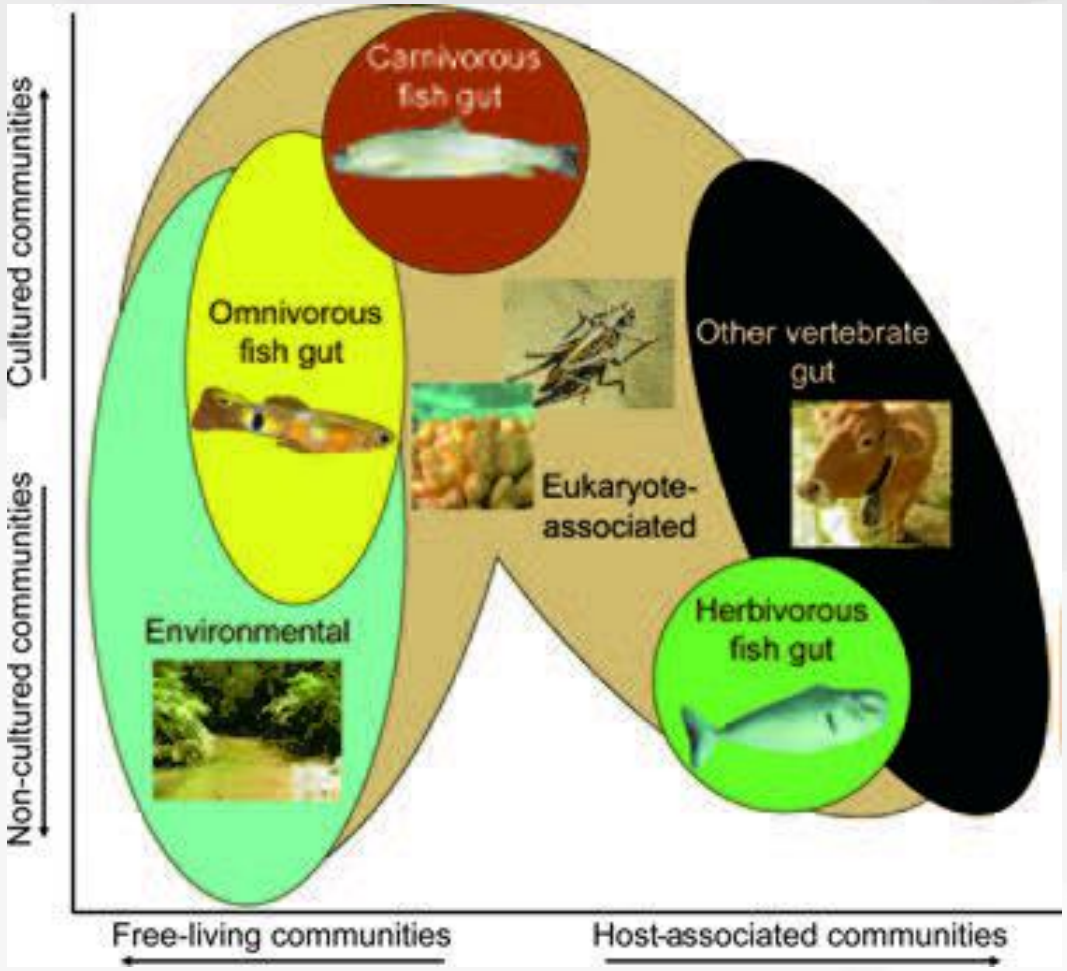
(Sullam et al. 2012)

This is consistent with a previous report showing that colonization of germ-free zebrafish with a Firmicutes phylum-dominant mouse gut microbiota results in enrichment of phylum Proteobacteria, which normally dominates zebrafish intestines (Rawls et al. 2006).

Additionally, in accordance with previous studies noting few differences between animals raised in artificial and natural environments (Ley et al. 2008a; Roeselers et al. 2011).

The analysis detected no significant effects of rearing environment on gut microbiota composition. (Sullam et al. 2012)

Together, these data support several emerging themes in fish gut microbial ecology: microbiota composition is strongly associated with host trophic level, habitat salinity and perhaps taxonomy, and but with relatively little impact of host provenance.



Surprising occurrence:

More than half the OTUs from herbivorous fishes were more closely related to bacteria in mammalian and bird intestines than to bacteria from fish intestines.

guidance

It will be important in the future to include additional freshwater herbivores and fish from taxa, trophic levels and water salinities not included in this study.

Moreover, the potential impact of other environmental parameters (e.g. water depth and temperature, diet

This information would provide an essential foundation for exploring the impact of gut microbiota composition and function on the ecology, fitness and evolution of their respective hosts.

