Supporting Information

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SI Text

Mature adults were collected during upstream spawning migration by personnel of the US Fish and Wildlife Service Marquette Biological Station and Canada Department of Fisheries and Ocean Sea Lamprey Control Center, Sault Ste. Marie, ON. Actively feeding parasites were collected from fishermen by US Geological Survey personnel, immediately transported to Michigan State University, and held at 5–8 °C until sampling. Larvae were obtained by Hammond Bay Biological Station (Millersburg, MI) staff and kept in tanks with sand and flow-through lake water. Newly transformed animals were caught during downstream migration before extensive feeding started. Animals were killed with 0.1% MS-222 (Sigma). Tissues were fixed in 4% PFA or snap frozen in liquid nitrogen. Frozen tissues were stored at −80 °C until use. Fixed tissues were processed by staff in the Investigative Histopathology Laboratory at Michigan State University.

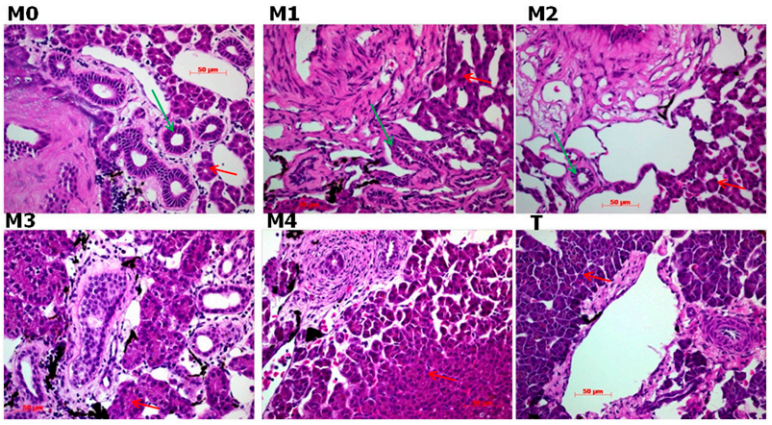

Fig. S1. Biliary atresia in metamorphic livers. H&E staining on paraffined liver sections. Selected stages of metamorphosis (M1–M4) are shown along with larval stage (M0), and newly transformed stage (T). Red arrows point to hepatocytes. Green arrows point to cholangiocytes.

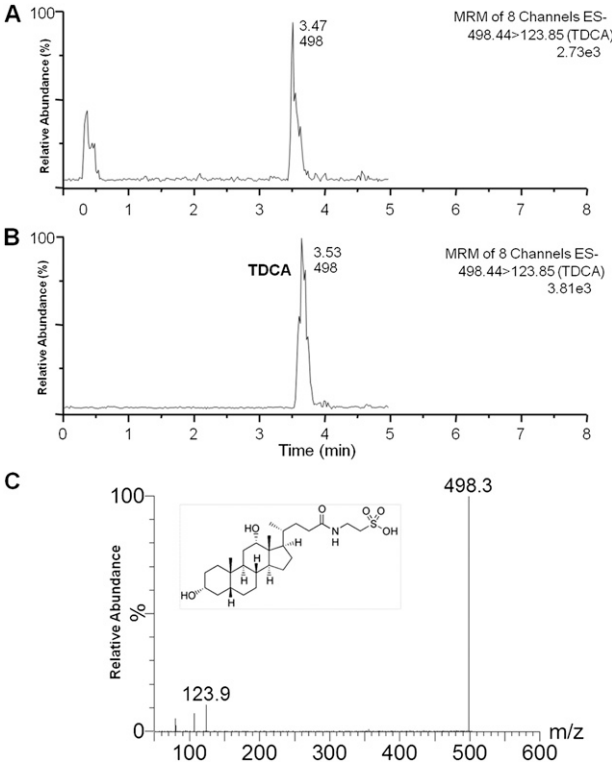

Fig. S2. Representative LC-MS/MS chromatograms of taurodeoxycholic acid (TDCA). (*A*) sample from sea lamprey liver at small parasite (SP) stage; (*B*) spiked standard solution containing TDCA. The best response of TDCA was observed in negative electrospray ionization multiple reaction monitoring (MRM) mode by monitoring the reaction *m*/*z* 498.44 > 123.85. The MRM transition as well as the cone voltage and collision energy voltage applied to the determination are displayed in Table S3. The peak observed in *A* has retention time comparable to the peak of standard in *B*. (*C*) Ion mass spectrum of TDCA separated from *A*. The product ion mass spectrum obtained showed parent/daughter ion pattern that confirmed the compound separated from *A* is TDCA.

