Plasticity in probabilistic reaction norms for maturation in a salmonid fish

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The relationship between body size and the probability of maturing, often referred to as the probabilistic maturation reaction norm (PMRN), has been increasingly used to infer genetic variation in maturation schedule. Despite this trend, few studies have directly evaluated plasticity in the PMRN. A transplant experiment using white-spotted charr demonstrated that the PMRN for precocious males exhibited plasticity. A smaller threshold size at maturity occurred in charr inhabiting narrow streams where more refuges are probably available for small charr, which in turn might enhance the reproductive success of sneaker precocious males. Our findings suggested that plastic effects should clearly be included in investigations of variation in PMRNs.

Keywords: mature male parr; phenotypic plasticity; threshold; probabilistic reaction norm

1. INTRODUCTION

The size at which organisms reach sexual maturity is one of the most important life-history traits affecting fitness. Evolutionary ecologists frequently focus on spatial–temporal variation in size at maturity to test evolutionary responses to selection (e.g. Aubin-Horth & Dodson 2004; Olsen *et al.* 2004). The relationship between body size and the probability of maturing, often referred to as the probabilistic maturation reaction norm (PMRN; Heino *et al.* 2002), has been increasingly used to quantify the maturation schedule. For example, some commercial fishes showed a decline in age-specific body size at which the probability of maturing is 50 per cent, a feature of the PMRN, supporting the idea of fisheries-induced evolution (e.g. Olsen *et al.* 2004). The possibility of unaccounted factors affecting plastic effects on PMRN, as well as the difficulty in disentangling phenotypic plasticity from genetic change, has been explicitly acknowledged and thoroughly discussed in many reports (e.g. Aubin-Horth & Dodson 2004; Olsen *et al.* 2004; Marshall & Browman 2007). However, few studies have experimentally demonstrated plasticity in the PMRN.

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This study examined plasticity in the PMRN for precocious males of white-spotted charr (*Salvelinus leucomaenis*) using a transplant experiment in a natural river. Similar to other salmonid fishes, charr exhibit alternative reproductive tactics. A portion of males become sexually mature at a younger age before migrating to the sea and sneak for access to females. In Atlantic salmon (*Salmo salar*), several studies have reported spatial variation in the PMRN for precocious males (Baum *et al.* 2004, 2005; Aubin-Horth *et al.* 2006). Our experimental design permits the examination of environmental conditions that might affect the plasticity of such spatial variation in the PMRN.

2. MATERIAL AND METHODS

To evaluate the plasticity in PMRNs in a natural environment, a transplant experiment was conducted in the Onbetsu River, eastern Hokkaido, Japan (figure 1). The experimental design was set up in a way to control for any potential genetic effects on PMRN. A total of 1500 fry (age 0+ year) were captured at a donor site using electrofishing and night-time hand netting on 10–11 August 2007. Fry captures were conducted in a river section approximately 1 km long. Captured fry were initially transferred to a single fish cage (60 × 50 × 50 cm), mixed well and then divided into five different cages. Each of the five cages held 300 fry. The donor site was located approximately 40 km upstream of the river mouth, where the main spawning ground of the anadromous white-spotted charr is situated (fig. 8 of Morita *et al.* 2009). Because the number of spawning adults at the donor site exceeded 1000 in the previous year, the collected fry represented a large sample of families. Three hundred fry were released at each of five different transplant sites on 11 August 2007. The fork lengths of 30 fry were measured at each transplant site before release, and no significant differences existed among sites (mean ± s.d., 54.1 ± 9.2 mm; analysis of variance, $F_{4,145} = 1.77$, $p > 0.05$). All transplant sites were separated by several impassable dams and lacked charr, probably due to local extinctions (Morita & Yamamoto 2002).

We sampled mature and immature age 1+ year charr in late September to early October 2008, during the breeding season of this species (table 1). In total, 178 charr (11.9%) were recaptured at the transplant sites, and 76 age 1+ year charr were captured at the donor site via electrofishing. The following variables were determined for these fish: fork length (millimetres), somatic weight (grams), sex and reproductive state by visual inspection of the gonads, and age confirmation using otolith analysis. Parr densities were estimated in a 100 m reach of each site using the three-pass removal method (model $M_{(b)}$, program CAPTURE, available at <http://www.mbr-pwrc.usgs.gov/software/index.html>). At the same time, four physical characteristics (river width, depth, velocity and substrate code) were measured in each 100 m reach of each site following Morita *et al.* (2004). Water temperatures were measured at each site at hourly intervals using data loggers (Stow-Away TidbiT, Onset Computer) from August 2007 to September 2008 (figure 1). Because one logger was lost in late April 2008, the mean temperatures from May to August 2008 were used as representative temperatures for each site.

