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Effects of hatchery rearing on brain structures of rainbow trout, *Oncorhynchus mykiss*

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Synopsis

In this study, we contrast brain morphology from hatchery and wild reared stocks to examine the hypothesis that in salmonid fishes, captive rearing produces changes in brain development. Using rainbow trout, *Oncorhynchus mykiss*, as a model, we measured eight regions of the salmonid brain to examine differences between wild and hatchery reared fish. We find using multiple analysis of covariance (MANCOVA), analysis of covariance (ANCOVA) and discriminant function analysis (DFA) that the brains of hatchery reared fish are relatively smaller in several critical measures than their wild counterparts. Our work may suggest a mechanistic basis for the observed vulnerability of hatchery fish to predation and their general low survival upon release into the wild. Our results are the first to highlight the effects of hatchery rearing on changes in brain development in fishes.

Introduction

The majority of wild stocks of Pacific salmon are exhibiting precipitous decline throughout their range (Moyle & Cech 2000). This is particularly evident in California where populations of three anadromous salmonids (Chinook salmon – *Oncorhynchus tshawytscha*, coho salmon – *O. kisutch* and steelhead trout – *O. mykiss*) are currently listed as state or federally endangered species (Yoshiyama et al. 2000). Some population declines have been quite dramatic. For example, the overall abundance of Chinook salmon in the Central Valley of California has decreased more than 75% since the 1950s (Yoshiyama et al. 1998, 2000, Yoshiyama 1999). In order to support continued economic harvest of these species, millions of dollars have been spent in rearing hatchery fish for wild stock enhancement. Yet the use of hatcheries to replace wild production of fishes has been widely criticized by ecologists and evolutionary biologists, partly because hatchery fish appear to be phenotypically less fit for survival in natural systems than wild fish (Brannon 1993).

Numerous studies have shown that brain structures often reflect the manner in which a species has adapted to a particular environment or selection regime (Masai et al. 1982, Brandstatter & Kotrschal 1990, Plognamm & Kruska 1990, Healy & Guilford 1990, Kotrschal & Palzenberger 1992, Huber & Rylander 1992, Ebinger & Rohrs 1995, Huber et al. 1997, Kotrschal et al. 1998, Ishikawa et al. 1999). Considerable progress has been made elucidating environmental effects on brain development in a variety of mammalian species, but few studies using animal models have placed these questions in an applied context. Fish brains are particularly amenable to relatively crude investigation since processing of sensory information occurs in large identifiable, anatomically distinct structures. These structures can be easily measured in the intact fish brain, and patterns can be

explored in relatively large data sets using multivariate statistical techniques (Brandstatter & Kotrschal 1990, Kotrschal & Palzenberger 1992, Huber & Rylander 1992, Huber et al. 1997, Kotrschal et al. 1998).

In this study, we adopted a simple ecomorphological approach to screen for possible influences of hatchery rearing practices on the brains of a rainbow trout, *Oncorhynchus mykiss*. We compared brain morphology from hatchery and wild reared stocks to examine the hypothesis that in salmonid fishes, captive rearing produces changes in brain development.

Methods

The study was conducted between October 1999 and June 2000. All fish were brought to the laboratory (John Muir Institute of Ecology at UC Davis) where they were held for less than one day. Scale samples and otoliths were removed for age identification of wild fish. Sex and age were determined upon dissection.

Animal collection

To control for genetic variability (Ishikawa et al. 1999), we sampled a total of 99 brains from two strains of hatchery fish and two geographically distant populations of wild fish. Whitney strain rainbow trout ($N = 35$) were obtained from Nimbus Trout Hatchery, Rancho Cordova, CA and Shasta strain rainbow trout ($N = 16$) were obtained from Darrah Springs State Fish Hatchery, Red Bluff, CA. Whitney strain has been in production continuously since 1917, whereas the Shasta strain has been propagated since the early 1950s (Barngrover 1990). The strains are thus considered to be genetically unique. Wild fish were obtained by backpack electrofishing in two creeks in Northern California, Big Valley Creek, Lake County, CA ($N = 37$), and Little Chico Creek, Butte County, CA ($N = 11$). The streams containing wild fish were chosen because they had not been stocked with hatchery fish according to regional experts (P. Moyle & P. Maslin personal communication). Big Valley Creek fish were collected between October and December 1999. Little Chico Creek fish were collected in early March 2000. The hatchery fish from both sources ranged in age from 1 to 2 years old, and the wild fish ranged in age from 1 to 4 years old. Hatchery fish tended to be larger (111–290 mm standard length) than wild fish (95–215 mm standard length).

