Brain development and predation: plastic responses depend on evolutionary history

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Although the brain is known to be a very plastic organ, the effects of common ecological interactions like predation or competition on brain development have remained largely unexplored. We reared nine-spined sticklebacks (Pungitius pungitius) from two coastal marine (predation-adapted) and two isolated pond (competition-adapted) populations in a factorial experiment, manipulating perceived predatory risk and food supply to see (i) if the treatments affected brain development and (ii) if there was population differentiation in the response to treatments. We detected differences in plasticity of the bulbus olfactorius (chemosensory centre) between habitats: marine fish were not plastic, whereas pond fish had larger bulbi olfactorii in the presence of perceived predation. Marine fish had larger bulbus olfactorius overall. Irrespective of population origin, the hypothalamus was smaller in the presence of perceived predatory risk. Our results demonstrate that perceived predation risk can influence brain development, and that the effect of an environmental factor on brain development may depend on the evolutionary history of a given population in respect to this environmental factor.

Keywords: competition; predation; brain plasticity; brain size; Pungitius; stickleback

1. INTRODUCTION

The vertebrate brain is an organ with great capacity for plastic neuro-anatomical changes (e.g. [1–3]). Brain parts that are likely to be important in a particular context develop more than those of less importance [4]. Further, as the brain is the most expensive tissue to develop and maintain [5], energetic constraints should impose strong selection against non-adaptive modifications. Surprisingly, studies on the effect of biotic environment on brain development are scarce [6–8]. In particular, studies in which the effects of common ecological interactions have been tested—such as predation and competition—are notably absent from the literature [9,10].

The evolution of the brain has attracted more scientific interest than brain plasticity induced by ecologically relevant environmental factors. However, most studies of brain evolution have relied on interspecific comparisons, looking for correlations between brain architecture and fitness-related traits [11–13]. Studies of interpopulation differences in brain architecture from an evolutionary perspective have started to appear only recently [14–16]. Still, research integrating plasticity into the evolutionary perspective, by studying population variation in environmentally induced brain plasticity, is scarce [8,17].

In this study, we aimed to investigate the effects of two important ecological factors—predation pressure and food limitation—on the brain development of different nine-spined stickleback (Pungitius pungitius) populations. Nine-spine sticklebacks living in isolated ponds (zero piscine predation) are often more aggressive, bolder, long-living giants with reduced body armour and increased costs of group living when compared with marine sticklebacks facing high piscine predation [8,18–21]. These patterns suggest that marine nine-spined sticklebacks are mainly adapted to avoid predation, whereas pond fish are adapted to intraspecific competition. Here, we compared the brain development of sticklebacks from two pond and two coastal marine populations in the presence or absence of predator chemical cues, and subjected to two different levels of food supply.

2. MATERIAL AND METHODS

Adult nine-spined sticklebacks were collected in 2009 from two isolated ponds (Abbotjärn, Sweden, 64°29' N, 19°26' E; Pyöreälampi, Finland, 66°15' N, 29°26' E; two low fertility, coastal marine habitats from the Baltic Sea (Nyköping, Sweden, 58°39' N; 17°06' E; Helsinki, Finland, 60°13' N, 25°11' E). Pond habitats were small (less than 5 ha) and contained no sympatric predatory fish, whereas marine environments are characterized by more diverse ecological communities, replete with several piscine predators. Sample sites were separated by more than 500 km. Crosses (six to eight per population) were done in vitro during July and August. Fish were reared individually in 1.41 plastic tanks within four Allentown Zebrafish Rack Systems (Aquaneering Inc., San Diego, CA, USA, hereafter ‘rack’), each equipped with physical, chemical, biological and UV filters and a closed water circulation system. All rearing took place in freshwater. The photoperiod was set to 14L:10D cycle (light:dark), and the water temperature was held constant at 12°C.

Fish were distributed into four treatment combinations of two perceived predation risk and two food levels in a full-factorial randomized block design. The water reservoir of each rack was connected to a separate 150 l tank. In the predation treatment, two 10–15 cm long perch (Perca fluviatilis) were placed in two randomly chosen tanks while the other two tanks contained only water. Hence, in the predation treatment olfactory cues from a fish predator abundant in the Baltic Sea and Fennoscandian freshwater habitats were either present or absent. In the food treatment, predation treatments were randomly divided into high (two ad libitum feedings per day) and low (one ad libitum feed per two days) food groups. Feeding was started with live brine shrimp nauplii (Artemia sp.), and was gradually changed to frozen bloodworms after 80 days. Each full-sib family was represented in each treatment combination.

