# Gill $\mathrm{Na}^{+} / \mathrm{K}^{+}$-ATPase in the threespine stickleback (Gasterosteus aculeatus): changes in transcript levels and sites of expression during acclimation to seawater APPENDIX 

Makoto Kusakabe ${ }^{1,2}$, Seiichi Mori ${ }^{3}$ and Jun Kitano ${ }^{4}$<br>${ }^{1}$ Department of Biological Sciences, Faculty of Science, Shizuoka University, Surugaku, Shizuoka, Japan, ${ }^{2}$ Department of Marine Bioscience, Atmosphere and Ocean Research Institute, University of Tokyo, Kashiwa, Chiba, Japan, ${ }^{3}$ Biological Laboratory, Gifu-keizai University, Ogaki, Gifu, Japan and ${ }^{4}$ Ecological Genetics Laboratory, National Institute of Genetics, Mishima, Shizuoka, Japan

Running heads
Left = Kusakabe et al.
Right $=$ Gill $\mathrm{Na}^{+} / \mathrm{K}^{+}-$ATPase in the threespine stickleback

Correspondence: Kusakabe.makoto@shizuoka.ac.jp
© 2019 Makoto Kusakabe

Appendix Figure 1. mRNA levels of the gill sodium-potassium ATPase $\alpha$-subunit (atp1a1a.4-201, atp1a1a.4-204, atp1a2, atp1a3a, and atp1a3b) of the (a-e) Ono and (f-j) Gifu threespine stickleback populations during the seawater acclimation process. The crossing point ( Cp ) values obtained from the real-time quantitative PCR were plotted without any corrections with internal controls to see the differences in overall expression levels among different transcripts. Cp values indicate how rapidly the PCR products are detected and represent an approximation of the overall transcript levels. Smaller Cp values indicate higher expression levels.

Appendix Figure 2. Boxplots of Reads Per Kilobase Million (RPKM) of four genes encoding ATP1A. RPKM data were obtained from Artemov et al. (2017). RNAsequencing was conducted in the gills of Russian freshwater and marine threespine stickleback ecotypes exposed either to freshwater or seawater.

Appendix Fig. 1


Appendix Fig. 2
ffow
f sw Freshwater ecotype under seawater
m_fw Marine ecotype under freshwater
m_sw Freshwater ecotype under seawater





