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Climbing experience in glass eels: A cognitive task or a matter of physical capacities?

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HIGHLIGHTS

- We studied phenotypic traits of eels climbing water obstacles.
- The analysis included muscle enzymatic activity and genes transcription in brain.
- Experienced fish up regulated genes associated with synapse and neurogenesis.
- Climbing water obstacles can implicate cognition-related traits in migrating fish.

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ABSTRACT

The European eel is a panmictic species, whose decline has been recorded since the last 30 years. Among human-induced environmental factors of decline, the impact of water dams during species migration is questioned. Indeed, water impoundments can be a severe obstacle for young eels trying to reach the upstream freshwater zones, even if they are equipped with fish-friendly passes. The passage by such devices could be an important event shaping the outcome of the future life and life history traits of eels. We studied what phenotypic traits were associated with the event of experience of passage by water obstacles. We analyzed specific enzyme activities and/or gene transcription levels in the muscle and brain to test whether the obstacle passage is rather a physical or cognitive task. We found that after a long period of maintenance under homogenous conditions, transcription levels of several genes linked to synaptic plasticity, neurogenesis and thyroid activity differed among the field-experience groups. In contrast, muscle gene transcription levels or enzymatic activities did not show any differences among fish groups. We suggest that cognitive processes such as learning and memory acquisition rather than swimming-related metabolic capacities are involved in passage of water obstacles by young eels.

1. Introduction

Anthropogenic activities are often associated with landscape restructuration for economic purposes. In aquatic ecosystems, landscape modifications involve the construction of water barriers such as weirs and dams. These changes strongly alter the longitudinal connectivity of water corridors and can negatively impact aquatic organisms [1–2]. Movements of migratory species are constrained by aquatic barriers, restricting their access to feeding or reproductive habitats [3–4]. Aquatic barriers can prevent the recruitment of fish into upstream freshwater habitats [5], hence forcing them to settle in the most downstream parts at high densities. Depending on the type and height of the barriers, environmental conditions and species physiology and behavior, obstacles may be impassable. Even though barriers are passable, they can delay migration [6], which in turn can lead to suboptimal colonization and modify life-history traits of animals and in fine impair reproductive capacity of genitors [7–8].

Many efforts have been undertaken to ease the passage of obstacles by installing fish-friendly devices such as fishways. However, even if equipped with fish passes, barriers still have an impact on fish passing through. Indeed, beyond quantitative effects being widely addressed in the literature [9], aquatic barriers could also have qualitative effects. Fish passage by fishway involves energy expenditure needed to find the entrance [10], especially when the fishway attractiveness is poor [11–12], and to ascend such devices. In addition, the ascension is often

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performed under harsh hydraulic conditions [13–14]. In this case, inter-individual variation in phenotypic traits at the intra-specific level could play an important role in the success of passage of animals through fishways. Among other traits, the capacity of individuals to perceive environmental cues as well as their overall activity could play an important role in their capacity to find the fishway [15]. The success of crossing the fishway could be also associated with the physiological status or swimming capacity of fish [9,16], and the fishway device could hence act as a selective filter or contribute to the modification of fish biology, physiology and behavior.

The impact of fishway passage on individual phenotypic traits can be particularly important in species with high phenotypic plasticity i.e. with a potential for environmentally induced wide range of reaction norms. One of the fish species with the widest range of variation in phenotypic traits is the European eel *Anguilla anguilla* [17–18]. The European eel is a catadromous species occurring in inland and estuarine ecosystems throughout Europe. It has a complex life cycle requiring two transatlantic migrations. The first migration from the Sargasso Sea, the unique spawning ground of the species, is done by a passive transport of leptocephali larvae towards the European continental shelf [19–20]. Once reached, the metamorphosis of leptocephali into glass eels occurs, and the colonization of continental waters can begin. Many young eels migrate upstream in search of optimal growth habitats to settle down as elvers or yellow eels.

Over the last 30 years, the European eel population has markedly declined throughout its whole repartition area and is considered as 'critically endangered' [21]. Many causes have been proposed (overfishing, man-introduced parasites and diseases, pollution, climate change), although the impact of anthropogenic barriers at both ontogenetic stages of fish (i.e. increased mortality during passage by hydropower plants in the case of spawning migration of silver eels, non-optimal access to freshwater habitats together with density dependent predation during glass eel upstream migration) has been suggested as one of the key factors contributing to the sharp decline of the European eel populations [22]. For eel species, the most efficient fish pass is ladder-type, based on a natural rheotaxy and climbing tendency of young eels. Yet, the efficiency of such fishways has been assessed only from a quantitative point of view. Currently, data are mainly restricted to the proportions of fish approaching the device and of those succeeding to pass the obstacle [8,23].