PART 2

Impact Factor: 5.578

SCIENTIFIC REPORTS

OPEN

The gut microbiome and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels

Received: 23 September 2015
Accepted: 16 March 2016
Published: 13 April 2016

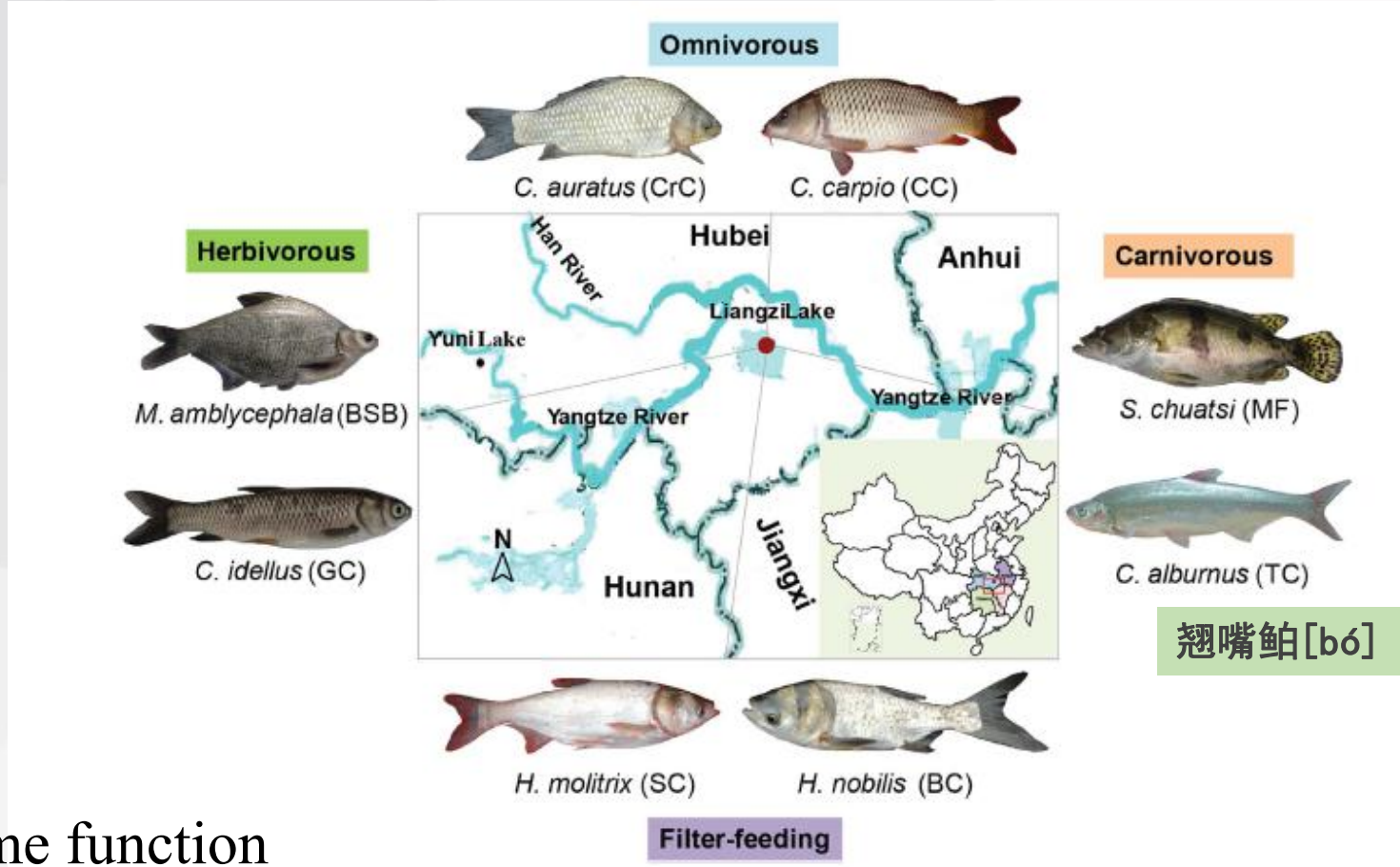
Han Liu[1], Xianwu Guo[2], Ravi Gooneratne[3], Ruifang Lai[1], Cong Zeng[1], Fanbin Zhan[1] & Weimin Wang[1,4,†]

8 fish species

985,000 quality-filtered sequences

24 16S rRNA libraries

PICRUSt predictions of metagenome function



The approaches to investigate the gut bacterial diversity:

- Isolation and cultivation approaches (Under laboratory conditions)
- PCR denaturing gradient gel electrophoresis (DGGE)
- Terminal restriction fragment length polymorphism (T-RFLP)
- Next-generation sequencing of 16S rRNA gene
- Single Molecule Real Time (SMRT™) DNA Sequencing
- Oxford Nanopore Technologies

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Some commercially viable fishes:

Rearing conditions

European sea bass , grass carp , perch , channel catfish and rainbow trout.

