1   Supplementary Files

2

3   Dynamics of miRNA transcriptome during gonadal development

4   of zebrafish

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6   **Babiak**

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8   Supplementary Figure S1. Read numbers and distribution

9   (A) The total number of mapped small RNA reads and their class distribution. (B) Length

10   distribution.

11

12   Supplementary Figure S2. Comparison of gonadal miRNA datasets between teleost species

13   (A) The number of miRNA common between mature gonadal miRNA datasets for olive

14   flounder [1], marine medaka [2], Nile tilapia [3], and rainbow trout [4], as well as zebrafish from the

15   present study at both 12 and 24 weeks post fertilization (wpf), and an additional study in

16   zebrafish [5]. The graph was produced using UpSet [6]. (B) A comparison of commonly abundant

17   gonadal miRNAs between teleost species and their frequency of presence in each dataset.

18

19   Supplementary Dataset S1. Sequence statistics

20   (Tab 1) The number of reads produced before and after quality trimming in each small RNA

21   library. (Tab 2) The total number of reads mapped to each class of RNA. (Tab 3) The

22   distribution of reads for each library by nucleotide length.

23

24   Supplementary Dataset S2. Low input RNA test

25 The absolute number of reads produced for individual miRNAs between different total RNA

26 inputs used during small RNA library preparation.

27

28 Supplementary Dataset S3. miRNA reads for mapped miRNAs in each small RNA library

29 The absolute (Tab 1) and DESeq2 normalized (Tab 2) number of reads for all miRNAs in

30 each library.

31

32 Supplementary Dataset S4. Differentially expressed miRNAs over time

33 Each tab displays differential expression analysis of DESeq2 normalized reads for different

34 time comparisons. All testis stages are compared to each other as well as 3 week

35 undifferentiated gonads. All ovary stages are compared to each other as well as 3 weeks

36 undifferentiated gonads.

37

38 Supplementary Dataset S5. Differentially expressed miRNAs between sexes

39 Each tab displays differential expression analysis of DESeq2 normalized reads at each

40 developmental stage between testis and ovary, as well as between gametes and sexually

41 mature gonads at 24 weeks post fertilization.

42

43 Supplementary Dataset S6. IsomiRs in zebrafish gonads

44 List of the relative percentage for each isomiR within the 27 most abundant zebrafish gonadal

45 miRNAs (Tab 1) and all sequenced reads for each isomiR detected (Tab 2).

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47 Supplementary Dataset S7. miRNA target prediction and GO analysis

48 (Tab 1) Target prediction using miRanda v. 21 for the 27 abundant miRNAs in zebrafish

49 gonads. (Tab 2) GO enrichment analysis for the 27 abundant miRNAs.

2

50   Supplementary References

51 1   Gu, Y., Zhang, L. & Chen, X. Differential expression analysis of *Paralichthys olivaceus*
52   microRNAs in adult ovary and testis by deep sequencing. *Gen. Comp. Endocrinol.* **204**, 181-
53   184, doi:10.1016/j.ygcen.2014.05.019 (2014).

54 2   Lau, K. *et al.* Identification and expression profiling of microRNAs in the brain, liver and
55   gonads of marine medaka (*Oryzias melastigma*) and in response to hypoxia. *PLoS ONE* **9**,
56   e110698, doi:10.1371/journal.pone.0110698 (2014).

57 3   Xiao, J. *et al.* Identification and characterization of microRNAs in ovary and testis of Nile
58   tilapia (*Oreochromis niloticus*) by using solexa sequencing technology. *PLoS ONE* **9**, e86821,
59   doi:10.1371/journal.pone.0086821 (2014).

60 4   Juanchich, A. *et al.* Characterization of an extensive rainbow trout miRNA transcriptome by
61   next generation sequencing. *BMC Genomics* **17**, 164, doi:10.1186/s12864-016-2505-9 (2016).

62 5   Vaz, C. *et al.* Deep sequencing of small RNA facilitates tissue and sex associated microRNA
63   discovery in zebrafish. *BMC Genom.* **16**, 1-16, doi:10.1186/s12864-015-2135-7 (2015).

