Comparative Biochemistry and Physiology, Part A 166 (2013) 522–527

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Male European eels are highly efficient long distance swimmers: Effects of endurance swimming on maturation

Erik Burgerhout [a,b,*], Sebastiaan A. Brittijn [b], Christian Tudorache [a], Daniëlle L. de Wijze [a], Ron P. Dirks [a,b], Guido E.E.J.M. van den Thillart [a,b]

[a] Molecular Cell Biology, Institute Biology Leiden, Leiden University, P.O. Box 9505, 2300 RA, Leiden, The Netherlands
[b] NewCatch B.V., J.H. Oortweg 19, 2333 CH, Leiden, The Netherlands

ARTICLE INFO

Article history:
Received 27 May 2013
Received in revised form 2 August 2013
Accepted 6 August 2013
Available online 17 August 2013

Keywords:
Anguilla anguilla
Migration
Swimming efficiency
Maturation
Dopaminergic inhibition
Gonadotropins

ABSTRACT

European eels (*Anguilla anguilla*) migrate ~6000 km towards their spawning area in the Sargasso Sea. Based on the recent discovery that males swim even more efficiently than females, it was predicted that males also would be able to swim ~6000 km within six months. Additionally, eels do not mature naturally in captivity due to strong neural inhibition. Earlier, it was hypothesized that swimming exercise is a natural trigger to induce sexual maturation and may even result in full maturation. In the present study two groups of farmed male silver eels were subjected to either endurance swimming or resting for up to 6 months. It was found that male eels were able to swim continuously for a total distance of 6670 km within 6 months. The body weight decrease in swimming and resting males after 6 months was similar (<30 g) underlining the extreme low energy cost of swimming. In contrast to our expectation long-term swimming did not induce sexual maturation in farmed silver eels, suggesting that swimming alone is not sufficient as a trigger for sexual maturation. In conclusion, male eels are efficient long distance swimmers and likely able to cover the distance to the Sargasso Sea within the expected time span of 6 months.

1. Introduction

Catadromous freshwater eels (*Anguilla* spp.) migrate long distances to their spawning grounds (Tsukamoto et al., 2002; Tesch, 2003; Aoyama, 2009; Tsukamoto, 2009). In the case of the European eel (*A. anguilla*) spawning migration covers a distance of more than 6000 km, from the European and North-African coasts to the Sargasso Sea (Schmidt, 1923). It is assumed that migrating this distance is accomplished in ca. 6 months, based on the time difference between the onset of the migration in autumn and the occurrence of larvae in spring (Schmidt, 1923; Tesch, 2003). During migration eels need to rely entirely on their energy stores as they cease feeding (Tesch, 2003). Several studies showed that female eels swim remarkably efficient as indicated by their low cost of transport (COT), i.e. the energetic costs per distance swum (van Ginneken and van den Thillart, 2000; van den Thillart et al., 2004; van Ginneken et al., 2005; A. Palstra et al., 2008). Calculations based on the optimal swimming speeds of female eels (ca. 0.65 m s^{-1}) indicate that females may reach the spawning area even in ca. 3.5 months (Palstra et al., 2008). In addition, it has been shown that farmed female European eels were able to swim continuously for 6 months covering a total of 5500 km (van Ginneken et al., 2007).

It is still unknown whether migrating male eels are able to swim a distance of ~6000 km within 6 months. Male European eels reach a body length of about 50% of that of the females and are approximately 5–10 times lower in body weight (Tesch, 2003). Based on studies regarding the relation between size and COT, it follows that the COT increases markedly when the body weight decreases (Schmidt-Nielsen, 1972; Beamish, 1978). Therefore one would assume that male eels must have a much higher COT than female eels at similar speeds. However, a recent study showed that farmed male eels are efficient swimmers by means of a relatively low minimum cost of transport at an optimal swimming speed of 0.5–0.6 m s^{-1} (ca. 1.5 BL s^{-1}; Burgerhout et al., 2013). The optimal swimming speed of males was found to be similar to that of females while the cost of transport was even lower. In addition, swimming in groups reduced the cost of transport further by ca. 30% (Burgerhout et al., 2013). Therefore, it is expected that also male eels would be able to cover the distance to the spawning area within 6 months.

At the onset of their reproductive migration eels are still in a prepubertal state; gonadal development remains inhibited (Dufour et al., 2003). Sexual maturation in female silver eels remains suppressed due to a deficit stimulation of gonadotropin-releasing hormone (GnRH) and dopaminergic inhibition of the pituitary, which blocks synthesis and release of luteinizing hormone (LH) (Dufour et al., 1988; Vidal et al., 2004; Weltzien et al., 2006, 2009). The induction of maturation must therefore occur during or after migration to the spawning area. Simulation of the migration by swimming exercise was hypothesized

* Corresponding author at: Molecular Cell Biology, Institute Biology Leiden, Leiden University, P.O. Box 9505, 2300 RA, Leiden, The Netherlands. Tel.: +31 6 18849427.
E-mail address: e.burgerhout@biology.leidenuniv.nl (E. Burgerhout).

