

Sources to the *Danio rerio* egg and larval microbiomes

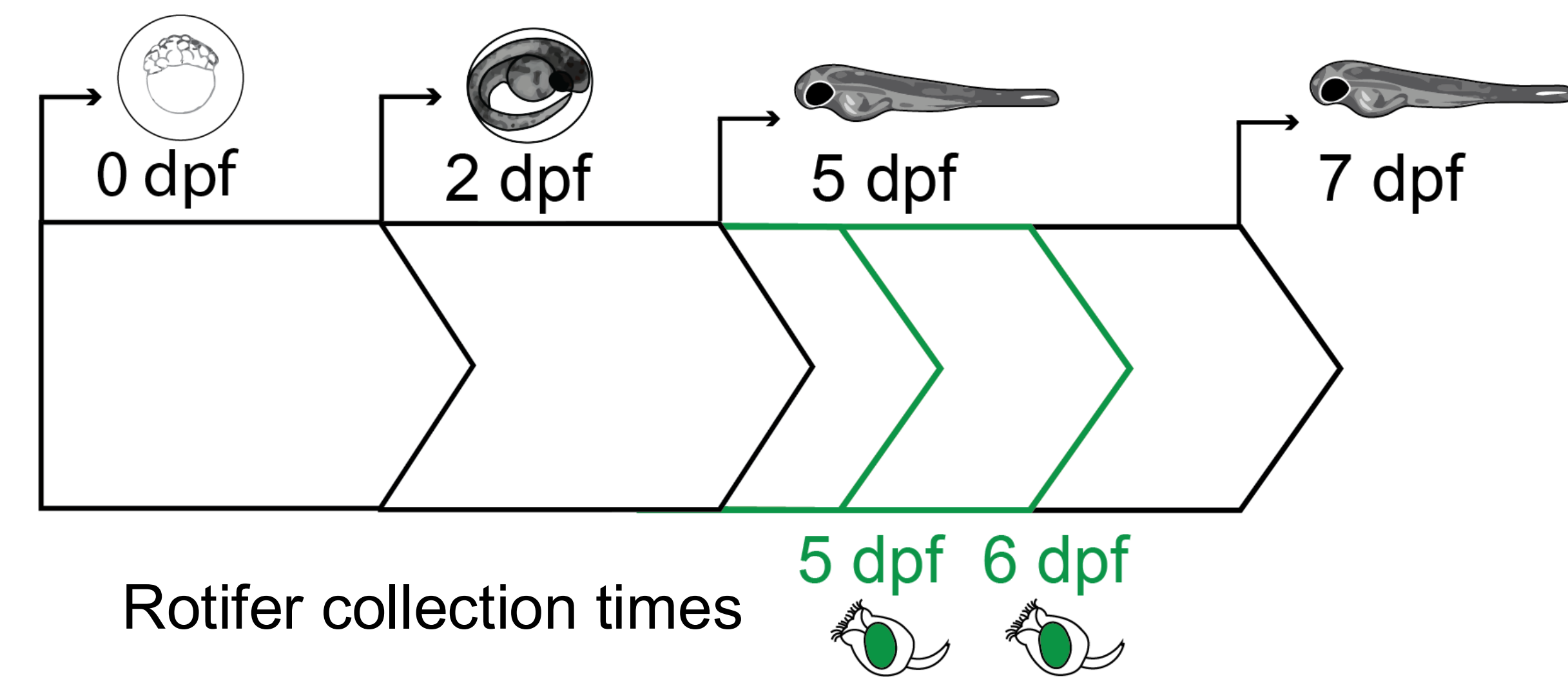
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BACKGROUND: The microbiome composition of zebrafish can affect host health and development. However, little is known about the sources to the early-life microbiome of zebrafish, or whether the microbiomes of the eggs shape the microbiomes composition of later development stages. Here, we use a source tracking algorithm (FEAST) to infer the contributions of parental and environmental sources to the egg and larval microbiomes.

METHODS:

