

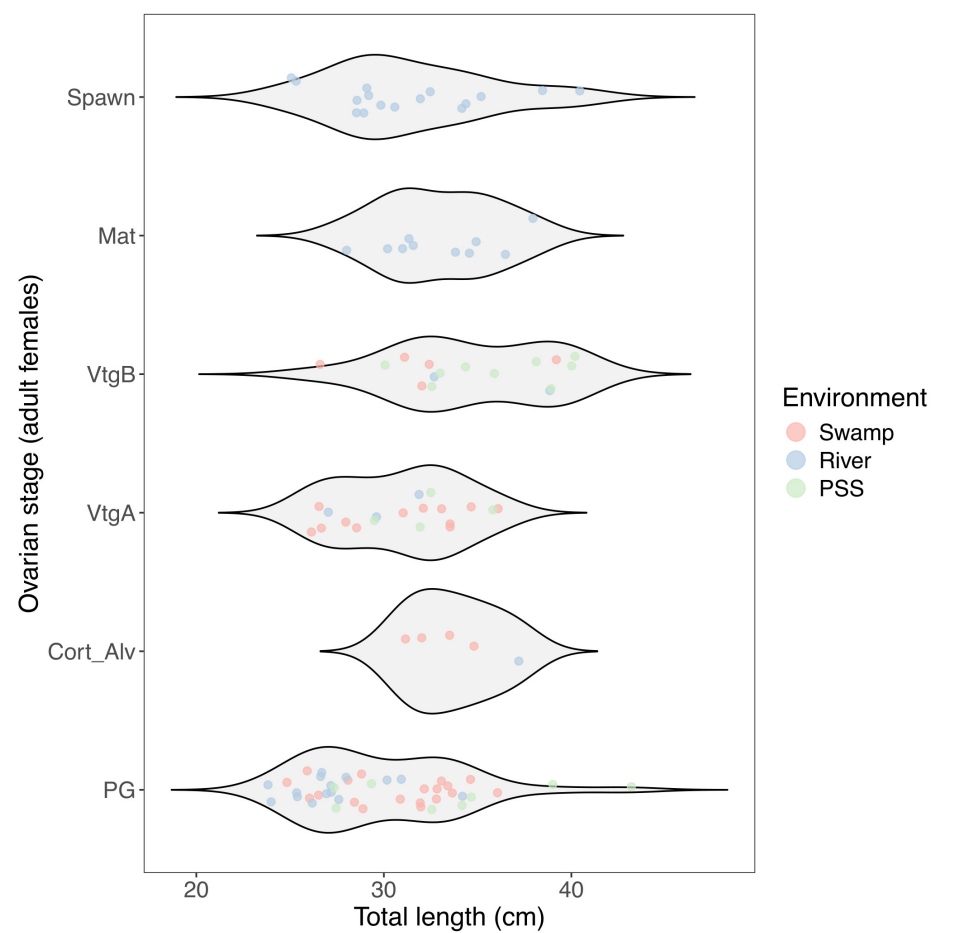


FIGURE S1 | Total length distribution of *Prochilodus magdalenae* females across ovarian developmental stages. Violin plots show the distribution of total length (cm) for each ovarian stage (PG, primary growth; Cort_Alv, cortical alveoli; VtgA, initial vitellogenesis; VtgB, final vitellogenesis; Mat, mature; Spawn, spawning), with individual observations overlaid. Most females fell within or above the reported size range at first sexual maturity for *P. magdalenae* (≈28–32 cm TL), consistent with sexually mature adult condition. A subset of smaller females in the primary growth stage (below 28 cm TL) may represent either reproductively resting adults or immature individuals; these cannot be distinguished histologically based on ovarian stage alone.

TABLE S2 | Number of samples collected each month in the three environments: Swamp, River, and Piscícola San Silvestre (PSS).