At the age of 34 weeks—when fish approached adult size—individuals were euthanized by MS-222, photographed under standardized conditions, and weighed to the nearest 0.01 g. Standard length was measured from pictures using tpsDig v. 2.15 [22]. Brains of the fish were dissected, fixed in 4 per cent formalin—0.1 M phosphate-buffered saline solution, and photographed with a digital camera connected to a dissecting microscope from dorsal, lateral and ventral viewpoints. Size of the brain and five brain parts (viz. bulbus olfactorius, telencephalon, tectum opticum, cerebellum and hypothalamus) were measured from the digital photographs with tpsDig v. 2.15 [22] (see also the electronic supplementary material). The volume of the total brain and the different brain parts was estimated according to the ellipsoid model, which estimates volume based on the length, width and height of the object using correction factors [8,14,15].
In total, 187 brains were analysed. Some individuals could not be included owing to random mortality (mainly at early life stages), fish escaping the system and problematic dissections. Hence, family effects were not analysed, but rather it was assumed that sampled fish represented an unbiased sample of each source population's genetic pool. All metric variables were log10 transformed. We used general linear mixed models (GLMMs) to analyse variation in brain size and the size of different brain parts. The models were built with habitat (marine versus pond), sex, predation (presence versus absence of perceived predation risk) and food treatments (high versus low) as fixed effects, with population nested in habitat as random factor. Standard length and body weight were both included as covariates because the study populations differ in relative weight [8,14,15]. In the analyses of brain parts, we also added total brain size as a covariate. In all models, we included simple effects will be reported, but not discussed here.

3. RESULTS
Brain plasticity was habitat-dependent (table 1): predation risk induced development of larger bulbous olfactorii in pond fish, but not in marine fish (figure 1). In general, marine fish had relatively larger bulbous olfactorii than pond fish (figure 1). Predation also had an effect on the development of the hypothalamus (table 1): independent of population, habitat or sex, fish developed smaller hypothalami in the presence of predator (figure 2). The food treatment did not affect brain development. The population effect was always non-significant \((p > 0.18)\).

4. DISCUSSION
To date, the effect of predation on brain development has only been assessed in a single population of anurans \([9,10]\). Here, we found that perceived predation risk in the absence of actual contact with the predator has a significant—and sometimes habitat-dependent—effect on brain development in nine-spined sticklebacks. Surprisingly, while marine sticklebacks had relatively larger bulbous olfactorii than pond fish, perceived predation risk induced plastic modification in the bulbous olfactorii only in the latter. Our results suggest that in marine environments under constant predation risk, a large relative size of bulbous olfactorii might have become fixed, while in ponds, plasticity of the relatively small bulbous olfactorii occurred. Taken together, predation induced bulbous olfactorii enlargement both on the evolutionary and ontogenetic scales. As the brain is an extremely expensive tissue both to develop and maintain \([5]\), the observed patterns support the conjecture that the bulbous olfactorii is an important sensory centre in predation avoidance. Given that predation-adapted sticklebacks are likely to represent the ancestral form, the fact that the plastic response appeared parallel to a decrease in bulbous olfactorii size in the piscine-predator free ponds warrants further investigations. We note that the predation treatment also reduced the aggression and risk-taking of our fish, demonstrating that sticklebacks identified olfactory cues from perch as predation risk \([23]\).

Independent of population, habitat and sex, fish developed smaller hypothalami in the presence of predatory cues than in their absence. The hypothalamus has a wide range of functions \([24]\). For instance, it regulates reproductive behaviour \([25]\), and it is also the centre of regulating feeding behaviour in fish \([26]\). Hence, based on our data, it is impossible to determine why perceived predation risk resulted in decreased hypothalamus size. However, considering

Table 1. Results of the GLMMs. F-statistics and degrees of freedom are shown. Note that sex and its interactions were only included in the model to control for sex-related variation, and they are not discussed further.