Fish species with distinct trophic levels from natural environments

Innovative Points of the Research

Herbivorous
Carnivorous
Omnivorous
Filter-feeding

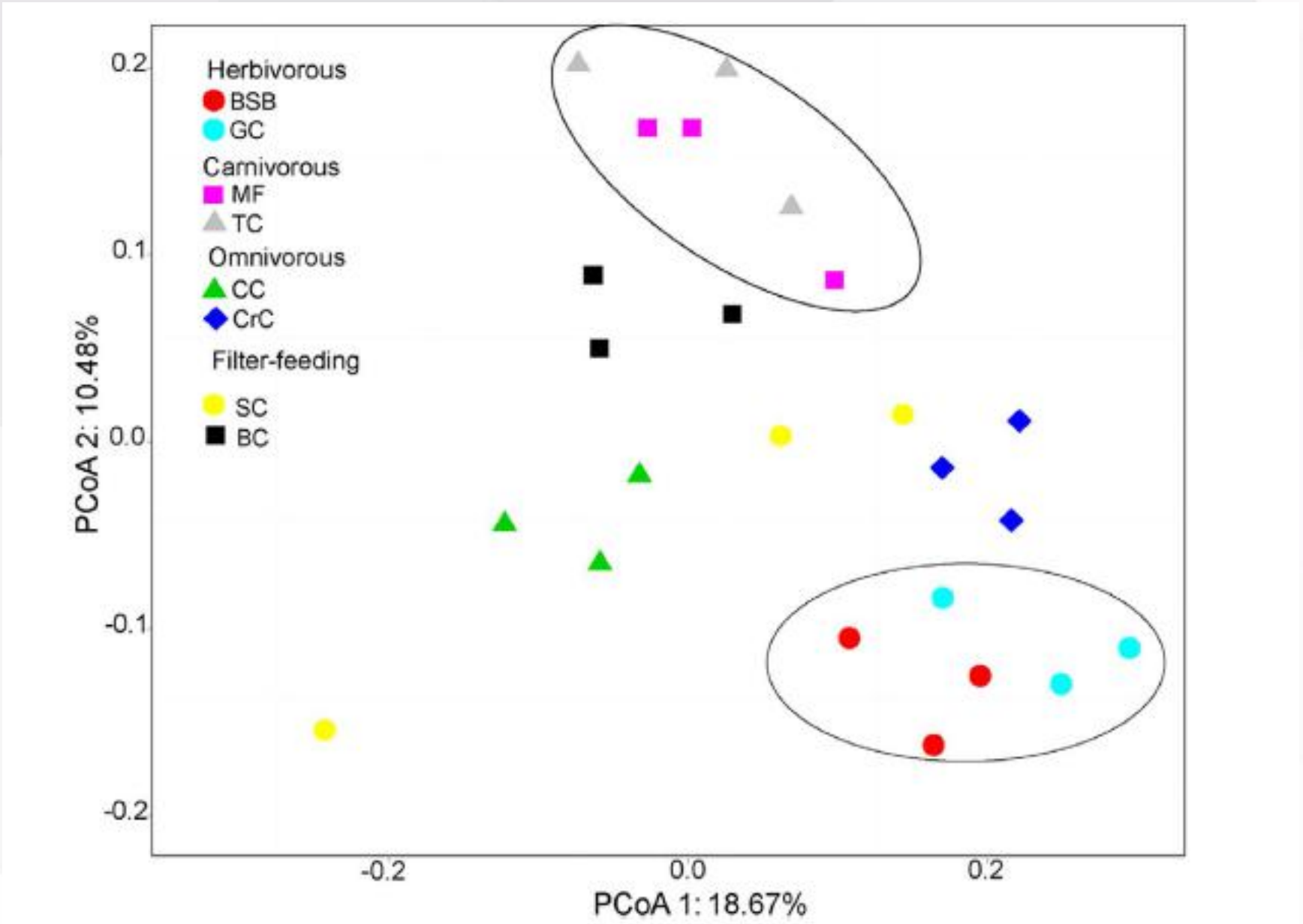
At the same time point
In the same water area