Tissue preparation

Each fish was deeply anesthetized (MS-222, buffered 3-Aminobenzoic acid ethyl ester), weighed (± 0.01 g) and measured (standard length, ± 0.5 mm) prior to perfusion. Fish were perfused intracardially with chilled heparinized PO_4-buffered saline followed by Bouin's fixative. The brains were removed and postfixed in Bouin's for 24 h and then photographed dorsally and ventrally using a Nikon HFX Microphotography Unit mounted on a dissecting scope (Wild M5A). Photographs were digitized and the linear dimensions of four brain divisions (olfactory bulb, telencephalon, optic tectum and cerebellum) were measured. As an additional potential indicator of olfactory system growth, we also measured the width of the olfactory nerve.

Measurements were taken from dorsal view photographs to the nearest 0.01 mm at 26 times enlargement using Scion Image for Windows (Meyer Instruments Inc. 1998, Houston, TX). The length of the cerebellum was not included in any analyses due to inabilities to produce accurate measurements from the photos. To decrease measurement error, all measurements were performed by a single person and measurements were done in triplicate. The numerical average of these three measurements was used for statistical analysis.

Statistical analysis

Recognizing that allometric measures of brain size are naturally correlated, we used a multiple analysis of covariance model (MANCOVA) with standard length, origin (hatchery or wild) and age to investigate differences between brain measurements from wild and hatchery reared fish.

For each of the eight independent brain measures, we then employed an analysis of co-variance (ANCOVA) model with standard length, origin (hatchery vs. wild), age, standard length $*$ origin, and age $*$ origin. We removed interaction terms that were not significant at $p \geq 0.05$. Data for the ANCOVA and MANCOVA models were $\ln(x + 1)$ transformed prior to statistical analysis.

We applied a discriminant function analysis (DFA) to the full brain data set to ask how well we could distinguish wild from hatchery fish based on the eight brain measures alone. To control for differences in overall size, brain measures were standardized to the fish's

standard length. All statistical analyses were performed using JMP for Windows (SAS Institute 1999).

Results

We found significant differences using all eight brain measures in the MANCOVA model (whole model: Wilks' lambda approx. $F = 9.11$, $p < 0.0001$) (Table 1). Least squares means from the ANCOVA's indicate in seven out of eight models that hatchery fish have smaller values for a particular brain measure than wild fish. For a graphical representation of the data, we plotted individual brain measures versus standard length and fitted linear regression lines separately for the hatchery and wild fish (Figure 1).

Using a MANCOVA model, we found no significant differences between the two strains of hatchery fish (whole model: Wilks' lambda approx. $F = 0.94$, $p = 0.504$). We did, however, find a significant difference between the two wild populations (whole model: Wilks' lambda approx. $F = 9.11$, $p < 0.0001$). Here, the Little Chico Creek population generally had larger measures than the Big Canyon Creek populations, but the direction of the difference from hatchery fish for both strains was the same (Figure 1).

We then used a DFA to ask how well we could distinguish wild from hatchery fish based on the brain measures alone. For hatchery fish we correctly predicted their origin in 28/30 fish (93%) and for wild fish we correctly predicted 35/36 fish (97%).

Discussion

These data suggest that hatchery-rearing practices influence the growth and development of brain structures to the extent that a different brain phenotype can be detected, even by simple anatomical measures. Brain structures that were most profoundly influenced included the optic tectum and the telencephalon, areas that are often linked to aggression, feeding behavior and reproduction in fishes. Deficiencies in these areas are consistent with the many behavioral and developmental abnormalities that have been attributed to captive or hatchery rearing, including changes in predator avoidance, growth and gaining access to appropriate mates (Jonsson et al. 1991, Fleming & Gross 1993, Unwin & Glova 1997, Jonsson 1997, Petersson & Jarvi 1997, Berejikian et al. 1997, Fleming et al. 1997, Gross 1998, Hard et al. 2000). Such differences have been shown to arise through the selection of traits that are specifically adapted for survival in the hatchery environment (Gross 1998). Selection for such traits is often unintentional, but occurs rapidly once wild fish are placed in captivity (Gross 1998, Fleming & Einum 1997). Farm reared Atlantic salmon, *Salmo salar*, for example, show measurable morphological and behavioral differences in aggression and predator avoidance in only seven generations (Fleming & Einum 1997).

