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INVITED PAPER

The Effects of Rearing Environment on Organization of the Olfactory System and Brain of Juvenile Sockeye Salmon, *Oncorhynchus nerka*

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Synopsis Pacific salmon (*Oncorhynchus* spp.) hatch and feed in freshwater habitats, migrate to sea to mature, and return to spawn at natal sites. The final, riverine stages of the return migrations are mediated by chemical properties of the natal stream that they learned as juveniles. Like some other fish, salmon growth is asymptotic; they grow continuously throughout life toward a maximum size. The continued growth of the nervous system may be plastic in response to environmental variables. Due to the ecological, cultural, and economic importance of Pacific salmon, individuals are often reared in hatcheries and released into the wild as juveniles to supplement natural populations. However, hatchery-reared individuals display lower survivorship and may also stray (i.e., spawn in a non-natal stream) at higher rates than their wild counterparts. Hatchery environments may lack stimuli needed to promote normal development of the nervous system, thus leading to behavioral deficits and a higher incidence of straying. This study compared the peripheral olfactory system and brain organization of hatchery-reared and wild-origin sockeye salmon fry (*Oncorhynchus nerka*). Surface area of the olfactory rosette, diameter of the olfactory nerve, total brain size, and size of major brain regions were measured from histological sections and compared between wild and hatchery-origin individuals. Hatchery-origin fish had significantly larger optic tecta, and marginally insignificant, yet noteworthy trends, existed in the valvula cerebelli (hatchery > wild) and olfactory bulbs (hatchery < wild). We also found a putative difference in olfactory nerve diameter (d_{min}) (hatchery > wild), but the validity of this finding needs further analyses with higher resolution methods. Overall, these results provide insight into the potential effects of hatchery rearing on nervous system development in salmonids, and may explain behavioral deficits displayed by hatchery-origin individuals post-release.

Introduction

Pacific salmon (*Oncorhynchus* spp.) are anadromous, semelparous teleost fish (Osteichthyes) native to the North Pacific Rim (Quinn 2018). Their ontogeny varies among species, but is characterized by several discrete morphological, physiological, and ecological stages: immediately after fertilization, females bury their embryos in the gravel of their natal streams or lakes and alevins (post-hatch embryos) complete yolk absorption in the gravel. Free-swimming fry then emerge from the gravel to feed, parr continue to feed in streams and lakes, until they migrate to sea as smolts, sub-adults feed and grow at sea, and maturing adults return to their natal freshwater habitats, where they spawn and die. Homing to natal sites for reproduction depends on the ability to recognize and respond to olfactory stimuli learned ("imprinted") as juveniles, prior to seaward migration (Dittman and Quinn 1996). Sensitive periods for olfactory imprinting have been demonstrated at the alevin stage and during parr-smolt transformation in many salmonids (Yamamoto et al. 2010; Bett et al. 2016; Havey et al. 2017; Armstrong et al. 2022). Indeed, the imprinting and homing processes are common to all salmonids; Pacific salmon differ in being

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semelparous, whereas the others are interoparous, and non-anadromous salmonids also imprint and home to natal streams within freshwater basins.

Pacific salmon represent one of the most economically valuable fisheries in North America (PACOFOC 2020). Sustaining healthy salmon populations is a primary concern from ecological, economic, and cultural standpoints. To supplement natural populations, juveniles are often reared in hatcheries and released into streams before seaward migration (Naish et al. 2007), although this practice has been met with some controversy (e.g., Hilborn 1992; Meffe 1992; Stewart 2015). Hatcheries greatly increase the survival of embryos, alevins, and juveniles prior to release, and thus typically produce more returning adults than would result from natural reproduction. However, hatcheries often house individuals at high density in physically barren environments (e.g., concrete raceways, as opposed to the complex habitat of streams and lakes occupied by wild fish), with constant, minimal current flow (Burrows and Combs 1968). These environmental differences, combined with genetic changes, cause wild- and hatchery-origin salmon to differ in such behavioral traits as aggression and competition (Berejikian et al. 1996; Tatara and Berejikian 2012), predator avoidance (Berejikian 1995), and migratory behavior (Goetz et al. 2015). There are also some indications that hatchery salmon have higher rates of straying (migration to non-natal spawning locations) than their wild counterparts (McIsaac 1990; Jonsson et al. 2003; Brenner et al. 2012), though many factors seem to affect straying (Labelle 1992; Unwin 1997; Pascual et al. 1995) and strict comparisons of straying between wild- and hatchery-origin conspecifics are difficult to conduct. Straying has natural advantages for salmonids, such as the colonization of new habitats (Milner and Bailey 1989) or avoidance of unfavorable environmental conditions (Leider 1989); however, individuals that stray may not supplement intended populations and can dilute the gene pools of native recipient populations (Quinn 1993). Consequently, interest in the development of the sensory systems associated with homing has great importance for salmon conservation, in addition to insights into neurobiology.

Studies on brain development among and within fish species may provide insights into differences between wild and hatchery-origin salmon. Brain size and organization (or the relative size of major brain regions) varies greatly among fish, and is associated with a range of ecological and behavioral parameters (e.g., Bauchot et al. 1988; Kotrschal and Palzenberger 1992; Yopak et al. 2007, 2009; Eifert et al. 2015; Salas et al. 2017; Axelrod et al. 2021). Similar interspecific variation is also documented in the peripheral nervous system, including the eye (Van Der Meer and Bowmaker 1995; Hasegawa et al. 2002; White et al. 2004) and olfactory rosette (Theiss et al. 2009; Atta 2013; Sarkar et al. 2014). According to the "Principle of Proper Mass," the size of major brain regions should reflect the relative importance of the functions that brain regions serves (Jerison 1973). Similarly, surface area (SA) of the olfactory rosette relative to body size may confer variation in olfactory capability (Theiss et al. 2009; Atta et al. 2013), although studies have yet to identify a direct link (Meredith and Kajiura 2010). Therefore, metrics including sensory SA, brain size, and size of brain regions are widely used as neuroanatomical proxies for sensory and/or behavioral specialization in fish (Triki et al. 2020, 2021).