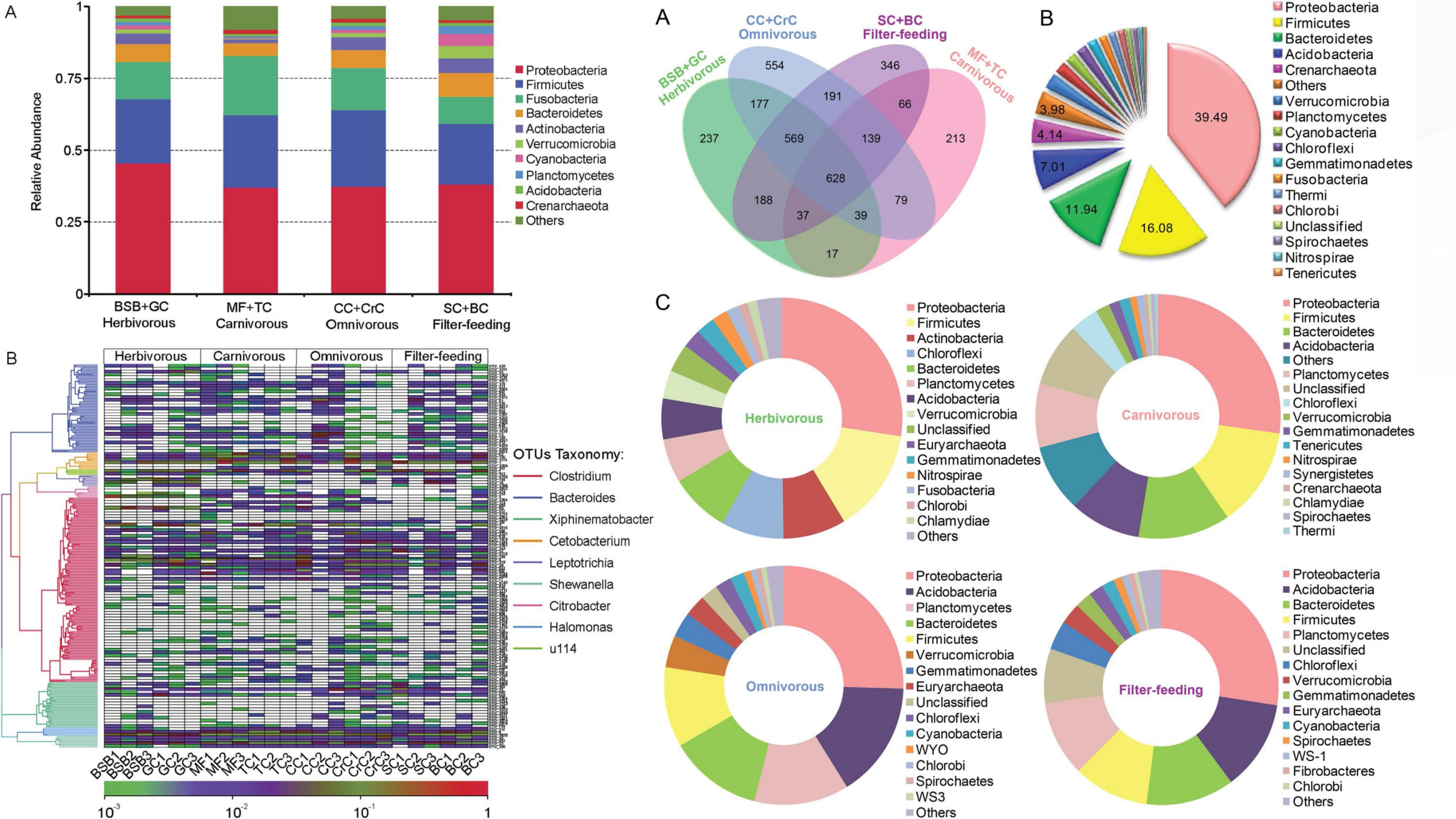
gut content :
cellulase/amylase/trypsin
enzyme activities

Cellulase

I. No endogenous genes coding cellulose-digesting enzymes were found in the genome of mammals. (Li, R. et al. 2010),but *Clostridium* group I (Zhu et al. 2011).

II. Cellulolytic enzyme-producing bacterial community: *Aeromonas, Enterobacter, Citrobacter, Bacillus, and Pseudomonas* (Ray et al. 2012, Li, H. et al. 2016).