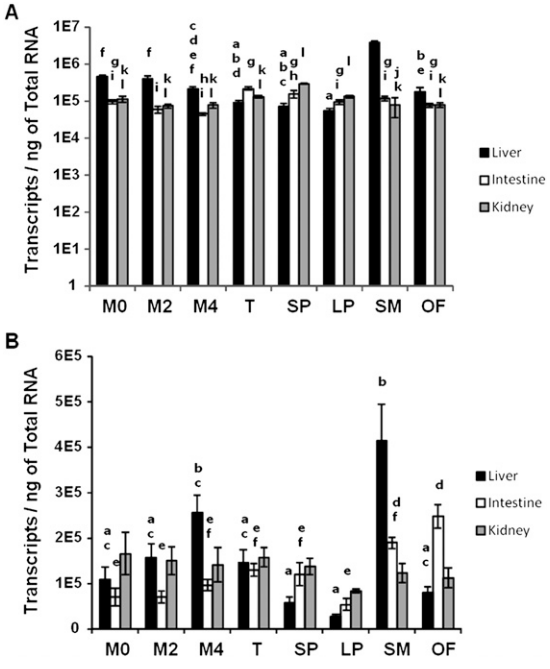

Fig. S3. Expression level of *cyp27a1* and *HMG-CoA reductase* in sea lamprey tissues during and after biliary atresia. (*A*) *cyp27a1* mRNA, *y* axis is in logarithmic scale. (*B*) *HMG-CoA reductase* mRNA, *y* axis is in linear scale. M0, larval; M2–M4, metamorphic stages 2–4; T, newly transformed; SP, small parasite; LP, large parasite; SM, spermiating male; OF, ovulatory female stages. Solid bars: liver; open bars: intestine; gray bars: kidney. Vertical lines represent mean ±1 SEM ($n \geq 3$ for all datapoints). Within each panel, bars with same lowercase labels indicate $P > 0.05$. In *B*, ANOVA showed $P > 0.05$ for kidney mRNA levels.

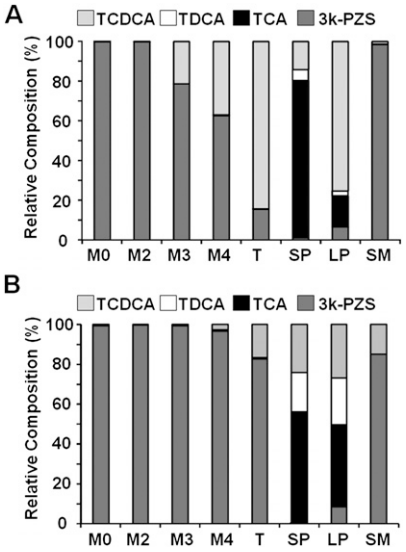

Fig. S4. Relative bile salt composition in sea lamprey liver and intestine during and after biliary atresia. Relative composition of the four bile salts is presented to show shifts of bile salt types through life stages. (*A*) Liver; (*B*) Intestine. 3-dehydro- petromyzonol sulfate (3k-PZS), taurocholic acid (TCA), taurochenodeoxycholic acid (TCDCA), taurodeoxycholic acid (TDCA).