It has been recognized that the rearing environment (RE), including varied sensory stimuli, can affect fish behavior and neural development in diverse and profound ways (Blaxter 1970; Ebbesson and Braithwaite 2012; Johnsson et al. 2014). In addition to interspecific variability in the brain, fish also exhibit indeterminate or asymptotic growth (Weatherley 1972; Sebens 1987) and lifelong neurogenesis (Zupanc 2006; Hinsch and Zupanc 2007; Ganz and Brand 2016), whereby brain and body grow continually throughout life, leading to a high degree of neural plasticity. Accordingly, ecological and life-history shifts (e.g., Bauchot et al. 1988; Salas et al. 2015; Edmunds et al. 2016; Laforest et al. 2020; Sauer et al. 2022) and varying environmental rearing conditions (e.g., Kihslinger et al. 2006; Na¨slund et al. 2019), often correlate with patterns of central nervous system organization. In particular, hatchery-reared fish often differ in nervous system growth and development compared to natural-origin conspecifics (Ebbesson and Braithwaite 2012). For example, hatchery-reared salmonids have smaller brains than wild counterparts (Marchetti and Nevitt 2003), even after controlling for artificial selection (Kihslinger and Nevitt 2005). Furthermore, alterations to the hatchery environment that more closely mimic natural conditions, including physical complexity (Kihslinger and Nevitt 2005; Kihslinger et al. 2006; Na¨slund et al. 2012) and fish density (Na¨slund et al. 2017; Na¨slund et al. 2019), positively correlate with brain size and cerebellum mass. These structural changes are also often accompanied by behavioral shifts, including alterations in locomotory behavior (Kihslinger and Nevitt 2005), further supporting the idea that changes in nervous system growth can affect function. The addition of structural enrichment in captive environments can also affect rates of brain cell proliferation in the forebrain in fish, including salmonids (Salvanes et al. 2013), although the drivers for this area unclear and results are inconsistent across species, enrichment type, and/or life stages (e.g., Lema et al. 2005; Kihslinger et al. 2006).

Hatchery and natural environments differ in many respects, but among them may be the nature and variability of chemical stimuli. Most studies of altered neural development have emphasized the effects of reduced or enhanced social and visual stimuli, but olfactory stimuli, such as alarm substances from injured conspecifics, can also affect development (Joyce and Brown 2020; Mokdad 2023). Many hatcheries incubate embryos partially or entirely on well or ground water because it is warmer and less variable in temperature in the winter than local river water, and often much lower in pathogens. The water may also be much lower in some chemical constituents that are abundant in streams, such as dissolved free amino acids, bile acids, and other organic compounds (Shen et al. 2015). However, no study to date had examined variation in the olfactory system between hatchery- and wild-origin salmonids to make inferences about differences in olfactory function between conspecifics.

This study compared morphological differences in the olfactory rosette SA, olfactory nerve diameter, brain size, and patterns of brain organization between hatchery-reared and wild sockeye salmon, *O. nerka*, fry. We test the hypothesis that wild-origin individuals would have larger brains than hatchery-reared conspecifics, especially in brain regions involved in olfactory processing.

Methods

Specimens were collected from the Cedar River and Cedar River Sockeye Salmon Hatchery, Washington, USA. As part of an integrated hatchery program, the hatchery and wild populations freely interbreed. Although the wild and hatchery fish sampled had different parents, the hatchery was founded by fish from the wild population, so differences related to genetic background were minimized. This hatchery incubates the embryos on groundwater piped from a nearby spring, whereas the river water drains a basin of mixed land use, including upper elevations protected from development to safeguard the city of Seattle's water supply, and residential areas, as it flows into Lake Washington. The Washington Department of Fish and Wildlife provided the wild-origin fish ($n = 8$) from their trap that routinely samples downstream migrating fry, directly after they emerged from the gravel. The average water temperature during incubation was 7.29°C ± 1.57°C. They also provided hatchery-origin fry ($n = 8$), where water temperature in the hatchery during incubation averaged 8.1°C ± 1.2°C. All specimens (wild- and hatchery origin) were collected on September 3, 2020. For all specimens, fresh body weight (mg) and fork length (FL; mm) were recorded. Upon collection, individuals were euthanized and immersion fixed in 10% neutral buffered formalin, and postfixed for up to 24 months.

For all wild and hatchery specimens, heads were dissected from the body just rostral to the pectoral fins and decalcified in 0.5 M ethylenediaminetetraacetic acid for a minimum of 2 days. Samples were then cryoprotected in 30% sucrose, embedded in Tissue-Tek optimal cutting temperature compound, and rapidly frozen in isopentane submerged in liquid nitrogen. Heads were subsequently cryosectioned coronally on a Leica CM 1860 from the anterior nares, including the olfactory rosette, to the caudal end of the medulla at a thickness of 30 µm, and every third section mounted onto gelatinized slides. After air drying, slide-mounted sections were then stained with 0.5% cresyl violet acetate solution, dehydrated through a graded ethanol series (95%, 95%, 100%, 100%), cleared (100% xylene, ×3) and cover-slipped with Permount mounting medium.

For the portions of the sample that included the olfactory nerve ($n = 7$ W, 8 H), tissue was sectioned serially at 20 µm and every section mounted onto gelatinized slides. The olfactory nerve for sample 3W was damaged during tissue processing and excluded from analyses. After air drying, olfactory nerve sections were dehydrated in a graded ethanol series (25%, 50%, 70%, 95%) and stained with 0.1% Luxol fast blue solution overnight at 60°C. Slides were then rinsed and differentiated in 0.05% lithium carbonate solution, cleared (100% xylene, ×3), and cover-slipped with Permount. All sections were photographed on a Leica DM1000 microscope equipped with an ICC50 HD camera (Fig. 1).

Olfactory nerve diameter was measured using QuPath image analysis software (Bankhead et al. 2017). Because sectioning could not be performed perfectly perpendicular to the nerve, the minimum diameter (d_{min}) of each section was measured. D_{min} for the left and right olfactory nerves were averaged for each section, and resultant values were averaged across all sections for each specimen. Olfactory rosette SA was also measured using Adobe Illustrator®. For each sample, the length of the epithelial surface was multiplied by the sum of section thickness and distance between successive sections (90 µm) for all sections of both the left and right rosette. These values were summed to get total SA values, which were averaged between both rosettes to get a final average rosette SA for each sample.

Brain subregions were identified using the criteria of Wullimann et al. (1996). These areas included the olfactory bulbs (OBs), telencephalon [divided into the area dorsalis, or dorsal telencephalon (DTe), and area ventralis, or ventral telencephalon (VTe)], diencephalon, optic tectum, valvula cerebelli, cerebellum (which includes all cerebellar tissue, excluding