<table>
<thead>
<tr>
<th>effect</th>
<th>total brain</th>
<th>bulbous olfactorii</th>
<th>telencephalon</th>
<th>tectum opticum</th>
<th>cerebellum</th>
<th>hypothalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td>habitat (H)</td>
<td>3.89 ((1,2.23))</td>
<td>29.24* ((1,3.15))</td>
<td>0.50 ((1,2.37))</td>
<td>(&lt;0.01 (1,2.39))</td>
<td>2.64 ((1,2.68))</td>
<td>0.96 ((1,3.08))</td>
</tr>
<tr>
<td>predation (P)</td>
<td>(&lt;0.01 (1,170))</td>
<td>1.97 ((1,170))</td>
<td>1.41 ((1,169))</td>
<td>0.02 ((1,169))</td>
<td>0.06 ((1,170))</td>
<td>3.89* ((1,171))</td>
</tr>
<tr>
<td>food (F)</td>
<td>0.34 ((1,170))</td>
<td>0.65 ((1,170))</td>
<td>0.79 ((1,169))</td>
<td>0.59 ((1,169))</td>
<td>0.38 ((1,170))</td>
<td>0.01 ((1,170))</td>
</tr>
<tr>
<td>sex (S)</td>
<td>124.54*** ((1,171))</td>
<td>6.09* ((1,171))</td>
<td>25.96*** ((1,170))</td>
<td>3.27 ((1,170))</td>
<td>2.44 ((1,170))</td>
<td>8.56*** ((1,171))</td>
</tr>
<tr>
<td>H × F</td>
<td>0.02 ((1,170))</td>
<td>4.14* ((1,171))</td>
<td>0.11 ((1,170))</td>
<td>0.10 ((1,170))</td>
<td>0.03 ((1,170))</td>
<td>0.40 ((1,171))</td>
</tr>
<tr>
<td>H × F</td>
<td>2.62 ((1,170))</td>
<td>0.05 ((1,169))</td>
<td>0.02 ((1,169))</td>
<td>0.27 ((1,169))</td>
<td>0.02 ((1,169))</td>
<td>0.04 ((1,169))</td>
</tr>
<tr>
<td>P × F</td>
<td>2.08 ((1,170))</td>
<td>0.28 ((1,171))</td>
<td>0.23 ((1,170))</td>
<td>1.86 ((1,170))</td>
<td>0.32 ((1,170))</td>
<td>0.10 ((1,171))</td>
</tr>
<tr>
<td>S × H</td>
<td>0.54 ((1,170))</td>
<td>0.30 ((1,167))</td>
<td>0.14 ((1,167))</td>
<td>0.26 ((1,167))</td>
<td>0.49 ((1,169))</td>
<td>0.32 ((1,170))</td>
</tr>
<tr>
<td>S × P</td>
<td>0.03 ((1,170))</td>
<td>0.56 ((1,167))</td>
<td>1.02 ((1,169))</td>
<td>0.13 ((1,169))</td>
<td>0.07 ((1,170))</td>
<td>0.01 ((1,170))</td>
</tr>
<tr>
<td>S × F</td>
<td>0.23 ((1,170))</td>
<td>7.52** ((1,169))</td>
<td>0.02 ((1,169))</td>
<td>0.11 ((1,169))</td>
<td>0.07 ((1,169))</td>
<td>0.15 ((1,169))</td>
</tr>
<tr>
<td>length</td>
<td>0.97 ((1,172))</td>
<td>3.87 ((1,155))</td>
<td>0.29 ((1,170))</td>
<td>1.76 ((1,171))</td>
<td>0.60 ((1,170))</td>
<td>1.15 ((1,150))</td>
</tr>
<tr>
<td>weight</td>
<td>86.74*** ((1,169))</td>
<td>1.91 ((1,165))</td>
<td>0.44 ((1,155))</td>
<td>1.29 ((1,158))</td>
<td>5.24* ((1,144))</td>
<td>1.32 ((1,54.4))</td>
</tr>
<tr>
<td>total brain</td>
<td>—</td>
<td>45.80*** ((1,158))</td>
<td>151.07*** ((1,171))</td>
<td>303.12*** ((1,171))</td>
<td>208.45*** ((1,171))</td>
<td>74.75*** ((1,153))</td>
</tr>
</tbody>
</table>

*\(p > 0.05; **p > 0.01; ***p > 0.001; *p = 0.0502\).
that feeding behaviour can be regulated by different stress factors such as predation [26], and that nine-spined stickbacks from the present experiment decreased their aggression and risk-taking levels in the presence of perceived predation risk [23], it seems possible that decreased hypothalamus size is somehow linked to the altered behaviour activity.

In summary, we found that perceived predation risk affected brain development, and that the effect can depend on a population’s evolutionary history with predation. Available energy did not affect brain development. Interestingly, stickback populations evolving under negligible predation had the ability to react to chemical predatory cues, while predation-evolving under negligible predation had the ability to develop. Interestingly, stickleback populations depend on a population’s evolutionary history with predation risk [23], it seems possible that decreased hypothalamus size is somehow linked to the altered behavioural activity.

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Our work was supported by the Academy of Finland (G.H. and J.M.), Finnish School in Wildlife Biology, Conservation and Management (A.G.) and Finnish Graduate School in Population Genetics (K.V.). The experiment was conducted under the license of the Finnish National Animal Experiment Board (Licence no. STH223A).


Figure 2. The effect of perceived predation risk on hypothalamus development. Least-squares means (from the GLMM) ± s.e. are shown.