Herbivorous
BSB
GC
Carnivorous
MF
TC
Omnivorous
CC
CrC
Filter-feeding
SC
BC
0.2
0.1
0.0
-0.1
-0.2
PCoA 2: 10.48%
-0.2
0.0
0.2
PCoA 1: 18.67%


A
Relative Abundance
1
0.75
0.5
0.25
0
BSB+GC Herbivorous
MF+TC Carnivorous
CC+CrC Omnivorous
SC+BC Filter-feeding
Proteobacteria
Firmicutes
Fusobacteria
Bacteroidetes
Actinobacteria
Verrucomicrobia
Cyanobacteria
Planctomycetes
Acidobacteria
Crenarchaeota
Others
B
Herbivorous
Carnivorous
Omnivorous
Filter-feeding
BSB1 BSB2 BSB3 GC1 GC2 GC3 MF1 MF2 MF3 TC1 TC2 TC3 CC1 CC2 CC3 CrC1 CrC2 CrC3 SC1 SC2 SC3 BC1 BC2 BC3
10-3
10-2
10-1
1
OTUs Taxonomy:
Clostridium
Bacteroides
Xiphinematobacter
Cetobacterium
Leptotrichia
Shewanella
Citrobacter
Halomonas
u114
A
CC+CrC Omnivorous
SC+BC Filter-feeding
BSB+GC Herbivorous
MF+TC Carnivorous
554
346
177
191
66
237
569
139
213
628
188
37
39
79
17
B
39.49
16.08
11.94
7.01
4.14
3.98
Proteobacteria
Firmicutes
Bacteroidetes
Acidobacteria
Crenarchaeota
Others
Verrucomicrobia
Planctomycetes
Cyanobacteria
Chloroflexi
Gemmatimonadetes
Fusobacteria
Thermi
Chlorobi
Unclassified
Spirochaetes
Nitrospirae
Tenericutes
C
Herbivorous
Proteobacteria
Firmicutes
Actinobacteria
Chloroflexi
Bacteroidetes
Planctomycetes
Acidobacteria
Verrucomicrobia
Unclassified
Euryarchaeota
Gemmatimonadetes
Nitrospirae
Fusobacteria
Chlorobi
Chlamydiae
Others
Carnivorous
Proteobacteria
Firmicutes
Bacteroidetes
Acidobacteria
Others
Planctomycetes
Unclassified
Chloroflexi
Verrucomicrobia
Gemmatimonadetes
Tenericutes
Nitrospirae
Synergistetes
Crenarchaeota
Chlamydiae
Spirochaetes
Thermi
Omnivorous
Proteobacteria
Acidobacteria
Planctomycetes
Bacteroidetes
Firmicutes
Verrucomicrobia
Gemmatimonadetes
Euryarchaeota
Unclassified
Chloroflexi
Cyanobacteria
WYO
Chlorobi
Spirochaetes
WS3
Others
Filter-feeding
Proteobacteria
Acidobacteria
Bacteroidetes
Firmicutes
Planctomycetes
Unclassified
Chloroflexi
Verrucomicrobia
Gemmatimonadetes
Euryarchaeota
Cyanobacteria
Spirochaetes
WS-1
Fibrobacteres
Chlorobi
Others