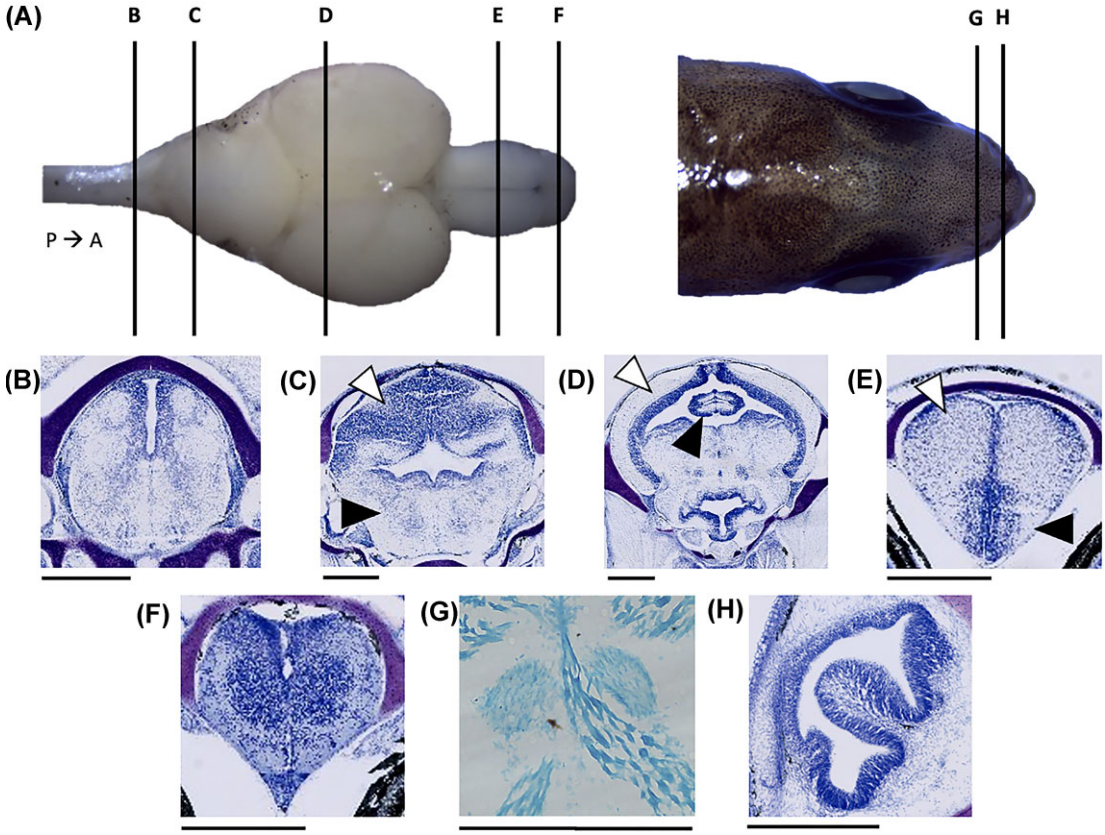

Fig. 1 Representativecoronal histological sections through the brain, olfactory nerve, and olfactory rosette of an *O. nerka* fry. (**A**) Dorsal schematic of the origin of sections B–H, with posterior (P) to anterior (A) orientation indicated. (**B**) Posterior medulla oblongata. (**C**) Posterior cerebellum (outlined arrow) and anterior medulla oblongata (solid arrow). (**D**) Optic tectum (outlined arrow), valvula cerebelli (solid arrow), and posterior diencephalon. (**E**) Telencephalon; area dorsalis (outlined arrow) and area ventralis (solid arrow). (**F**) Olfactory bulbs. (**G**) Olfactory nerve. Black arrow indicates left olfactory nerve. (**H**) Rosette with primary lamella (solid arrow).

the valvula, including corpus and vestibulocerebellum), and the medulla oblongata. The cross-sectional area of each brain structure was digitally traced using Adobe Photoshop. Total volume of each structure was calculated by multiplying the cross-sectional area of each section by the sum of section thickness and thickness of skipped sections (90 μm) and summing the results for each sample (Rosen and Harry 1990). Total brain volume was calculated as the sum of the volume of all brain structures. Because the brain was sectioned within the head, fresh brain volume could not be obtained and used to correct for the volumes of brain regions after ethanol dehydration. Therefore, a uniform tissue shrinkage was assumed across all samples and brain volume was not corrected for fixation.

Statistical analysis

Both body mass (one-tailed Welch's *t*-test, $t = 4.95$, $P = 0.001$) and FL (one-tailed *t*-test, $t = 4.35$, $P < 0.001$) differed significantly between groups (see Supplementary Fig. S1a and b). Body mass and FL were correlated (OLS regression, $F_{0.05}(1,14) = 68.9$, $P = < 0.001$; Supplementary Fig. S1c), and this relationship did not differ between REs (two-way ANCOVA, $F_{0.05}(1,14) = 1.37$, $P = 0.264$).

The best linear model (linear or log-transformed) was determined using Akaike Information Criterion (AICc) scores, which are best for small sample sizes to correct for bias (Cavanaugh 1997). The AICc was designed to minimize Kullback–Leibler information between the model generating the data and a fitted candidate model (Kullback and Leibler 1951). The model yielding the lowest AICc is considered to be the best fit (Lavin et al. 2008) and differences in AICc values (ΔAICc, or the difference between the best-fit model and the alternative) within 1–2 units can also be considered as having substantial support (Burnham and Anderson 1998). For all metrics, log-transformation was the best fit.

All data were then $\log_{10}$-transformed and OLS regressions were used to assess scaling relationships between olfactory structure metrics (rosette SA and d_{min}) and brain volume with body mass. OLS regressions were

Table 1 OLS regression output for combined, wild-only, and hatchery-only linear models for $\log_{10}$(olfactory structure) ~ $\log_{10}$(dependent variable) for rosette SA and d_{min} predicted from body mass, brain mass, and rosette SA, where appropriate

Model	**Rosette SA ~ Body size**				**d_{min} ~ Body size**			
Dataset	**Slope**	**Int.**	**Adj. R^2**	***P***	**Slope**	**Int.**	**Adj. R^2**	***P***
Combined	0.912	3.807	0.807	**<0.001**	0.308	1.44	0.414	**0.00574**
Wild	0.479	4.72	−0.148	0.765	0.818	0.338	−0.0399	0.420
Hatchery	0.595	4.55	0.777	**0.00227**	0.109	1.91	−0.0960	0.557
Model	**d_{min} ~ Brain volume**				**d_{min} ~ Rosette SA**			
Dataset	**Slope**	**Int.**	**Adj. R^2**	***P***	**Slope**	**Int.**	**Adj. R^2**	***P***
Combined	0.360	−1.43	0.199	0.0540	0.343	0.127	0.572	**<0.001**
Wild	−0.00226	2.12	−0.200	0.995	0.574	−1.20	0.470	0.0534
Hatchery	0.129	0.877	−0.103	0.579	0.178	1.10	−0.0842	0.524

Combined models included rearing environment (RE) as a categorical factor. Slope, intercept, adjusted R^2, and *P*-values are shown for each model. Bold indicates significant results.

also used to assess the relationship between brain region volume and the brain volume remainder (BVR), which equals the total brain volume minus the volume of the dependent variable. This mitigated the bias that can exist when a specific brain region of interest is scaled against total brain volume (which includes the volume of the region of interest) (Deacon 1990). Across the full dataset, there was a significant relationship between rosette SA and body mass [$F_{0.05}(1,14) = 63.54$, $P < 0.001$], olfactory nerve d_{min} and body mass [$F_{0.05}(1,13) = 10.9$, $P < 0.01$], total brain volume and body mass [$F_{0.05}(1,14)$, $P < 0.001$], and for all brain regions and BVR except for the OBs (see Tables 1 and 2). Therefore, allometric relationships were used to determine the effects of RE on all dependent variables.