Month	Swamp	River	PSS
Jul	9	8	13
Aug	4	6	15
Sep	7	9	18
Oct	5	15	13
Nov	6	14	16
Dec	11	16	8
Jan	10	10	12
Feb	6	5	4
Mar	14	0	8

TABLE S3 | Non-parametric analysis of gonadosomatic index (GSI) among environments and ovarian developmental stages. Differences among GSI groups were evaluated using non-parametric Kruskal-Wallis tests, followed by Dunn's multiple comparisons with Bonferroni correction. The table reports pairwise comparisons, adjusted p-values, and factor. Adjusted p-value, Bonferroni-adjusted p-value; Cort_Alv, cortical alveoli; GSI, gonadosomatic index; Mat, mature; PG, primary growth; PSS, Piscícola San Silvestre; Spawn, spawning; VtgA, initial vitellogenesis; VtgB, final vitellogenesis.

Pairwise comparison	Adjusted p-value	Factor
PSS - River	0.00019577	Environment
PSS - Swamp	2.29E-09	Environment
River - Swamp	0.05069909	Environment
Cort_Alv - Mat	1	Stage
Cort_Alv - PG	0.44930459	Stage
Mat - PG	1.7373E-10	Stage
Cort_Alv - Spawn	1	Stage
Mat - Spawn	0.01979889	Stage
PG - Spawn	0.00378164	Stage
Cort_Alv - VtgA	1	Stage
Mat - VtgA	0.00159131	Stage
PG - VtgA	0.04741451	Stage
Spawn - VtgA	1	Stage
Cort_Alv - VtgB	1	Stage
Mat - VtgB	1	Stage
PG - VtgB	7.0913E-22	Stage
Spawn - VtgB	0.01549156	Stage
VtgA - VtgB	0.00032766	Stage
Aug - Dec	7.0051E-05	Month
Aug - Feb	0.10843914	Month
Dec - Feb	1	Month
Aug - Jan	0.00043192	Month
Dec - Jan	1	Month
Feb - Jan	1	Month
Aug - Jul	1	Month
Dec - Jul	9.3862E-05	Month
Feb - Jul	0.18077731	Month
Jan - Jul	0.00064044	Month
Aug - Mar	1	Month
Dec - Mar	0.00197527	Month
Feb - Mar	0.67450749	Month
Jan - Mar	0.00891825	Month
Jul - Mar	1	Month
Aug - Nov	1	Month
Dec - Nov	0.00013566	Month
Feb - Nov	0.26939668	Month
Jan - Nov	0.00096683	Month
Jul - Nov	1	Month
Mar - Nov	1	Month
Aug - Oct	1	Month
Dec - Oct	1.1404E-10	Month



TABLE S3 | (Continued)

Pairwise comparison	Adjusted p-value	Factor
Feb - Oct	0.00023312	Month
Jan - Oct	3.5267E-09	Month
Jul - Oct	1	Month
Mar - Oct	0.59659491	Month
Nov - Oct	0.67324961	Month
Aug - Sep	1	Month
Dec - Sep	3.1619E-05	Month
Feb - Sep	0.12450987	Month
Jan - Sep	0.00025911	Month
Jul - Sep	1	Month
Mar - Sep	1	Month
Nov - Sep	1	Month
Oct - Sep	1	Month