A PMRN method based on logistic regression (Heino *et al.* 2002) was used to analyse the relationships between body size and the probability of becoming mature for age 1+ year male parr. Because no charr mature at age 0+ year (Morita *et al.* 2009), maturing charr of age 1+ year were considered to be first-time maturers. The logistic regression model for this study was described as

$$\text{logit}(p) = a + b_1 l + c_{\text{site}}, \quad (2.1)$$

or alternatively, with the site effect replaced by appropriate environmental factors

$$\text{logit}(p) = a + b_1 l + b_2 E_2 + b_3 E_3 + \dots + b_n E_n, \quad (2.2)$$

where p is the probability of becoming mature, a is the regression constant, b_n are regression coefficients, c_{site} is a site-specific constant, l is body size (fork length or weight) and E_n represents potential environmental factors: river width, depth, velocity, substrate code, temperature and density.

The model parameters were estimated using maximum likelihood methods. The significance of the model parameters was evaluated using the likelihood-ratio test and the Akaike information criterion (AIC). Details of the analyses are described in the electronic supplementary material. Because the use of fork length and somatic

Figure 1. Study area indicating the donor and five transplant sites (with hourly temperatures) for the charr transplant experiments.

weight yielded the same qualitative results and the use of length resulted in lower AIC values, only length-related results are reported below.

3. RESULTS

The logistic regression analysis identified significant effects of body size and site on the probability of maturing for male age 1+ year charr, indicating that the probability of maturing increased with increasing body size; however, these relationships differed across the transplant and donor sites (table 2). By replacing the site factor with various environmental factors, step-wise logistic regression ($p < 0.05$ to add and $p > 0.10$ to remove) identified three variables: fork length, river width and temperature, as the best predictors of the probability of maturing, although the effect of temperature was marginal (table 2). The model including these three variables exhibited the smallest AIC and thus represents the best model considered here. The resulting logistic model predicted 83.6 per cent of the maturation. The regression coefficient for river width was negative, while those for fork length and temperature was positive (table 2). Thus, the probability of

maturing for male age 1+ year charr increased with increases in fork length and temperature but decreased with increases in river width (figure 2). In other words, threshold size at maturity increased with increasing river width and decreased with increasing temperature (electronic supplementary material).

4. DISCUSSION

Our transplant experiment using white-spotted charr demonstrated that the PMRN, i.e. the relationship between fish body size and the probability of becoming mature at a given age, exhibited plasticity. Furthermore, two environmental variables, river width and temperature, were related to changes in the threshold size at maturity of precocious males. Although the relative importance of genetic versus plastic effects on changes in PMRNs remains controversial, recent studies have increasingly incorporated both types of effects into a general understanding of changes in the PMRN (Kuparinen & Meriä 2007; Marshall & Browman 2007). Our findings support this approach; in particular, plastic effects should clearly be included in investigations of variation in PMRNs.

Table 1. Environmental variables at a donor and five transplant sites and body sizes and maturity rates of age 1+ year charr at the end of experiments. All values are means $\pm$ s.d.

	donor site	transplant sites				
	main stream	Tounpe	Itsukanbetsu	Sen	Muri	Yamabe
river width (m)	5.7 $\pm$ 1.4	2.7 $\pm$ 0.7	3.7 $\pm$ 1	2.1 $\pm$ 0.9	4.3 $\pm$ 1.2	2.3 $\pm$ 0.6
depth (cm)	18 $\pm$ 10	14 $\pm$ 6	13 $\pm$ 5	13 $\pm$ 13	12 $\pm$ 12	9 $\pm$ 4
velocity (cm s ⁻¹)	44 $\pm$ 23	29 $\pm$ 16	43 $\pm$ 24	19 $\pm$ 11	41 $\pm$ 16	26 $\pm$ 12
substrate code ^a	2.7 $\pm$ 0.6	3.1 $\pm$ 0.5	3.0 $\pm$ 0.7	2.3 $\pm$ 0.6	2.9 $\pm$ 0.9	3.0 $\pm$ 0.6
temperature (°C)	12.5 $\pm$ 3.1	9.4 $\pm$ 0.5	11.3 $\pm$ 2.5	12.8 $\pm$ 3.4	12.4 $\pm$ 2.9	10.3 $\pm$ 2.9
fork length (mm)	118 $\pm$ 16	130 $\pm$ 19	201 $\pm$ 18	153 $\pm$ 23	183 $\pm$ 23	182 $\pm$ 20
somatic weight (g)	15 $\pm$ 6	21 $\pm$ 9	85 $\pm$ 24	36 $\pm$ 16	64 $\pm$ 25	59 $\pm$ 19
sample size (male/female)	42/34	11/13	24/23	25/30	17/19	9/7
male maturity rate ^b	0.071	0.273	0.833	0.760	0.765	0.778
parr density (n m ⁻²)	0.463	0.067	0.014	0.206	0.019	0.031
sympatric fishes ^c	<i>Oncorhynchus masou</i> ⁺⁺ <i>Barbatula toni</i> ⁺⁺⁺ <i>Cottus nozawae</i> ⁺⁺ <i>Lampetra</i> sp. ⁺	none	<i>Barbatula toni</i> ⁺⁺ <i>Cottus nozawae</i> ⁺⁺	<i>Barbatula toni</i> ⁺⁺⁺ <i>Cottus nozawae</i> ⁺ <i>Lampetra</i> sp. ⁺	<i>Barbatula toni</i> ⁺⁺ <i>Cottus nozawae</i> ⁺⁺	<i>Cottus nozawae</i> ⁺⁺