For each component of the olfactory system (rosette SA and olfactory nerve d_{min}), two separate models were constructed across the combined dataset, assuming slopes were equal between wild- and hatchery-origin individuals. First, olfactory parameters were scaled against brain volume or body mass alone (model 1: rosette SA ~ body mass, d_{min} ~ body mass, d_{min} ~ total brain volume, and d_{min} ~ rosette SA). Then, from model 1, standardized residuals, or vertical deviations from the predicted slope, were calculated using the car package in R (Fox and Weisberg 2019). After confirming that residuals were not correlated with the relevant independent variable, residuals were then compared between groups (hatchery vs. wild origin) using one-tailed *t*-tests. Then, to assess effects of RE, a second model was run, which included RE as an explanatory variable (model 2: rosette SA ~ body mass + RE, d_{min} ~ body mass + RE, d_{min} ~ total brain volume + RE, and d_{min} ~ rosette SA + RE). From model 2, any significant effects of RE were analyzed using Tukey's honest significant difference (HSD) test. The dataset was then divided into wild and hatchery-origin individuals to determine scaling relationships between olfactory metrics within each group.

A similar approach was employed to examine variation in scaling relationships in the brain. Total brain volume was scaled against body mass (model 1: brain volume ~ body mass) and each brain region was scaled against BVR (model 1: brain region volume ~ BVR). Then, from model 1, standardized residuals were calculated. After confirming that residuals were not correlated with the relevant independent variable, they were compared between groups using one-tailed *t*-tests. Then, models with the inclusion of RE for brain volume (model 2: brain volume ~ body mass + RE) and brain region volume (model 2: brain region volume ~ BVR + RE) were analyzed. From model 2, any significant effects of RE were analyzed using Tukey's HSD test. The dataset was then divided into wild and hatchery-origin individuals to determine brain-body and brain region-BVR scaling relationships within each group.

Results

Olfactory nerve and rosette

Olfactory rosette SA scaled significantly with body mass across the full dataset (OLS regression, $y = 0.912x + 3.807$, $n = 16$; $r^2 = 0.807$, $P = 1.43e{-}08$), but there were no significant differences in residuals between wild- and hatchery-origin fry (one-tailed *t*-test, $t = -0.970$, $P = 0.168$) (Fig. 2a and c). Similarly, there was no significant effect of RE when it was added to the model ($P = 0.0622$), though it was marginally insignificant ($P < 0.1$). Olfactory nerve d_{min} also scaled significantly with body mass (OLS regression, $y = 0.308x + 1.44$, $n = 15$; $r^2 = 0.414$, $P = 0.006$) (Fig. 2b and d), and rosette SA ($y = 0.343x + 0.127$, $n = 15$; $r^2 = 0.572$, $P < 0.001$),

Table 2 OLS regression output for combined, wild-only, and hatchery-only regression models for $\log_{10}$(subregion volume) ~ $\log_{10}$(BVR) across all eight brain regions examined in this study (olfactory bulbs, dorsal telencephalon, ventral telencephalon, diencephalon, optic tectum, valvula cerebelli, cerebellum, and medulla oblongata)

Model	**Brain ~ Body**				**Olfactory bulbs ~ BVR**			
Dataset	**Slope**	**Int.**	**Adj. R^2**	***P***	**Slope**	**Int.**	**Adj. R^2**	***P***
Combined	0.470	8.84	0.525	**<0.001**	1.11	−2.98	0.181	0.0568
Wild	−1.45	13.0	0.122	0.210	0.350	4.52	−0.150	0.778
Hatchery	0.573	8.60	0.435	**0.0449**	2.33	−15.1	0.619	**0.0126**
Model	**Dorsal telencephalon ~ BVR**				**Ventral telencephalon ~ BVR**			
Dataset	**Slope**	**Int.**	**Adj. R^2**	***P***	**Slope**	**Int.**	**Adj. R^2**	***P***
Combined	0.814	0.618	0.474	**0.00192**	0.880	−0.355	0.242	**0.0306**
Wild	1.22	−3.33	0.325	0.0816	0.334	5.00	−0.148	0.765
Hatchery	0.965	−0.881	0.606	**0.0139**	0.843	0.0274	0.267	0.109
Model	**Diencephalon ~ BVR**				**Optic tectum ~ BVR**			
Dataset	**Slope**	**Int.**	**Adj. R^2**	***P***	**Slope**	**Int.**	**Adj. R^2**	***P***
Combined	1.27	−3.35	0.604	**<0.001**	0.709	2.46	0.580	**<0.001**
Wild	0.552	3.69	−0.0403	0.426	1.03	−0.639	0.756	**0.00311**
Hatchery	1.63	−6.82	0.778	**0.00233**	0.381	5.69	0.373	0.0634
Model	**Valvula cerebelli ~ BVR**				**Cerebellum ~ BVR**			
Dataset	**Slope**	**Int.**	**Adj. R^2**	***P***	**Slope**	**Int.**	**Adj. R^2**	***P***
Combined	1.21	−3.69	0.202	**0.0461**	0.978	−0.688	0.465	**0.00362**
Wild	0.584	2.43	−0.129	0.669	0.613	−2.89	−0.0722	0.495
Hatchery	0.441	4.000	−0.0920	0.546	1.12	−2.08	0.594	**0.0154**
Model	**Medulla oblongata ~ BVR**							
Dataset	**Slope**	**Int.**	**Adj. R^2**	***P***				
Combined	0.796	1.60	0.576	**<0.001**				
Wild	0.648	3.04	0.384	0.0598				
Hatchery	1.10	−1.40	0.582	**0.0169**				

Slope, intercept (int.), adjusted R^2, and *P*-values are shown for each model. Bold indicates significant results.

but not total brain volume ($y = 0.360x - 1.43$, $n = 15$; $r^2 = 0.199$, $P = 0.054$). No significant differences existed in residuals from any of these models between wild- and hatchery-origin individuals, except for d_{min} residuals corrected for total brain volume (W < H, one-tailed *t*-test, $t = -2.10$, $P = 0.035$), but, as the linear model had a marginally insignificant correlation, this must be met with caution. Similarly, only the model predicting d_{min} from total brain size showed a significant effect of RE ($P = 0.026$), but this effect was minimized during the multiple comparisons correction in the Tukey's HSD test (W < H, $P = 0.064$).

Brain size and organization

Total brain size varied significantly with body mass across the dataset (OLS regression, $y = 0.470x + 8.84$, $n = 16$; $r^2 = 0.525$, $P < 0.001$). In addition, residuals did not significantly differ between hatchery- and wild-origin fry (Fig. 3). RE also did not have a significant effect when added to the model ($P = 0.786$).