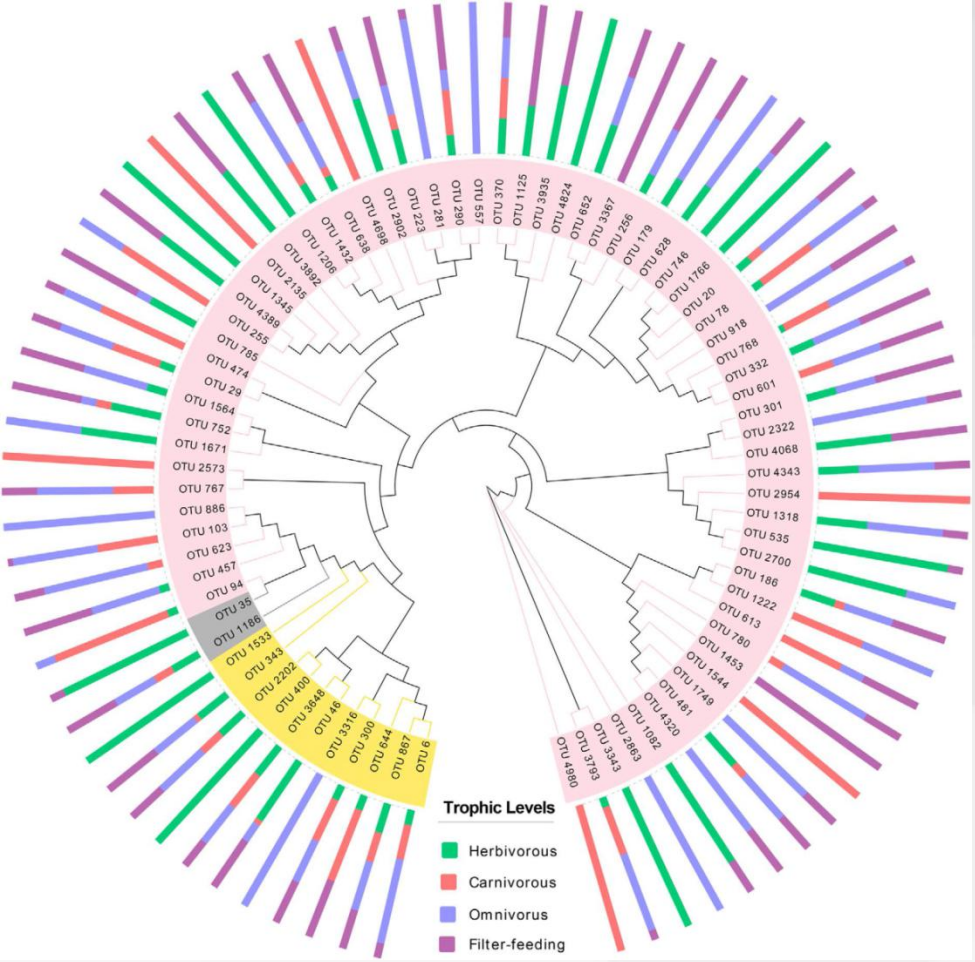
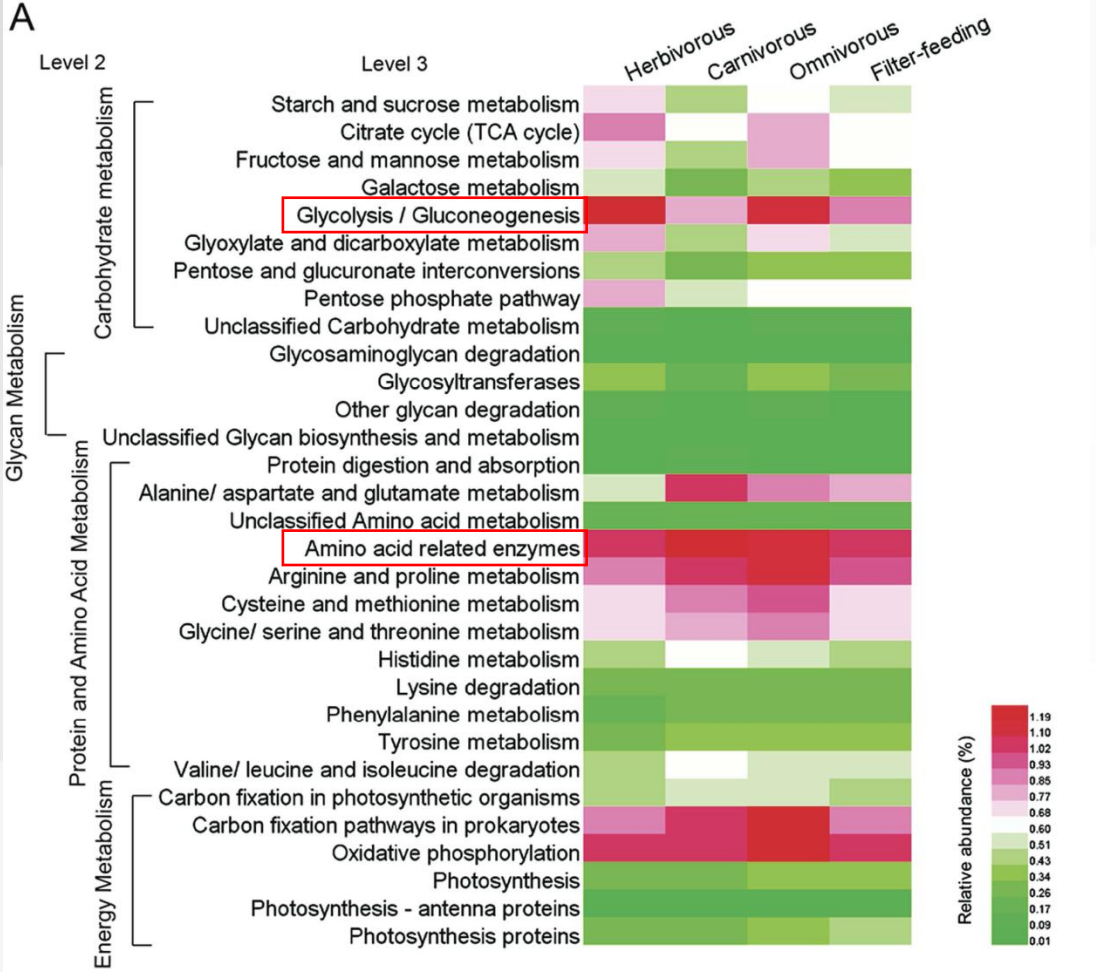


Figure 5. Dendrogram of cellulose-degrading represented OTUs and their host occurrence patterns. Bars show the proportion of fish samples with different trophic levels in which the given OTUs is present. Circles indicate the phylogenetic relationship of 13 kinds of cellulolytic species.