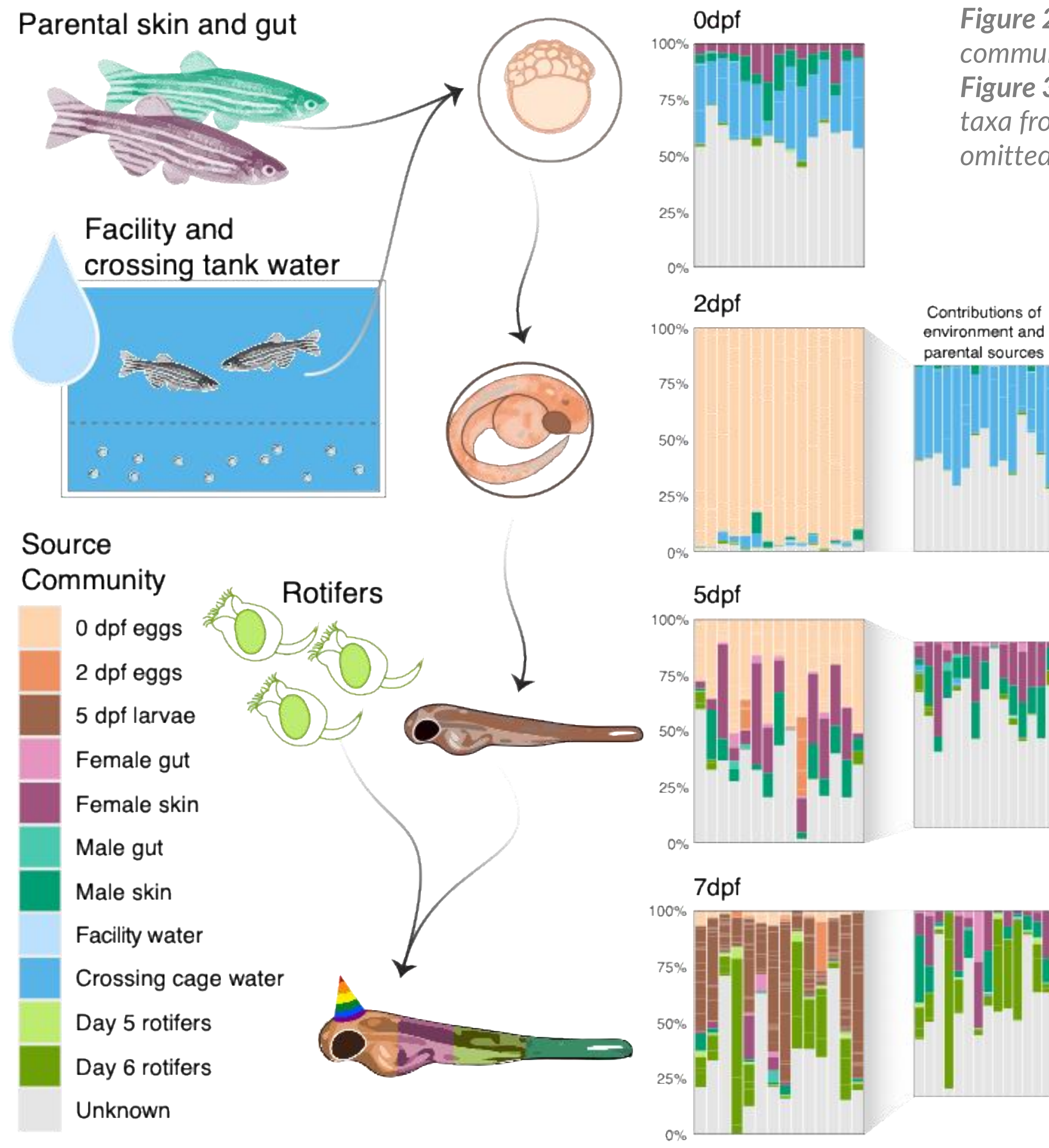
- We crossed adult ABCxTu zebrafish reared at the Huestis Zebrafish Facility (University of Oregon, Eugene, USA).
- We sampled adult tissues (skin and gut), facility water, crossing tank water, rotifers (at day 5 and 6 feedings), and offspring (egg and larvae; Figure 1) for DNA extraction.
- We PCR amplified the V4 region of the 16S rRNA gene and sequenced amplicons using Illumina MiSeq.
- Quality filtering, amplicon sequence variant inference and taxonomic assignment was performed using R package *dada2*.
- FEAST estimated the relative contributions of the various source communities to the microbiomes of the sink communities.
- Indicator Species Analyses were performed to identify amplicon sequence variants (ASVs) that are likely transmitted from source community to eggs and larvae

Figure 1: Sample collection times:



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Microbial Heritage: Parents and the egg are important contributors to the gut microbiome of larval zebrafish.