For all brain regions except the OBs ($y = 1.11x - 2.98$, $n = 16$; $r^2 = 0.181$, $P = 0.057$), volume scaled with BVR across the full dataset (see Table 2). DTe ($y = 0.814x + 0.618$, $n = 16$; $r^2 = 0.474$, $P = 0.002$), VTe ($y = 0.880x - 0.355$, $n = 16$; $r^2 = 0.242$, $P = 0.031$), diencephalon ($y = 1.27x - 3.35$, $n = 16$; $r^2 = 0.604$, $P < 0.001$), optic tectum ($y = 0.709x + 2.46$, $n = 16$; $r^2 = 0.580$, $P =$ XX), valvula ($y =$ s1.21$x - 3.69$, $n = 16$; $r^2 = 0.202$, $P = 0.046$), cerebellum ($y = 0.978x - 0.688$, $n = 16$; $r^2 = 0.465$, $P = 0.004$), and medulla ($y = 0.796x + 1.60$, $n = 16$; $r^2 = 0.576$, $P < 0.001$) all increase significantly with BVR (Fig. 4). Although allometric relationships could not always be recovered within groups, particularly in wild-origin fry (see Table 2), this is likely due to small sample sizes.

There was a significant difference in residuals between hatchery- and wild-origin individuals for the optic tectum (wild < hatchery, one-tailed *t*-test, $t = -2.63$, $P = 0.014$), and marginally insignificant differences ($0.05 < P < 0.1$) existed for the valvula (wild < hatch-

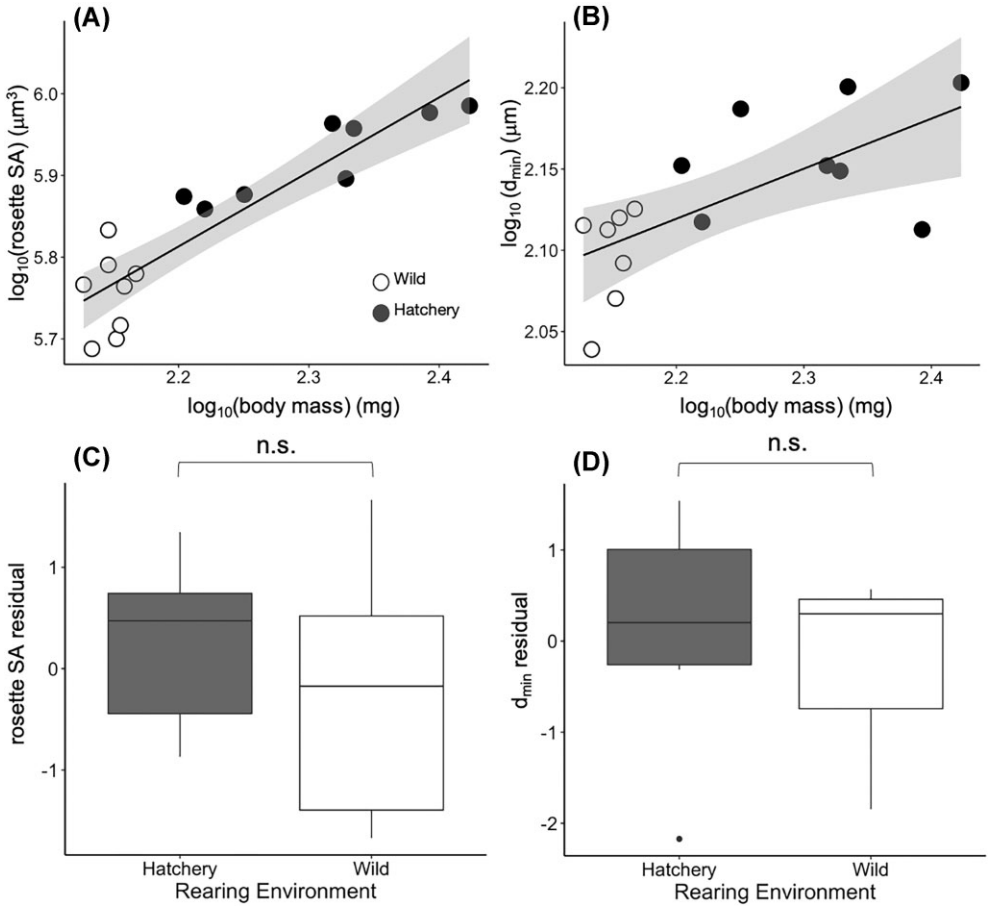

Fig. 2 OLS regressions of rosette SA **(A)** and olfactory nerve d_{min} **(B)** on body mass. Both metrics scaled significantly with body mass (rosette SA: $y = 0.912x + 3.807, n = 16; r^2 = 0.807, P = 1.43e{-}08$; d_{min}: $y = 0.308x + 1.44, n = 15; r^2 = 0.414, P = 0.00574$). Differences in residuals between REs were tested for both the rosette **(C)** and d_{min} **(D)** models. No significant differences in residuals between REs existed for either model.

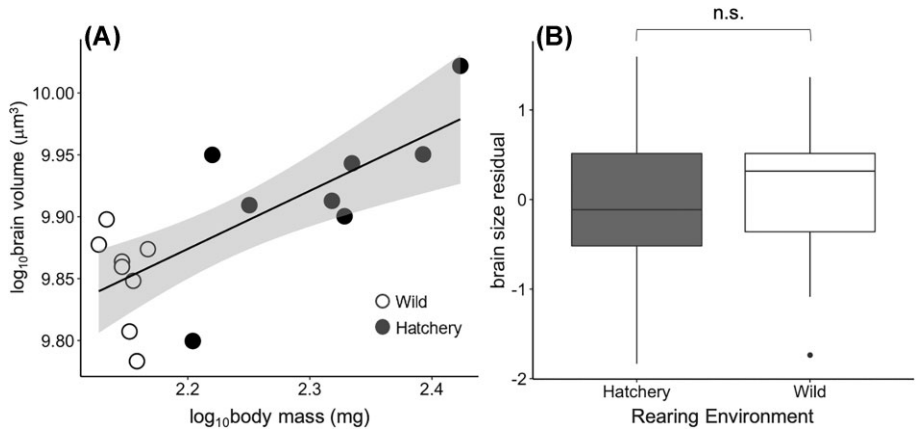

Fig. 3 (**A**) Linear relationship of $\log_{10}$(brain volume) and $\log_{10}$(body mass). A significant relationship existed for these two variables (OLS regression, $y = 0.470x + 8.84, n = 16; r^2 = 0.525, P = 0.0009$). **(B)** Residuals did not significantly differ between REs for this model.

ery, $t = -1.79$, $P = 0.052$) and OBs (wild < hatchery, $t = 1.46$, $P = 0.082$) (Fig. 4). Correspondingly, when added to the linear models, RE had a significant effect for the optic tectum (Tukey HSD, wild < hatchery, $P = 0.021$), and marginally insignificant effects for the valvula (wild < hatchery, $P = 0.0546$; Tukey HSD, $P = 0.107$) and OBs (wild > hatchery, $P = 0.0942$; Tukey HSD, $P = 0.173$).