Degree of accuracy gt 85%-90%



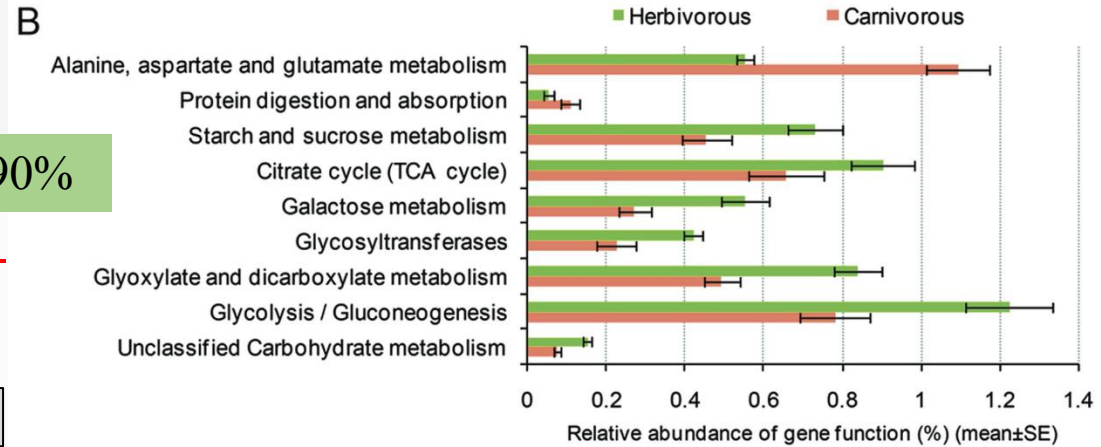


Figure 6. Comparison in the relative abundance of PICRUSt-generated functional profile of gut microbiota among four trophic levels. (A) Heat map shows the relative abundance changes in fishes with four trophic levels. **(B)** Significant differences in gene categories at level 3 (t-test, $P < 0.05$) between the herbivorous and the carnivorous.

PICRUSt: Phylogenetic Investigation of Communities by Reconstruction of Unobserved States

Trophic levels	Species	Gut content enzymes activities		
		Cellulase	Amylase	Trypsin
Herbivorous	BSB	16.55 ± 4.55^{abc}	263.55 ± 52.17^{a}	9.25 ± 1.69^{cd}
	GC	20.08 ± 6.45^{a}	252.62 ± 51.59^{a}	6.83 ± 1.35^{d}
Carnivorous	MF	8.69 ± 1.61^{bc}	12.93 ± 2.87^{c}	13.13 ± 1.17^{b}
	TC	6.78 ± 0.81^{c}	27.75 ± 7.69^{c}	19.63 ± 2.12^{a}
Omnivorous	CC	8.90 ± 1.82^{bc}	222.01 ± 71.83^{ab}	12.49 ± 1.98^{bc}
	CrC	16.70 ± 5.16^{ab}	237.67 ± 42.07^{a}	15.27 ± 2.91^{b}
Filter-feeding	SC	11.56 ± 1.68^{abc}	354.17 ± 68.91^{a}	9.56 ± 0.84^{cd}
	BC	8.61 ± 1.48^{bc}	93.69 ± 19.83^{bc}	6.85 ± 1.12^{d}

Table 2. Fish gut content enzymes activities (U/mg protein). The means (mean ± SE) with different letters in each enzyme indicate significant differences. ANOVA was followed by Tukey' s test, $P < 0.05$.