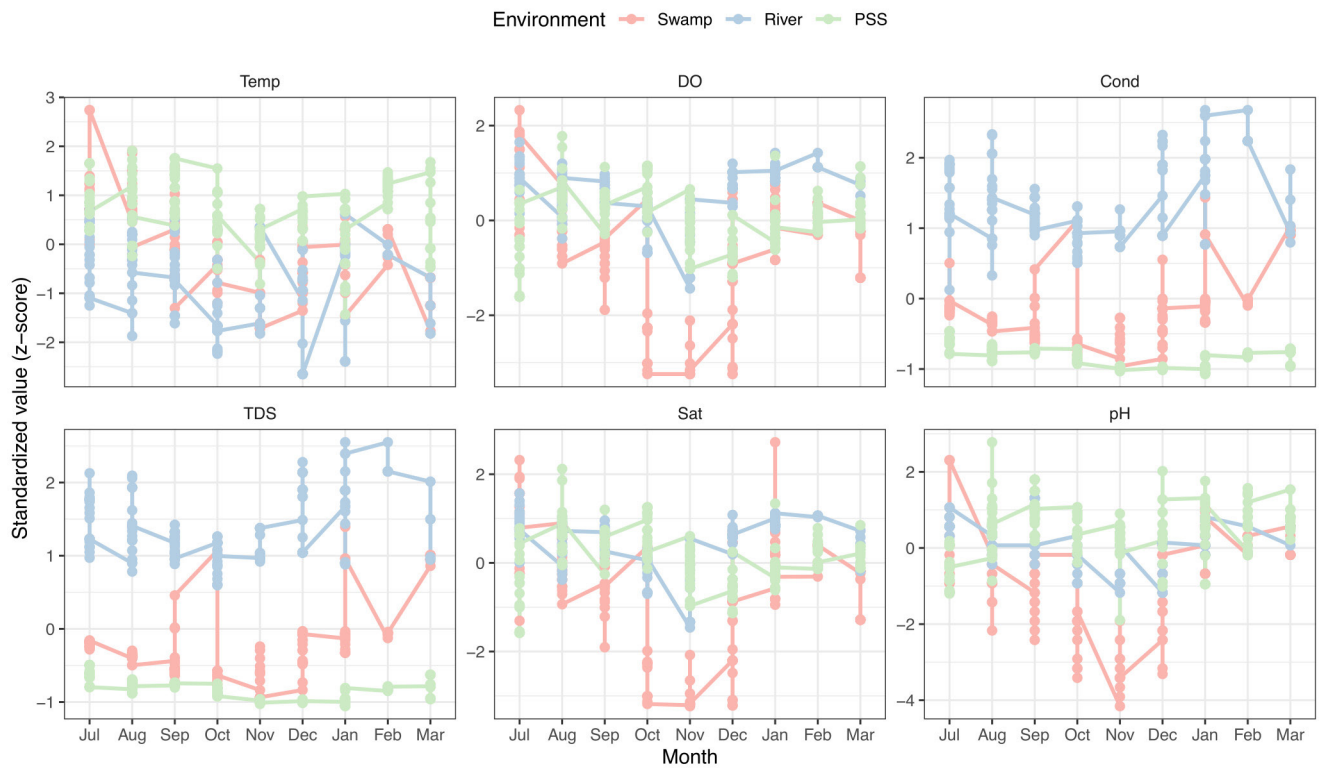


FIGURE S4 | Standardized (z-score) monthly profiles of water physicochemical parameters across the three study environments (Swamp, River, and Piscícola San Silvestre, PSS). Panels show water temperature (Temp), dissolved oxygen (DO), electrical conductivity (Cond), total dissolved solids (TDS), oxygen saturation percentage (Sat), and pH. Points represent individual measurements and lines connect monthly means for each environment. Standardization was performed within each variable to facilitate comparison of temporal variability among environments. The PSS environment exhibited reduced month-to-month variability across most parameters compared with the Swamp and River, indicating greater physicochemical stability during the study period.

TABLE S5 | Non-parametric analysis of plasma estradiol (E_2) concentrations among environments and ovarian developmental stages. Differences in E_2 levels among groups were evaluated using Kruskal-Wallis tests, followed by Dunn's multiple comparisons with Bonferroni correction. The table reports pairwise comparisons, adjusted p-values, and factor. Adjusted p-value, Bonferroni-adjusted p-value; Cort_Alv, cortical alveoli; E_2 , plasma estradiol; Mat, mature; PG, primary growth; PSS, Piscícola San Silvestre; Spawn, spawning; VtgA, initial vitellogenesis; VtgB, final vitellogenesis.