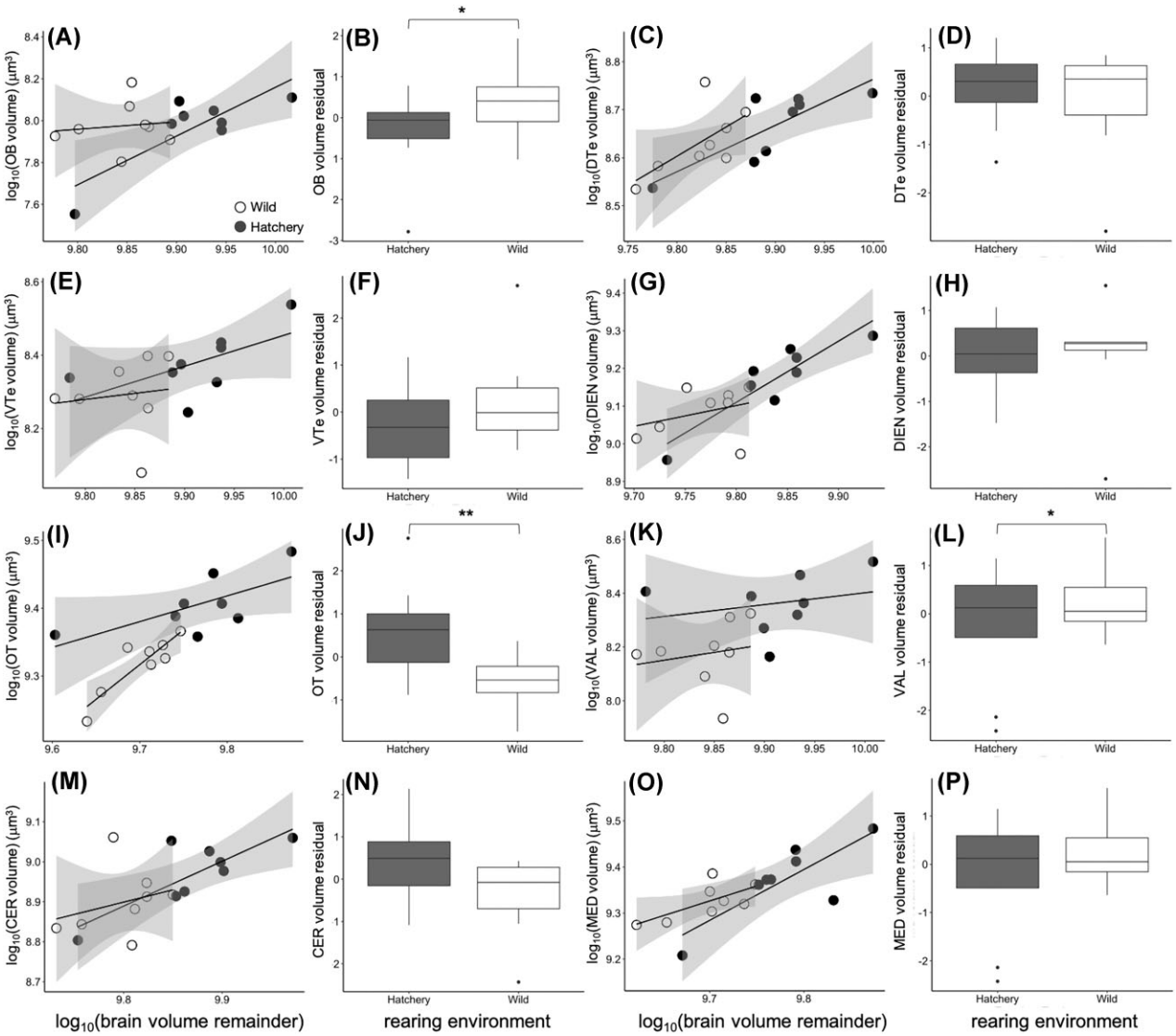

Fig. 4 Linear relationships between log-transformed brain region volumes and brain volume remainder for all brain regions, including the (**A**) olfactory bulbs, OBs; (**C**) dorsal telencephalon, DTe; (**E**) ventral telencephalon, VTe; (**G**) diencephalon, DIEN; (**I**); optic tectum, OT; (**K**) valvula cerebelli, VAL; (**M**) cerebellum, CER; and (**O**) medulla oblongata, MED (**O**). Residuals were compared between REs for the (**B**) OBs, (**D**) DTe, (**F**) VTe, (**H**) DIEN, (**J**) OT, (**L**) VAL, (**N**) CER, and (**P**) MED. A significant difference in residuals existed for the optic tectum ($W < H$, one-tailed *t*-test, $t = -2.63$, $P = 0.0136$), and marginally insignificant differences ($0.05 < P < 0.1$) existed for the valvula ($W < H$, $t = -1.79$, $P = 0.0523$) and olfactory bulbs ($W < H$, $t = 1.46$, $P = 0.0823$). $^{**}P < 0.05$; $^{*}0.05 < P < 0.01$.

Discussion

Numerous studies have sought to understand the impacts of RE on brain growth, particularly in salmonids, which may confer changes in cognitive and/or sensory capabilities (e.g., Marchetti and Nevitt 2003; Kihslinger and Nevitt 2005; Kihslinger et al. 2006; Na¨slund et al. 2019). However, to date, none have collectively examined morphometric differences in the peripheral olfactory system and across all brain areas between wild and hatchery-origin salmonids. Using OLS regressions, we found that hatchery-reared sockeye salmon fry had larger optic tecta. Further, statistically insignificant yet compelling trends ($0.05 < P < 0.1$) suggest that RE also affects OB (wild > hatchery) and valvula size (wild < hatchery). We discuss effects with $P < 0.1$ with caution, but recognize that the low sample size of this study may mask significant trends, which warrant future study. Although this study was not a functional analysis, the results may provide insight into the sensory and cognitive differences between individuals from different REs, which may influence behavior, including olfaction-mediated imprinting and homing. Rearing conditions in the hatchery have been implicated in behavioral deficits, as they lack many of the natural stimuli that fish would experience in streams, from physical structure to sensory inputs.