Our study aimed at testing whether phenotypic variation of specific traits in glass eels can be associated with their experiential differences in passing water obstacles. We investigated whether fish with different climbing experiences acquired in the field express differences in phenotypic traits after a common garden period. We previously showed that brain gene expression was related to different behaviors of fishway passage during in situ investigations [30]. However, these differences were observed in fish directly analyzed after field sampling, thus preventing the detection of delayed and long-term impacts on fish. In the present study, we aimed at expanding these preliminary findings by assessing the relative long-term impacts of obstacle crossing on cognitive abilities, often associated with exploratory behavior [24–25] in contrast to swimming performances [26–27]. Moreover, thyroid metabolism and body condition were assessed, as they were suggested to play a role in glass eel freshwater migration [28–29]. We therefore investigated differences in the gene transcription level and in vitro enzyme activity in two tissues -the brain and the muscle.

2. Material & methods

All procedures used in this study were approved by the Aquitaine fish–birds ethic committee (a committee approved and registered by the French Ministry of Higher Education and Research under number 73).

2.1. Sampling

Eels were collected using electric fishing during two consecutive days (16–17 of July 2013) under similar climatic and hydrological conditions in the Canal des Etangs, an artificial freshwater corridor in South-Western France (44.75–44.95 N, 1.1–1.2 W). The river line is linear, whereas the water flow remains homogenous and controlled by a series of weirs. Three successive low-distanced obstacles were built along the river length, all equipped with a fish pass delimiting three successive river segments. The most downstream dam is equipped with a glass eel-specific pass, and the two other are equipped with an eel pass (Fig. 1). Thirty individuals were sampled from each of three sites, according to their body size (between 67 and 98 mm) and health status (no externally visible pathogens). By sampling three linear dammed sites, we ensure that certain fish have already expressed different climbing behaviors in the field (with expectedly no climbing event, one climbing event or two climbing events, hereafter labeled respectively as 0C, 1C and 2C). In the two most downstream segments (i.e. 0C and 1C), individuals were sampled below the obstacle, close to the fishway entry. Fish from the most upstream segment (2C) were sampled directly on the fishway slope, as water depth below the obstacle precluded the use of electric fishing. All fish were brought alive to the laboratory for the common garden experiment.

2.2. Common garden

After a prophylactic treatment (H_2O_2, 250 ppm, 60 min), eels were individually marked by inserting a 6 mm long RFID device (NONATEC, Lutronics) in the peritoneal cavity. Tagging was undertaken under fish anesthesia (eugenol). After one week recovery, all the fish were placed into the same tank and reared for two months at low density (1 fish/100 L) in the same controlled conditions of light, food, temperature and water flow (T = 22 °C, pH = 6, 12/12 h light cycle, feed with Chironomidae ad libitum). After this period, body weight and body length were measured and monthly growth rates were calculated. Then, fish were sacrificed by severing the *medulla oblongata*. The whole brain and a sample of muscle were dissected and stored in RNALater buffer (Qiagen) for gene transcription analyses. Additional samples of muscle were dissected and stored in liquid nitrogen for further enzymatic activity analyses.

2.3. Gene transcription analysis

Gene sequences were chosen to specifically target the cognitive function, thyroid activity and aromatase activity in the brain and the swimming capacity of fish muscle (Table 1). A total of 9 genes are associated with neurogenesis and synaptic plasticity, both involved in cognitive processes such as perception, learning and memorization [25,30]: Glutamate receptor ionotropic, NMDA1 (*grin1*), c-*Jun* (*jun*), cofilin-1 (*cfl1*), CREB binding protein (*crebbp*), Thy-1 membrane glycoprotein (*thy1*), disintegrin and metalloproteinase domain-containing protein 10 (*adam10*), protein S100B (*s100b*), glutamate receptor 3 (*gria3*), and neurogenic differentiation factor 1 (*neurod1*). The gene of iodothyronine deiodinase 2 (*dio2*) was chosen due to its implication in thyroid hormone metabolism, and the gene of aromatase (*cyp19a1*) due to its involvement in neurogenesis [31] and brain sexualization [32]. Concerning muscle analyses, aerobic and anaerobic metabolic pathways were targeted and both gene transcription levels and enzymatic activities of lactate dehydrogenase (gene = *ldh*/enzyme = LDH), citrate synthase (*cs*/CS), cytochrome c oxidase (*cc*/CCO) and pyruvate kinase (*pk*/PK) were analyzed (Table 1). The type of metabolic activity provides insights into physiological performance of fish during two swimming modes: cruise swimming and burst swimming [27,33].

For each gene, specific primer pairs were designed using the EelBase [34] and the Primer3Plus software [35] and were purchased from Sigma Aldrich. All primer pairs are reported in Table 1. Samples