64 6   Lex, A., Gehlenbord, N., Strobelt, H., Vuillemot, R. & Pfister, H. UpSet: visualization of
65   intersecting sets. *IEEE Trans. Vis. Comput. Graph.* **20**, 1983-1992,
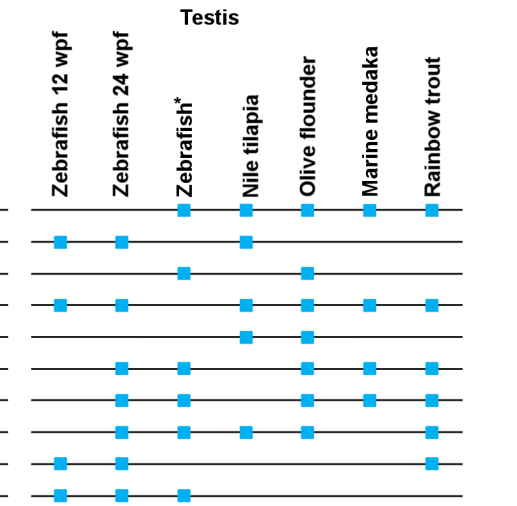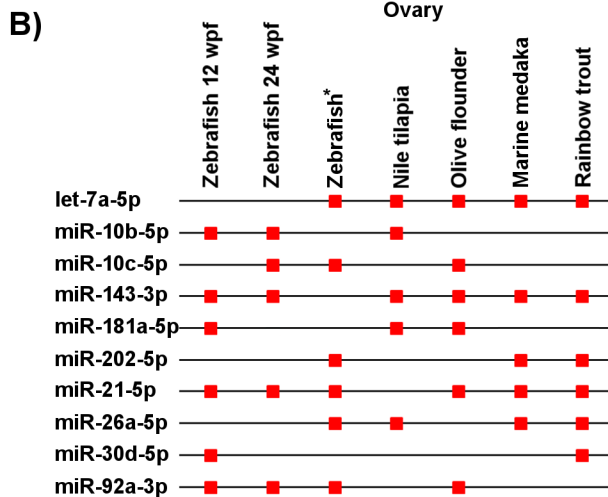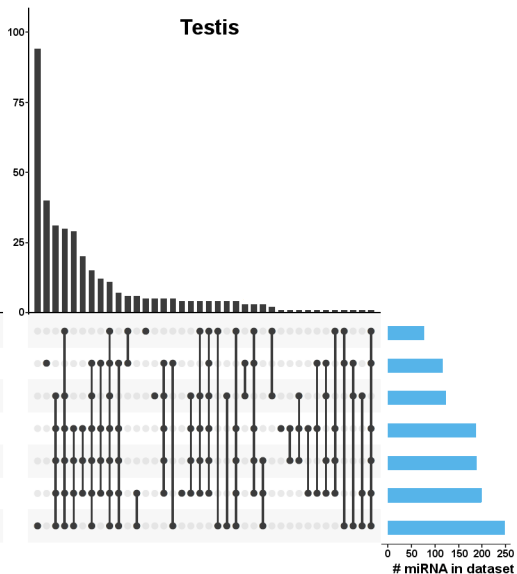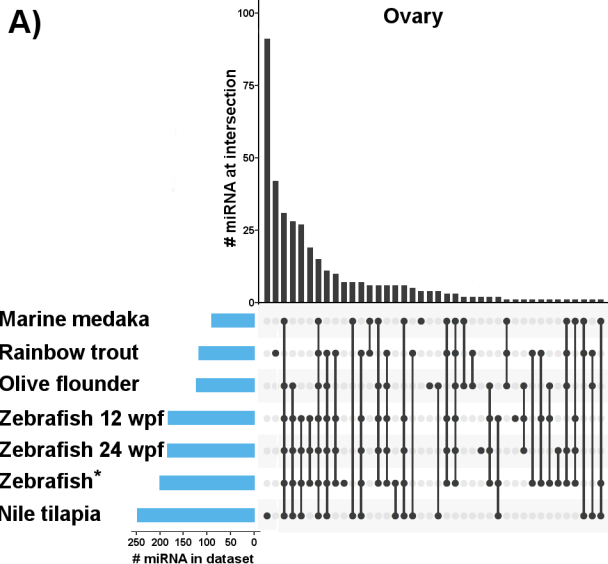66   doi:10.1109/tvcg.2014.2346248 (2014).

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a) Summary of small RNA reads
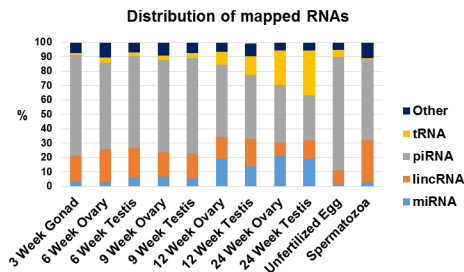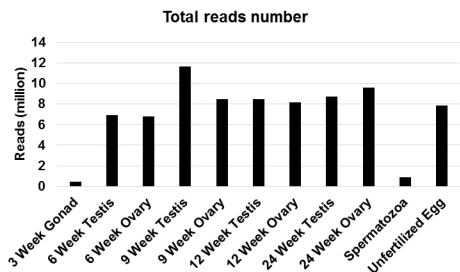
b) Read length distribution

A)

B)
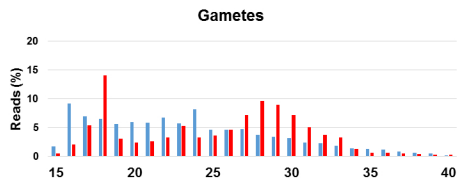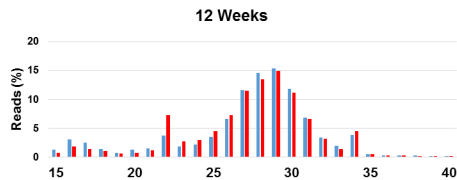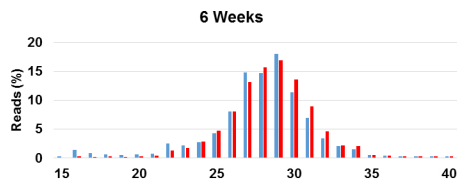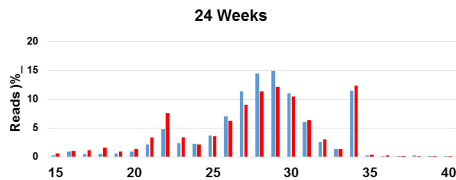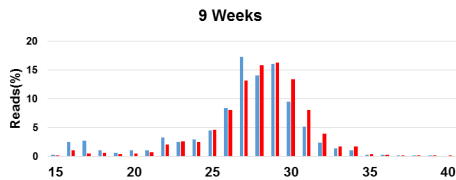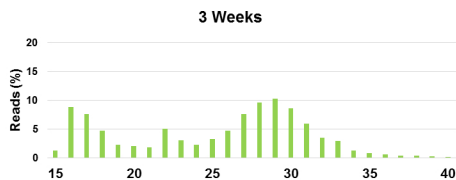
*Zebrafish dataset from Vaz et al. (2015)