Olfactory system

The teleost nervous system is plastic, especially the olfactory system, including changes in lamellae number, epithelial morphology, olfactory sensory neuron (OSN) density, and patterning of synaptic connections in the OB that have been related to discrete imprinting and homing in salmonids (e.g., Kalinina et al. 2005; Kudo et al. 2009; Ochs et al. 2017; Rheinsmith et al. 2023). However, no study to date has examined differences in the peripheral olfactory system in hatchery-reared vs. wild salmonids. Previous studies have used epithelial SA as proxy for olfactory capability in fish (e.g., Schluessel et al. 2008; Theiss et al. 2009; Atta 2013; Ferrando et al. 2019). Theoretically, a larger SA in sockeye salmon fry may house more OSNs, which bind water-borne odorants, as their olfactory rosette is almost exclusively covered in sensory epithelium (Rheinsmith et al. 2023). In the current study, rosette SA did not differ between hatchery-origin and wild individuals (Fig. 2) However, our data suggest that RE may affect d_{min}, with hatchery individuals having larger residuals predicted from total brain volume. As there was no difference in brain size between hatchery and wild-origin individuals (Fig. 3), this may represent an interesting difference between these two groups. The olfactory nerve is comprised of axons of the OSNs, which synapse with mitral cells in the OB of the brain (reviewed by Kermen et al. 2013). A larger nerve diameter suggests a higher number of OSNs in the olfactory epithelium, which may affect olfactory capacity (Hamdani and Doving 2007; Camilieri-Asch et al. 2020). These data suggest that hatchery individuals may possess a higher number of OSNs; however, given d_{min} was not significantly larger in hatchery-origin fry when scaled against body size (Fig. 2b and d) or rosette SA, and rosette SA did not significantly differ between the two groups (Fig. 2), as one would expect whether hatchery-origin sockeye salmon truly had a higher number of OSNs in the epithelium, the biological relevance of this difference is unclear.

Differences in OSN number between hatchery- and wild-origin sockeye salmon fry would suggest differences in olfactory detection and/or processing capabilities between the two groups, whereby environmental cues during early life stages may modulate cell fates in the olfactory epithelium. The lifespan of vertebrate OSNs is only months (Mackay-Sim and Kittel 1991), as OSNs are exposed and vulnerable to the surrounding environment (Moulton 1974). In salmonids, olfactory receptor gene expression is sensitive to changes in developmental and environmental cues (Johnstone et al. 2011; Bett et al. 2018; Madsen et al. 2019). Patterns of OSN plasticity may, therefore, be partially influenced by olfactory experience (Wilson et al. 2004). In hatcheries, it is a common practice to incubate embryos in well water, and at least some part of the water used for subsequent rearing is often from spring or wells, as it is pathogen-free and thus requires less rigorous sterilization than normal stream water (Burrows and Combs 1968). However, well water can have lower levels of amino acids (potential odorants) present in associated streams (Dittman, unpublished data; Shen et al. 2015). Such a lack of chemical complexity in groundwater-based hatchery water may affect growth of the salmon olfactory system, and could hinder subsequent homing, a logic that contradicts what our data may suggest. However, as d_{min} is a rough metric, future work should examine OSN subtype distribution in the epithelium via immunohistochemistry (e.g., Gayoso et al. 2011; Braubach et al. 2012) or stereologically estimate of OSN number from transmission electron microscopy images of the olfactory nerve (Camilieri-Asch et al. 2020). These can then be coupled with olfactory tracking trials (e.g., Yamamoto et al. 2010), to confirm whether RE truly impacts olfactory detection and processing.

Encephalization and brain organization

Brain/body relationships have been established for nearly all vertebrate groups (e.g., Crile and Quiring 1940; Jerison 1973; Tsuboi et al. 2018), though we know far less about intraspecific variability in the brain and the drivers of plasticity within species. Although contentious, encephalization (or a larger than expected brain for a given body size) may predict cognitive ability and/or behavioral flexibility within species (Jerison 1973). Indeed, in the guppy (*Poecilia reticulata*), individuals artificially selected for a 5–10% increase in brain size relative to body size displayed higher proficiency in associative learning (Kotrschal et al. 2013) and spatial search learning tasks (Kotrschal et al. 2015). However, artificial selection likely creates a more dramatic difference in brain size than what may be created environmental differences alone. In the present study, there was no difference in relative brain size between hatchery- and wild-origin sockeye salmon fry. This differs from what previous work has shown when comparing brain organization in between wild and hatchery salmonids, where wild *O. mykiss* had larger brains than hatchery-reared conspecifics (Marchetti and Nevitt 2003). Similar differences have also been documented in other captive teleosts, including Atlantic cod (*Gadus morhua*) (Mayer et al. 2011) and mahseer (*Tor putitora*) (Ullah et al. 2021), although the causes for brain size reductions are unclear. Despite the phenotypic plasticity associated with RE, changes in the teleost brain are often impermanent, suggesting that plasticity may occur

without dependence on a developmental critical period. For example, increasing structural complexity of the RE resulted in larger brains in *S. salar* alevins, but this effect disappeared upon sampling of parr of the same group several months later that had been moved to barren tanks (Näslund et al. 2012). Impermanence of environmentally induced plastic changes in the brain may reduce their functional consequences over time. The wild-origin fish in our study were collected soon after emergence from the gravel, meaning that they experienced many of the external factors of their environments for a short time. If RE affects brain size, as previous studies suggest, changes in brain growth may require a critical period of exposure that was not captured in our narrow time window post-emergence. Future work should also examine sockeye salmon at later stages of development (parr and smolt), to determine whether brain size variation might be additive ontogenetically.

Previous work has shown that rearing salmonids in an enriched environment, including structural complexity and conspecific density, is associated with larger relative volume of the OBs, telencephalon, optic tectum, and/or cerebellum (Näslund et al. 2012; 2017; 2019), even when controlling for artificial selection and genetic variation (Kihslinger and Nevitt 2005; Kihslinger et al. 2006), which may have significant functional implications. However, many existing studies have focused on a small number of brain regions and/or have approximated brain region size from photographs (e.g., Marchetti and Nevitt 2003; Kihslinger and Nevitt 2005; Kihslinger et al. 2006). To date, no study has histologically examined how most major brain regions vary between wild vs. hatchery-origin salmonids; thus, important differences may have gone undetected. Estimation of brain volume from linear measurements can be highly problematic, and fail to take into account variation in the ventricles, leading to an over- or underestimation of the size of major brain regions (see Ullmann et al. 2010). In particular, over-estimation of optic tectum volume may be of particular concern; Pollen et al. (2007) showed the ellipsoid method, (which is based on linear measurements of major brain regions; Wagner 2001), can overestimate whole brain, telencephalon, and cerebellum by 24–33%, but can overestimate optic tectum volume by as much as 107% when compared to histological sections, which excludes the tectal ventricles. While some of this variation is likely due to dehydration during the staining process, this suggests that the comparison of brain volumes using these methods should be approached with caution, as it is unclear whether differences documented in previous studies have erroneously reported variation in ventricular volume rather than true tectal variation.