^a1, sand-silt (<2 mm); 2, gravel (2–16 mm); 3, pebble (17–64 mm); 4, cobble (65–256 mm); 5, boulder (>256 mm).

^bNo female matured.

^cTotal number of fishes observed within 100 m reach: +, <10; ++, 10–100; +++, >100.

Table 2. Results of selected logistic regression models of the effect of fork length, site and physical parameters on the probability of maturing for age 1+ year male charr. (ln *L*, log likelihood for the full model; *G*², statistic of the likelihood-ratio test; ΔAIC, Akaike information criterion measured relative to the best model.)

model	variable	ln <i>L</i>	<i>G</i> ²	d.f.	coefficient	<i>p</i>	ΔAIC
1	fork length	−56.4	64.6	1	0.052	<0.001	9.8
	constant				−8.050		
2	fork length	−49.4	13.0	1	0.049	<0.001	5.8
	site		14.1	5	0 to 2.574	0.015	
	constant				−8.716		
3	fork length	−49.5	41.5	1	0.047	<0.001	0
	river width		13.2	1	−0.676	<0.001	
	temperature		2.8	1	0.380	0.095	
	constant				−9.228		
4	fork length	−50.9	41.6	1	0.046	<0.001	0.8
	river width		11.0	1	−0.592	<0.001	
	constant				−4.945		
5	fork length	−49.4	−55.9	1	0.049	<0.001	5.8
	river width		−49.6	1	−2.227	0.504	
	temperature		−49.5	1	1.855	0.554	
	depth		−49.4	1	0.122	0.725	
	substrate		−49.5	1	8.743	0.641	
	density		−49.5	1	7.004	0.669	
	constant				−48.470		

Male salmonid fishes exhibit two alternative mating tactics: sneaking and fighting (Gross 1991). In general, small males sneak, whereas larger males fight for access to females. In our study system, the breeding population structure includes precocious and larger anadromous charr (mean fork length $\pm$ s.d., precocious males: 155 $\pm$ 40 mm, *n* = 148; anadromous males: 440 $\pm$ 92 mm, *n* = 57; anadromous females: 519 $\pm$ 96 mm, *n* = 52). Therefore, precocious male charr require a sneaking tactic. In addition to body size, several other environmental factors could affect the reproductive success of sneakers, e.g. refuge availability, frequency of sneakers and water level (Gross 1991).

The unexpected finding in this study was that the river width was the most important environmental factor affecting the probability of maturing at a given size for precocious males. Increasing river width was associated with lower probabilities of maturing at a given size. This finding was similar to the spatial variation in threshold size at maturity observed in Atlantic salmon: threshold size at maturity decreases in the upstream direction (Baum *et al.* 2004; Aubin-Horth *et al.* 2006). We deduced that the greater proportion of edge habitat with overhanging cover in narrow streams (cf. Kozel & Hubert 1989) might be positively associated with refuge availability for sneakers.

Figure 2. Fitted logistic regressions of the probability of maturing based on fork length of age 1+ year male charr for different temperatures and river widths. Regressions were plotted for the range of fork lengths, temperatures and river widths used. Black dashed line, temp. 9.5°C, width 2.5 m; black solid line, temp. 9.5°C, width 5.0 m; grey dashed line, temp. 12.5°C, width 2.5 m; grey solid line, temp. 12.5°C, width 5.0 m.

Gross (1991) suggested that the reproductive success of sneakers increases with greater availability of refuges. Therefore, a smaller threshold size at maturity, i.e. a higher maturity rate at a given size, may constitute adaptive phenotypic plasticity for charr inhabiting narrow streams. Such patterns are generally assumed to reflect adaptive phenotypic plasticity, but whether this assumption is correct is largely unknown. In addition, the proximate cue of river width remains unclear.

Our results also imply that the probability of maturing at a given size tended to increase with increasing temperature. This implication contradicts that of a previous study of Atlantic salmon (Baum et al. 2005), but is consistent with the well-established general prediction that higher temperatures result in earlier maturation at smaller sizes in ectotherms (cf. Dhillon & Fox 2004). Because maturation is a physiological process associated with endocrine changes, temperature probably affects maturation regardless of body size.