Pairwise comparison	Adjusted p-value	Factor
PSS - River	0.00043866	Environment
PSS - Swamp	0.00532108	Environment
River - Swamp	1	Environment
Cort_Alv - Mat	1	Stage
Cort_Alv - PG	1	Stage
Mat - PG	0.16394556	Stage
Cort_Alv - Spawn	1	Stage
Mat - Spawn	0.39468374	Stage
PG - Spawn	1	Stage
Cort_Alv - VtgA	1	Stage
Mat - VtgA	0.06147167	Stage
PG - VtgA	1	Stage
Spawn - VtgA	1	Stage
Cort_Alv - VtgB	1	Stage
Mat - VtgB	1.4765×10^{-06}	Stage
PG - VtgB	2.6572×10^{-05}	Stage
Spawn - VtgB	3.0474×10^{-06}	Stage
VtgA - VtgB	0.00010005	Stage
Aug - Dec	1	Month
Aug - Feb	1	Month
Dec - Feb	1	Month
Aug - Jan	1	Month
Dec - Jan	1	Month
Feb - Jan	1	Month
Aug - Jul	0.82133686	Month
Dec - Jul	1	Month
Feb - Jul	0.46783175	Month
Jan - Jul	1	Month
Aug - Mar	1	Month
Dec - Mar	0.89729004	Month
Feb - Mar	1	Month
Jan - Mar	1	Month
Jul - Mar	0.00118008	Month
Aug - Nov	1	Month
Dec - Nov	1	Month
Feb - Nov	1	Month
Jan - Nov	1	Month



TABLE S5 I (Continued)

Pairwise comparison	Adjusted p-value	Factor
Jul - Nov	1	Month
Mar - Nov	0.9707818	Month
Aug - Oct	1	Month
Dec - Oct	1	Month
Feb - Oct	1	Month
Jan - Oct	1	Month
Jul - Oct	0.57134341	Month
Mar - Oct	1	Month
Nov - Oct	1	Month
Aug - Sep	1	Month
Dec - Sep	1	Month
Feb - Sep	1	Month
Jan - Sep	1	Month
Jul - Sep	0.07967093	Month
Mar - Sep	1	Month
Nov - Sep	1	Month
Oct - Sep	1	Month

TABLE S6 I Significance of smooth terms for environmental variables included in the Generalized Additive Model (GAM) explaining 79% of the deviance in plasma estradiol concentrations. Abbreviations: Cond, water conductivity; EDF, effective degrees of freedom of the fitted smooth term; Ref.df, reference degrees of freedom; Sat, oxygen saturation percentage; TDS, Total dissolved solids; Temp, water temperature. p-value < 0.05, significant values of the explanatory variables.

Environmental cue	EDF	Ref.df	Chi ²	p value
TDS	1.00	1.00	5.40	0.022
Temp	1.00	1.00	4.54	0.036
Sat	2.75	3.37	3.07	0.025
Cond	4.88	5.93	3.43	0.004

TABLE S7 | Sensitivity analysis of smooth terms in the ordinal GAM for oogenesis. Significance of smooth terms across the base ordinal Generalized Additive Model (GAM) and alternative sensitivity scenarios evaluating the treatment of the mature ovarian stage (Mat). Sensitivity scenarios included S1, reassignment of Mat to VtgB; S2, exclusion of Mat; and S3, collapsing VtgB and Mat into a single category. Reported values correspond to approximate p-values derived from the smooth terms of the GAM. Seasonal interactions represent season-specific smooth functions. Cort_Alv, cortical alveoli; DO, dissolved oxygen; EDF, effective degrees of freedom of the fitted smooth term; GAM, Generalized Additive Model; Mat, mature; PG, primary growth; Spawn, spawning stage; TDS, total dissolved solids; Temp, water temperature; VtgA, early vitellogenesis; VtgB, late vitellogenesis; X^2 , approximate chi-square statistic.