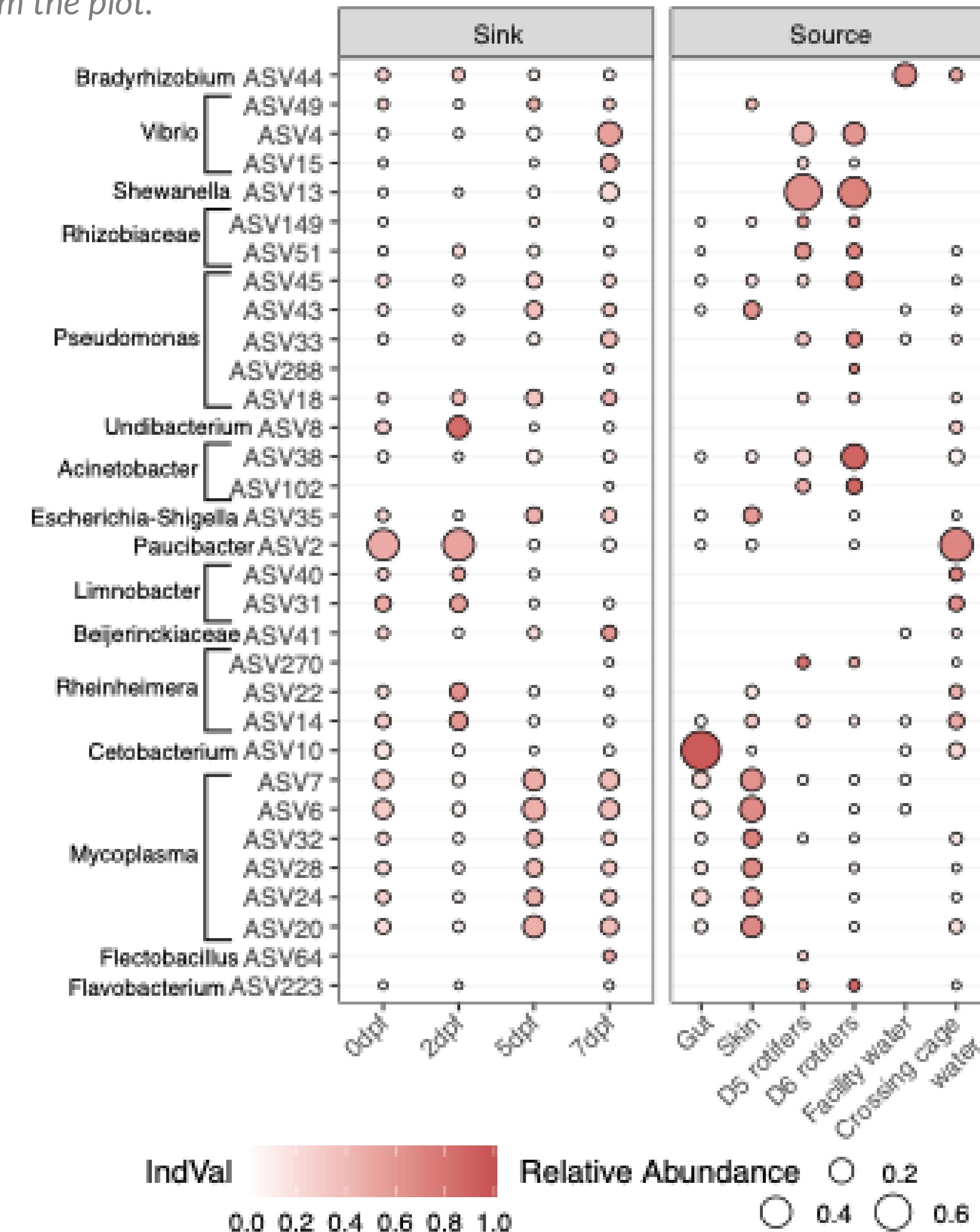


RESULTS: At 0 days post fertilization (dpf), about 14% of the egg microbiome is derived from parental sources and 9% from crossing cage water. Parental contributions decrease to less than 1% at 2 dpf and increase at 5 dpf (28%). These contributions decrease again at 7 dpf (18%), after the larvae are fed rotifers (Figure 2). There is no significant difference between male and female parent contributions. Adult skin microbiomes contribute more to the offspring microbiomes than the adult gut microbiomes. The egg microbiome (particularly at 0 dpf) is a significant source to later development stages (94% for 2 dpf eggs and 36% for 5 dpf larvae). The microbiome of previous development stages also contributes substantially (nearly 50%) to the 7 dpf larval microbiome (0 dpf = 4%; 2 dpf = 7.5%; 5 dpf = 35%). The egg facilitate the transmission of parental microbes to later development stages, since the only exposure to parents occurs at reproduction.

The day 6 rotifer culture contribute approximately 20% to the 7 dpf larval microbiomes, potentially displacing parentally transmitted microbes.

Figure 2 (left): FEAST estimates the relative contributions of various source communities to the microbiomes of zebrafish eggs and larvae.

Figure 3 (below): Indicator Species Analysis reveals putatively transmitted taxa from sources to eggs and larvae. ASVs not detected in a sink or source are omitted from the plot.



We used an indicator species analysis to identify ASVs that are likely transmitted from the various source communities to the sink communities (eggs and larvae; Figure 3). Generally, indicator ASVs to source communities that contribute to the egg and larval microbiomes according to FEAST (Figure 2-3). Notably, ASVs belonging to *Mycoplasma* are indicators to the skin, 0 dpf eggs, 5 dpf larvae and 7 dpf larvae. Since adult skin is a major source to the offspring microbiome at these ages, *Mycoplasma* is likely transmitted from the adult skin to the offspring at reproduction and can colonize the gut post-hatching.

IMPORTANCE: Zebrafish are often rendered germ-free in microbiome studies, erasing any contributions of the parents and environment on the microbiome at later development stages. These results show that adult zebrafish contribute substantially to the egg and larvae microbiomes. Additionally, the egg microbiome can shape the microbiome at later development stages and should be considered in future microbiome studies in zebrafish.



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