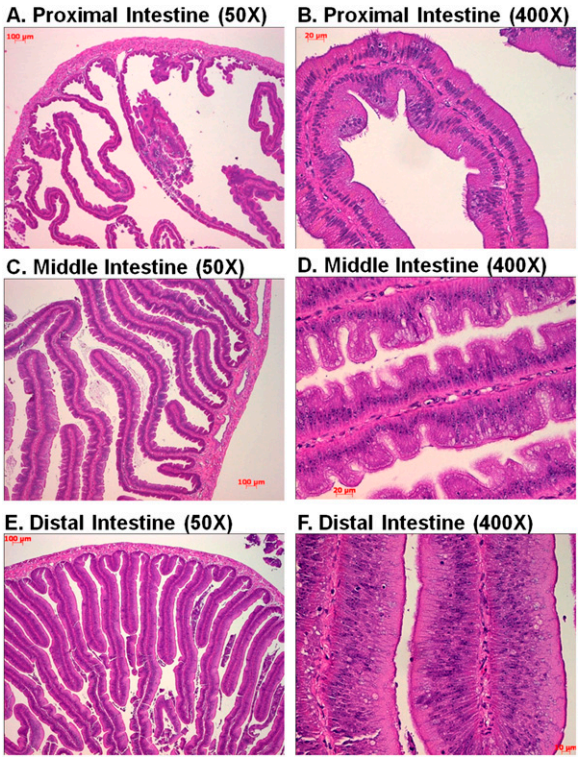

Fig. S5. H&E of Intestine tissue after ex vivo incubation. Intestinal tissue was fixed in 4% PFA after 24 h of incubation in whole intestine sac experiment. (*A*, *C*, and *E*) 50× magnification of proximal, middle, and distal segment, respectively; (*B*, *D*, and *F*) 400× magnification of proximal, middle, and distal segment, respectively.

Table S1. Bile salt intermediates in intestine of parasites

Bile salt intermediates	7α-Hydroxycholesterol	7α-Hydroxy-4-cholesten-3-one	7α-Hydroxy-5β-cholestan-3-one	5β-Cholestane-3α, 7α, 12α, 27α-tetrol	3α,7α,12α-Trihydroxy-5β-cholestanoic acid
MW	402.66	400.64	402.66	436.67	450.33
SP	+	±	+	±	–
SP	+	±	+	+	–
LP	+	+	+	±	–
LP	+	+	+	±	–

Intestine extracts were submitted to full-scan ES ± for detection of bile salt intermediates. LP, large parasite (n = 2); SP, small parasite (n = 2) Total sample size of parasitic intestine is 4. +, detection of this molecular weight occurred in ES+ mode; –, detection of this molecular weight occurred in ES– mode. ±, detection of this molecular weight occurred in both ES+ and ES– modes.

Table S2. Quantitative RT-PCR related sequences

Gene ID	Sequence for quantitative RT-PCR
cyp7a1	CAACATGTCGGCGCTCATCGC***CCTCCGAATACAACTCA***ATGACACGCTGTCTCGCATG
cyp27a1	TCTGGCCAAAATGTCATTCCTT***AAGGCTGTCATCAAAG***AGATTCTCAGACTGTATCCAGTGGTGCC
HMG-CoA Reductase	AGGTGTCCGGCAGTTGGAGA***TCATGTGCTCATTTGG***TTGCTTCTCCATCCTGGCC

Sequences listed are partial sequences selected as amplicons in quantitative RT-PCR experiments. The underlined sequences indicate 5′ and 3′ primers of each amplicon. Sequences used for probes are in both bold and italic font.

Table S3. Optimum of UPLC-MS/MS parameters for each analyte

Compound	MRM *m/z*	Cone (V)	Collision energy (V)	Dwell (s)	Retention time (min)
TCA	514.4 > 123.9	60	50	0.1	3.03
3k-PZS	471.3 > 96.8	60	50	0.1	3.15
TDC	498.4 > 123.9	60	50	0.1	3.47
TCDC	498.0 > 123.0	50	50	0.1	3.68
[$^2H_5$]3k-PZS	476.3 > 97.8	60	50	0.1	3.15
[$^2H_4$]TCA	518.4 > 123.6	58	46	0.1	3.03

A Waters Quattro Premier XE tandem quadruple mass spectrometer coupled to a Waters Acquity ultraperformance liquid chromatography (UPLC) system was used. Separation was achieved by using a Waters C18 column (ACQUITY UPLC BEH 1.0 × 50 mm, 1.7-μm particle size) with oven temperature at 50 °C. The mobile phase was a gradient established between solvent A (10 mM TEA in H_2O) and solvent B (MeOH) at a flow rate of 0.150 mL/min. Baseline separation was achieved by using the gradient started at 88% of A and 12% of B and followed by linear increasing of B to 50% in 3.0 min and 70% in 4.0 min. Data were collected in centroid mode with a scan range of 50–1,000 *m/z*. MRM measurements of the analytes and internal standards were performed using individually optimized cone voltage and collision energy.