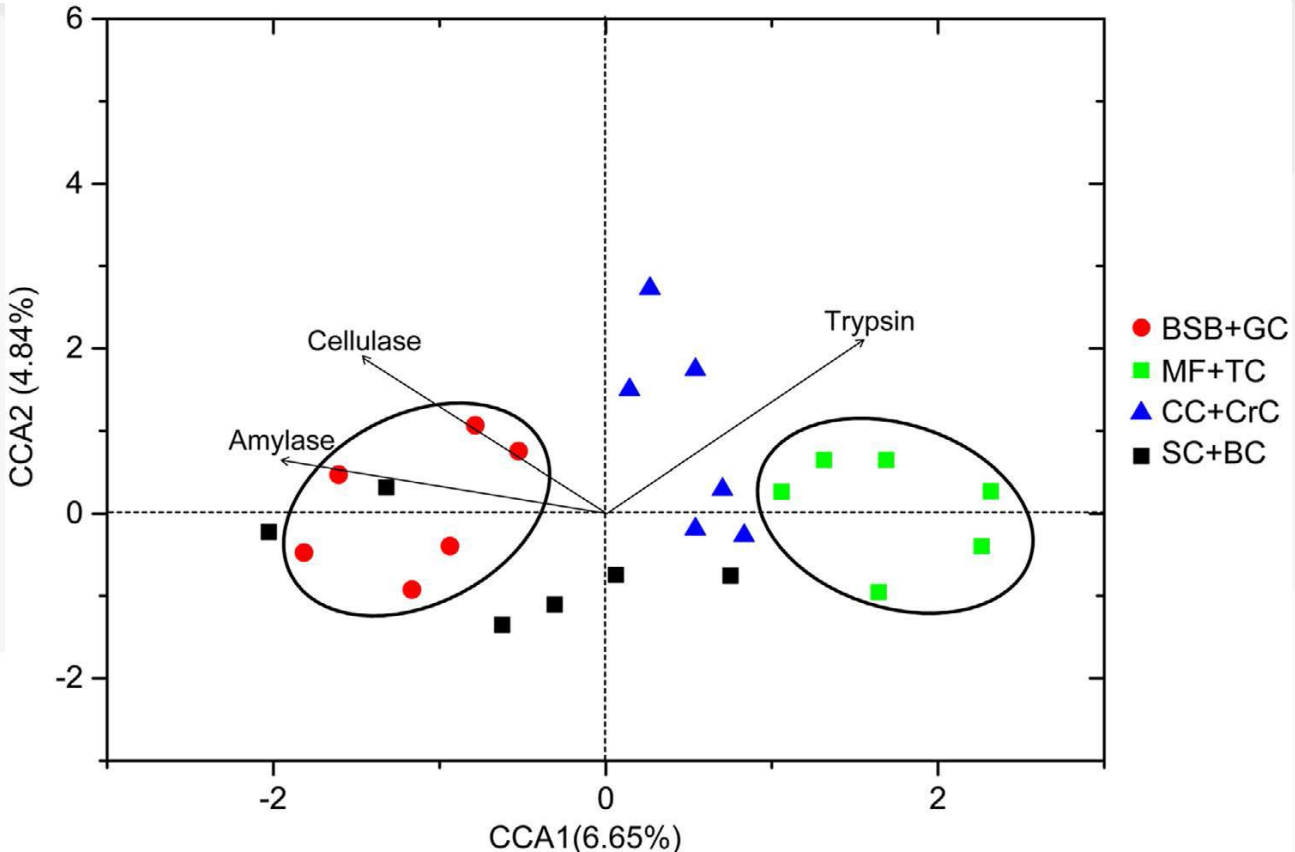


Figure 7. Canonical correspondence analysis (CCA) showing the correlation between the gut microbial compositions of eight fish species and their enzyme activities.

Sample collection:

Prior to dissection, fishes were euthanized with an overdose of tricaine methanesulfonate (dissolved in water). All procedures for handling and euthanasia of wild freshwater fish species were approved by institution animal care. To help eliminate transient bacteria, the whole intestinal tract of individual fish was dissected with sterile instruments and washed in 70% ethanol and sterile water. Then the gut content from the midgut region to the hindgut region were squeezed out and mixed thoroughly, and then collected into sterile tubes and immediately stored at liquid nitrogen.

DNA extraction, amplification and sequencing :

200 mg sample

Homogenized using a three-minute **bead beating** procedure at 30 Hz

QIAamp DNA Stool Mini Kit (Qiagen, Valencia, USA)

Electrophoresis in 1% agarose gel with Tris-acetate-EDTA (TAE) buffer

NanoDrop ND-2000 spectrophotometer (Thermo Scientific)

Illumina MiSeq sequencing platform

V4 hypervariable region (515F and 806 R) of the 16S rRNA gene

The reverse primer contained a 6-bp error-correcting barcode

Novogene Bioinformatics Technology

Taxonomic analyses of sequenced reads. ▲

Analysis of Enzyme activities.

200 mg sample、2mL 0.1 M phosphate buffer on ice (PBS, pH 6.8, 1:20 w/v)

Hand-held glass homogenizer

Be centrifuged at 12,000 × g for 20 min at 4 °C

The supernatant was divided into four Eppendorf tubes and then stored at − 40 °C

All enzymatic assays were conducted within 3 days after extraction.

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The Revelations to Me

1. What can I do now?
2. Which I need to do now?
3. How to sample and handling the fish gut?
4. How to analysis the data sets?
5. How to create the connection between the different results?

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THANKS

2016-05-21