While captivity and subsequent domestication may alter brain phenotype over generations (Masai et al. 1982, Ishikawa et al. 1999), both classic (e.g., Wiesel 1982) and more recent studies in other animal models suggest that the monotony of the hatchery environment itself is likely to impact neural plasticity and development more directly (Kempermann et al. 1997a,b, Kempermann & Gage 1999, VanPraag et al. 1999, Jacobs et al. 2000). The present study does not discriminate between genetic and environmental effects. However, environmental enrichment has recently been shown to promote neural growth and proliferation in the dentate-gyrus of the mouse hippocampus, while stress apparently suppresses the rate of cell proliferation in a number of mammalian species (reviewed by Jacobs et al. 2000). Such processes may be even more exaggerated in fishes, where neurogenesis continues throughout life, thus allowing experience and environmental enrichment to play a more proximate role in shaping brain phenotype (Kotrschal et al. 1998).

These issues are particularly important when endangered fishes are taken out of the wild to be maintained in captive rearing programs (Philippart 1995, Snyder et al. 1996). The environmental landscape of a typical fish hatchery is deprived of many of the natural sensory inputs a wild fish would encounter. Standard captive rearing environments lack temporally and spatially changing olfactory and visual cues; fish experience little or no contact with living organisms other than conspecifics or hatchery workers. There are no predators to avoid or live prey to pursue. Such programs tend to be designed to preserve genetic strains of fish without regard to the critical role phenotypic plasticity may play in the life history of the species (Shumway 1999), or the potential impact of domestication when animals must be maintained for several generations (Philippart 1995).

By illustrating phenotypic differences between hatchery and wild reared fish in an organ as fundamental to behavior as the brain, the present study adds to a growing body of literature in providing a dramatic illustration for why such practices need to

Table 1. MANCOVA and ANCOVA results. MANCOVA F values are Wilks' lambda approximations. Dashes indicate the factor was not included in the model. All brain measures are ln(x + 1) transformed. Five of the eight models included origin as significant (optic tectum length, cerebellum width, telencephalon length, although olfactory bulb width was nearly significant at $p = 0.059$). Four measures included a standard length ∗ origin interaction term as significant (optic tectum length, cerebellum width, telencephalon width and olfactory bulb width). By itself, age was significant in one measure (optic tectum width) but was included in three models as an origin ∗ age interaction term (optic tectum width, telencephalon width and olfactory bulb width).

Factor	Adjusted R^2	Origin		Standard length		Age		Origin × standard length		Origin × age		Least squares means for origin	
		F value	p value	F value	p value	F value	p value	F value	p value	F value	p value	Hatchery	Wild
MANCOVA (whole model) with age		8.27	<0.001	126.68	<0.001	2.10	0.052	9.29	<0.001	—	—	—	—
Optic tectum width	0.853	0.32	0.574	535.03	<0.001	1.04	0.31	—	—	5.00	0.028	2.030	2.115
Optic tectum length	0.936	21.43	<0.001	745.22	<0.001	21.31	<0.001	22.77	<0.001	—	—	1.812	1.784
Cerebellum width	0.886	9.45	0.003	459.43	<0.001	0.73	0.394	8.82	0.004	—	—	1.292	1.307
Telencephalon width	0.777	0.42	0.519	314.19	<0.001	2.08	0.153	—	—	6.15	0.015	1.492	1.564
Telencephalon length	0.754	5.95	0.017	208.40	<0.001	3.05	0.084	4.47	0.037	—	—	1.303	1.373
Olfactory bulb width	0.820	3.67	0.059	279.29	<0.001	1.98	0.163	3.20	0.077	—	—	1.244	1.271
Olfactory bulb length	0.512	15.34	<0.001	83.98	<0.001	0.22	0.643	—	—	9.39	0.003	0.757	0.832
Olfactory nerve width	0.674	10.70	0.002	137.08	<0.001	0.60	0.440	—	—	—	—	0.443	0.504