Base model (six-stage ordinal response):

PG, Cort_Alv, VtgA, VtgB, Mat, Spawn

Smooth term	EDF	X^2	p-value
s(TDS)	1.78	0.68	0.739
s(TDS):Jul-Aug	4.64	20.99	5.5×10^{-4}
s(TDS):Sep-Nov	1.00	~0	1.000
s(TDS):Dec-Mar	2.79	4.96	0.213
s(Temp)	2.16	59.45	<0.001
s(Temp):Jul-Aug	~0	>10 ⁵	<0.001
s(Temp):Sep-Nov	1.00	31.63	<0.001
s(Temp):Dec-Mar	3.70	18.38	0.0018
s(DO)	1.00	5.72	0.017
s(DO):Jul-Aug	1.63	0.40	0.776
s(DO):Sep-Nov	3.31	15.08	0.0048
s(DO):Dec-Mar	~0	>500	<0.001

S1: Mat recoded as VtgB

Smooth term	edf	X^2	p-value
s(TDS)	1.64	1.22	0.573
s(TDS):Jul-Aug	4.77	20.12	0.032
s(TDS):Sep-Nov	~0	~0	0.500
s(TDS):Dec-Mar	2.81	6.67	0.099
s(Temp)	2.80	6.18	0.190
s(Temp):Jul-Aug	~0	~0	0.999
s(Temp):Sep-Nov	1.00	3.14	0.076
s(Temp):Dec-Mar	3.31	12.60	0.016
s(DO)	1.00	4.52	0.033
s(DO):Jul-Aug	2.64	4.07	0.364
s(DO):Sep-Nov	3.30	14.04	0.007
s(DO):Dec-Mar	~0	~0	0.999

S2: Mat excluded

Smooth term	edf	X^2	p-value
s(TDS)	1.00	4.63	0.031
s(TDS):Jul-Aug	3.53	20.50	9.9×10^{-4}
s(TDS):Sep-Nov	1.00	4.30	0.038
s(TDS):Dec-Mar	3.07	18.36	0.0014
s(Temp)	5.02	33.29	1.1×10^{-5}
s(Temp):Jul-Aug	1.05	~0	0.991
s(Temp):Sep-Nov	1.00	10.41	0.0013
s(Temp):Dec-Mar	~0	>10 ⁵	<0.001
s(DO)	1.00	0.21	0.643
s(DO):Jul-Aug	2.40	2.38	0.394
s(DO):Sep-Nov	2.39	3.45	0.281
s(DO):Dec-Mar	1.00	0.07	0.794

S3: VtgB and Mat collapsed

Smooth term	edf	X^2	p-value
s(TDS)	1.64	0.48	0.780
s(TDS):Jul-Aug	3.77	7.46	0.111
s(TDS):Sep-Nov	1.00	~0	1.000
s(TDS):Dec-Mar	2.81	5.05	0.210
s(Temp)	2.80	54.52	<0.001
s(Temp):Jul-Aug	~0	>10 ⁵	<0.001
s(Temp):Sep-Nov	1.00	15.97	6.4×10^{-5}
s(Temp):Dec-Mar	3.31	9.62	0.051
s(DO)	1.00	6.57	0.010
s(DO):Jul-Aug	2.64	4.53	0.310
s(DO):Sep-Nov	3.30	16.28	0.0027
s(DO):Dec-Mar	~0	>800	<0.001

TABLE S8 | Sensitivity analysis of model performance for the ordinal GAM of ovarian development. Summary statistics of alternative ordinal Generalized Additive Models (GAMs) fitted to ovarian developmental stage under different treatments of the mature ovarian stage (Mat). Scenarios include the base six-stage model, S1, conservative reclassification of Mat as vitellogenic B (VtgB), S2, exclusion of Mat, and S3, collapsing of VtgB and Mat into a single category. Reported metrics include sample size (n), percentage of deviance explained, Akaike Information Criterion (AIC), and Bayesian Information Criterion (BIC). Together with the smooth-term results presented in Tab. S4, these metrics indicate that model performance and inference were robust to alternative treatments of the mature ovarian stage.

Scenario	n	Deviance explained (%)	AIC	BIC
Base (six-stage response)	225	45.34	491.12	617.24
S1: Mat reclassified as VtgB	225	43.09	446.13	573.84
S2: Mat excluded	211	42.96	418.50	535.96
S3: VtgB and Mat collapsed	225	43.09	444.13	568.43

HOW TO CITE THIS ARTICLE

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