Genetic factors affecting the PMRN or threshold size at maturity should not be ruled out. Recently, Piché et al. (2008) demonstrated that genetic variability in the probability of maturing at a given size for male Atlantic salmon parr existed across geographically separated rivers. By contrast, spatial variation in the PMRN for precocious male charr within a river may be explained by phenotypic plasticity (Morita & Fukuwaka 2007). Moreover, in a common garden experiment, the PMRNs for precocious male charr did not significantly differ across five localities within the Ken-ichi River system (K. Morita 2009, unpublished data from a reanalysis of data in Morita et al. in press). While it is important to seek evidence of both genetic variability and phenotypic plasticity of PMRNs, it is also necessary to address the relative contribution of these effects on variation in PMRNs in natural systems.

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- Aubin-Horth, N. & Dodson, J. J. 2004 Influence of individual body size and variable thresholds on the incidence of a sneaker male reproductive tactic in Atlantic salmon. *Evolution* **58**, 136–144.
- Aubin-Horth, N., Bourque, J. F., Daigle, G., Hedger, R. & Dodson, J. J. 2006 Longitudinal gradients in threshold sizes for alternative male life history tactics in a population of Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **63**, 2067–2075. (doi:10.1139/F06-103)
- Baum, D., Laughton, R., Armstrong, J. D. & Metcalfe, N. B. 2004 Altitudinal variation in the relationship between growth and maturation rate in salmon parr. *J. Anim. Ecol.* **73**, 253–260. (doi:10.1111/j.0021-8790.2004.00803.x)
- Baum, D., Laughton, R., Armstrong, J. D. & Metcalfe, N. B. 2005 The effect of temperature on growth and early maturation in a wild population of Atlantic salmon parr. *J. Fish. Biol.* **67**, 1370–1380. (doi:10.1111/j.0022-1112.2005.00832.x)
- Dhillon, R. S. & Fox, M. G. 2004 Growth-independent effects of temperature on age and size at maturity in Japanese Medaka (*Oryzias latipes*). *Copeia* **2004**, 37–45. (doi:10.1643/CI-02-098R1)
- Gross, M. R. 1991 Salmon breeding behavior and life history evolution in changing environments. *Ecology* **72**, 1180–1186. (doi:10.2307/1941091)
- Heino, M., Dieckmann, U. & Godø, O. R. 2002 Measuring probabilistic reaction norms for age and size at maturation. *Evolution* **56**, 669–678.
- Kozel, S. J. & Hubert, W. A. 1989 Factors influencing the abundance of brook trout (*Salvelinus fontinalis*) in forested mountain streams. *J. Freshwater Ecol.* **5**, 113–122.
- Kuparinen, A. & Merilä, J. 2007 Detecting and managing fisheries-induced evolution. *Trends Ecol. Evol.* **22**, 652–659. (doi:10.1016/j.tree.2007.08.011)
- Marshall, C. T. & Browman, H. I. 2007 Disentangling the causes of maturation trends in exploited fish populations. *Mar. Ecol. Prog. Ser.* **335**, 249–251.
- Morita, K. & Fukuwaka, M. 2007 Why age and size at maturity have changed in Pacific salmon. *Mar. Ecol. Prog. Ser.* **335**, 289–294. (doi:10.3354/meps335289)
- Morita, K. & Yamamoto, S. 2002 Effects of habitat fragmentation by damming on the persistence of stream-dwelling charr populations. *Conserv. Biol.* **16**, 1318–1323. (doi:10.1046/j.1523-1739.2002.01476.x)
- Morita, K., Tsuboi, J. & Matsuda, H. 2004 The impact of exotic trout on native charr in a Japanese stream. *J. Appl. Ecol.* **41**, 962–972. (doi:10.1111/j.0021-8901.2004.00927.x)
- Morita, K., Morita, S. H. & Yamamoto, S. In press. Effects of habitat fragmentation by damming on salmonid fishes: lessons from white-spotted charr in Japan. *Ecol. Res.*
- Olsen, E. M., Heino, M., Lilly, G. R., Morgan, M. J., Brattey, J., Ernande, B. & Dieckmann, U. 2004 Maturation trends indicative of rapid evolution preceded the collapse of northern cod. *Nature* **428**, 932–935. (doi:10.1038/nature02430)
- Piché, J., Huchings, J. A. & Blanchard, W. 2008 Genetic variation in threshold reaction norms for alternative reproductive tactics in male Atlantic salmon. *Salmo salar. Proc. R. Soc. B* **275**, 1517–1574. (doi:10.1098/rspb.2008.0251)