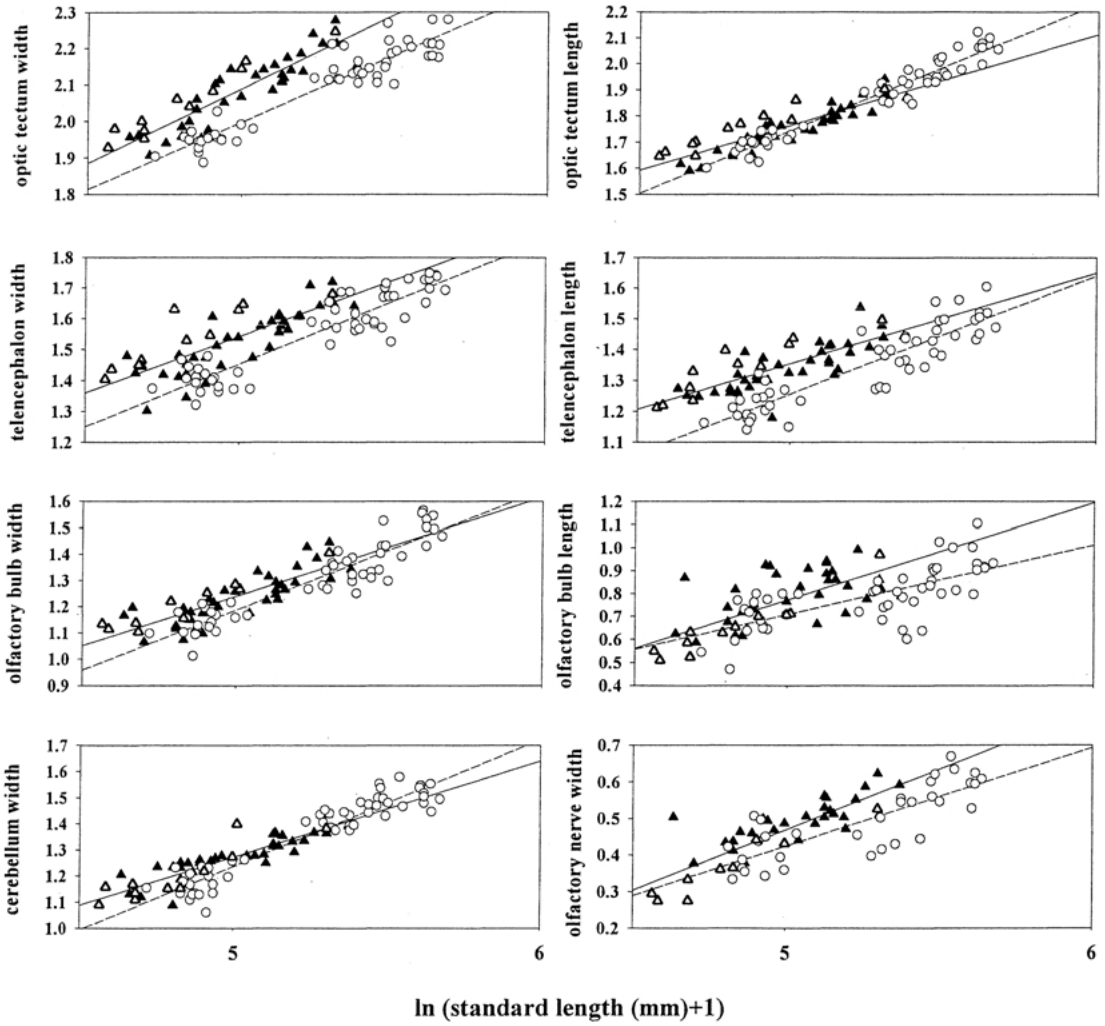

Figure 1. Eight separate brain measures [ln(x + 1) transformed measurements] are plotted relative to fish standard length. Fish origin (hatchery or wild) and linear regressions are indicated for clarity. Wild fish are represented by triangles and solid lines: solid triangles = fish from Big Valley Creek, empty triangles = fish from Little Chico Creek. Hatchery fish are represented by open circles and dashed lines. Hatchery fish (N = 51) were obtained from two state run hatcheries in Northern California. Wild fish (N = 48) were obtained by backpack electrofishing from two streams in Northern California. Due to statistical equivalency both hatchery strains are shown together.

be revisited in conservation efforts to preserve wild salmon. Understanding how environmental enrichment or captive rearing practices influences neural proliferation and development may well be a topic of concern for hatchery managers and conservation biologists of the future.

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