The present study excluded the ventricles from our measurements of the optic tectum and found a significant effect of RE on scaling of the optic tectum with the rest of the brain, with a significantly larger optic tectum in hatchery-origin individuals (Fig. 4I–J). This contradicts previous studies, where hatchery-reared *O. mykiss* and *T. putitora* have smaller body size-corrected optic tecta than wild conspecifics (Marchetti and Nevitt 2003; Ullah et al. 2022). The optic tectum receives the majority of retinal ganglion cell afferents, and is commonly associated with vision, visual processing, and sensory integration (reviewed by Northmore 2011). While wild environments are presumably richer in visual stimuli, diel light levels and visual complexity of the hatchery where our sockeye salmon were reared were not directly measured. However, point sources of light and/or abnormal light/dark cycles in hatchery environments may be driving tectal development. Alternatively, visual input is limited for wild salmonids at the alevin stage, as individuals are submerged in stream gravel, until emerging as fry (Quinn 2018). Thus, the optic tectum up to the fry stage in wild-origin salmon may not be fully developed and warrants further investigation.

Another interesting trend was seen in the valvula, where hatchery individuals had marginally larger valvula volumes than wild-origin fry (Fig. 4k and l). Although the function of this structure is not fully resolved, the valvula is involved in behavior patterns such as reflex conditioning, avoidance conditioning, and dorsal light response (Aronson and Herberman 1960; Kaplan et al. 1969; Yanagihara et al. 1993), and may also serve to process and integrate non-motor information (Chang et al. 2021). Its involvement in the dorsal light response is particularly interesting, as the same wild < hatchery trend was shown for the optic tectum, suggesting that the optical environment of hatcheries may differ significantly enough from that of natural streams, whereby related neural processing centers may be affected. Detection of visual cues is important in natal homing (Yano and Nakamura 1992), but it is unclear how variation in optic tectum and valvula volume may affect visual processing tasks between rearing habitats. As straying and post-release mortality is higher in hatchery-origin individuals, a putative increase in visual system development in hatchery fish may not be sufficient to counteract the other drawbacks of hatchery rearing.

The OBs receive primary projections from the olfactory epithelium, whereby OSN axons synapse with large mitral cells in topographically arranged glomeruli in fish (Laberge and Hara 2001; Hamdani and Døving 2007), and are associated with processing odors. In many vertebrates, the size of the OBs correlates with olfactory capability (Zelenitsky et al. 2011; Yopak et al.

2015), which suggests variation in OB size, to some extent, confers olfactory capacity (e.g., Gonzalez-Voyer et al. 2009; Yopak et al. 2010, 2015). Changes in OB size intraspecifically have been proposed to reflect changes in the relative importance of olfaction throughout life (e.g., Salas et al. 2017; Laforest et al. 2020). In the present study, a marginally insignificant, but noteworthy, difference in OB size (wild > hatchery) existed between hatchery- and wild-origin sockeye salmon fry, suggesting that hatchery rearing may have a negative impact on OB development. Although the effect was not significant ($P = 0.094$), low sample size in our study could have masked genuine, underlying effects. Similar trends in the OBs have been documented in Chinook salmon (*O. tshawytscha*) and rainbow trout (*O. mykiss*) (Marchetti and Nevitt 2003; Kihslinger et al. 2006), although differences were less localized and seen across most major brain regions examined. As OBs have been shown to scale hyperallometrically with the rest of the brain in some fish throughout life (e.g., Wagner 2003; Laforest et al. 2020), these differences may be more dramatic at other life stages. Larger OBs in wild-origin sockeye salmon suggest some degree of enhanced olfactory processing, which may be related to the increased chemical complexity of the rearing habitat. However, the DTe, which receives secondary and tertiary olfactory projections (Folgueira et al. 2004a, b), did not vary between groups. This brain region serves important roles in spatial learning and memory tasks in fish (Savage 1980; Rodriguez et al. 2002; Emmanuvel Rajan et al. 2011) and has been implicated in recognition of imprinted natal stream water in sockeye salmon (Bandoh et al. 2011). While trends might emerge with a larger sample size (see Fig. 4d), a lack of significant variation in the DTe makes it difficult to draw conclusions between brain organization and olfactory imprinting.

While differences in the nervous system between REs were identified in sockeye salmon, it is important to consider the limitations of this study. Although the histological approach to calculating brain volume is more accurate, excluded ventricular spaces, and allows for the assessment of a higher number of brain subregions, it is more time consuming and therefore resulted in smaller sample sizes. In particular, wild-origin individuals were very close in body size, which may not allow for an appropriate demonstration of true allometric relationships. Larger sample sizes (over a greater range of overlapping body mass between groups) would have improved our ability to detect differences between these groups and assess broader patterns of brain/body and brain region/brain scaling. Future work should consider comparing rates of growth of the brain and its subregions between REs over a longer period of time and across life stages, as the olfactory system and brain change throughout ontogeny in sockeye salmon (Rheinsmith et al. 2023) and other salmonids (Kudo et al. 2009). This would provide greater insight into the existence of an environment-associated critical period for brain development in salmonids (Naslund et al. 2012), and whether the impacts of hatchery-rearing are compounded over time.

Conclusions

This study provides key insights into the potential links between RE and brain and olfactory system organization in *O. nerka*. This species is subject to diverse behavioral and sensory demands throughout its life cycle, which involves long-distance migrations and dramatic habitat shifts. Previous work has shown low survivorship and high rates of straying in post-release, hatchery-origin *O. nerka*, and our results suggest this may be due, in part, to differences in brain development. Hatchery-origin fish had significantly larger optic tecta, and marginally larger valvula cerebelli (hatchery > wild), with larger d_{min} relative to brain size. In contrast, wild-origin sockeye salmon had marginally larger OBs (hatchery < wild), perhaps reflecting differences in sensory and/or functional capacity between the two groups. These findings contradict previous studies, which note enlargement of several key brain regions in wild-origin salmonids, which may be related to sample size or methodology used to assess the brain, where finer resolution techniques might help to detect more nuanced differences in brain morphology in relation to RE. As the Pacific salmon fishery represents one of the most valuable fisheries in North America, optimal post-release performance of hatchery-reared individuals is paramount from both an economic and ecological standpoint. Optimizing and enriching hatchery environments to more closely mimic wild habitats may support neural development and is a promising strategy to ensure *O. nerka* populations, as well as other Pacific salmon, will find their way home.

Author contributions

Conceptualization: T.P.Q., A.H.D., and K.E.Y.; Methodology: R.H.W., T.P.Q., A.H.D., and K.E.Y.; Data collection: R.H.W.; Formal analysis: R.H.W. and K.E.Y.; Visualization: R.H.W.; Supervision: K.E.Y.; Writing—original draft: R.H.W.; Writing—review & editing: R.H.W., T.P.Q., A.H.D., and K.E.Y.

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Supplementary data

Supplementary Data available at *ICB* online.

Conflict of interest

Authors declare that they have no competing interests.

Data availability

All data needed to evaluate the conclusions in the paper are present in the main text and/or the Supplementary Materials. Detailed numerical data will be made available to individuals upon request.

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