1095-6433/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cbpa.2013.08.002

as the natural trigger releasing the dopaminergic inhibition and thereby inducing maturation (Palstra et al., 2007; van den Thillart et al., 2007; van Ginneken et al., 2007).

Although the response to swimming exercise in female eels appears to have an inhibiting effect (Palstra et al., 2008, 2010), wild male eels showed a different response. Palstra et al. (2008) found a significantly higher GSI in males after three months of swimming, covering ca. 900 km (average velocity of 0.12 m s^{-1}). In addition, the expression of *lhβ* subunit (*lhβ*) in the pituitary was two- to three-fold higher as compared to resting males. As spermatogenesis was also stimulated, it was suggested that swimming exercise stimulates sexual maturation in male silver eels, and that full maturation may occur after swimming for a longer period or distance (Palstra et al., 2008). In the same study, treatment with GnRH-agonist (GnRHa) resulted in significant growth of the testes which led the authors to conclude that the dopaminergic inhibition such as that found in female eels is not effective in male eels.

During the present study the effects of endurance swimming on maturation in farmed male silver eels was investigated. A long term swimming trial was performed with farmed male silver eels to test two hypotheses: 1) male eels are able to cover ~6000 km within 6 months, and 2) male silver eels are stimulated to full maturation when subjected to continuous long term swimming.

2. Materials and methods

2.1. Animals and housing

Farmed male silver eels (*Anguilla anguilla*; n = 70; 119.9 ± 2.2 g; 38.2 ± 0.3 cm; average ± SE) were obtained from a commercial eel farm (Nijvis-Holding B.V., Nijmegen, The Netherlands), where they were kept in fresh water at 24 °C. Prior to the trial, the eels were acclimated for two weeks and housed in a ca. 2500 L recirculation system, supplied with well oxygenated natural seawater (32 ± 1 ppt) at 18 ± 0.5 °C. The fish were kept under red light conditions (670 nm, bandwidth 20 nm), 12:12 L:D. This wavelength is likely invisible for silver eels as during silvering eye pigment changes to a blue spectral band (Pankhurst and Lythgoe, 1983). Light intensity above the experimental set-up was 0.06 lx (Voltcraft MS-1500). As silver eels cease feeding at the onset of the migration, they were not fed. Black conservation sacks (120 × 80 cm, Spro Strategy Conservation Sack) were added as shelter. The experiments complied with the Dutch law on animal experiments and were approved by the animal ethical committee of Leiden University (DEC #09020).

2.2. Swimming exercise

Prior to the trial, morphometric data — including body length (BL), body weight (BW), eye diameter horizontal and vertical (Edh and Edv, respectively) and the pectoral fin length (PFL) — of all eels were obtained. Data were used to calculate the eye index following Pankhurst (1982) and the silver index following Durif et al. (2005). All males used in this study were assigned as silver eels.

Thereafter, eels were randomly divided into seven groups ($n = 10$ per group). One group was sampled (see 2.3.) as initial control ($t = 0$), the other six groups were each introduced into six 127 L Blazka-type swimming tunnels (described by van den Thillart et al., 2004). The swimming tunnels were covered with plastic sheets which reduced the light intensity to 0.02 lx.

Of the remaining six groups, three groups were subjected to a water velocity of 0.57 m s^{-1} (swim group), which is the optimal swimming speed found for males in a recent study under similar conditions (Burgerhout et al., 2013). The other three groups were kept resting (rest group) at a water velocity of 0.05 m s^{-1}. The latter flow rate was necessary to keep the water well mixed and oxygenized, while low enough for the eels to remain rested. After 1.5, 3, and 6 months (corresponding to 46, 91 and 182 days), eels from the respective swim and rest groups were removed from the tunnel, euthanized, dissected and sampled (see Section 2.3).

After 26 days swimming at 0.57 m s^{-1} (ca. 1280 km), four males died (drop-outs) and were removed from the tunnels within ca. 15 minutes. As the swimming velocity of 0.57 m s^{-1} might have been too high for long-term endurance swimming. The velocity was directly decreased to 0.40 m s^{-1}, which is the estimated minimum speed to cover 6000 km within 6 months.

2.3. Sampling procedure

The eels were sacrificed using an overdose of clove oil (1:10 dissolved in 96% ethanol, dose 5 mL L^{-1}), followed by decapitation. Blood was obtained from the tail (caudal vein), using a heparin rinsed needle and syringe (10.000 IU heparin in 0.9% saline). Blood was centrifuged for 5 min at 16,000*g* at 4 °C) to obtain the blood plasma, which was stored at −80 °C until further analysis.

Gonad, liver, and digestive tract were dissected and subsequently weighed to calculate the respective somatic indices ((tissue weight/body weight) × 100). A testis sample (left side) for histological analysis was fixed overnight in 4% paraformaldehyde (PFA), and afterwards stored in 70% ethanol at 4 °C.

2.4. Blood analysis

The levels of the gonadotropins, follicle stimulating hormone (FSH) and LH were measured in blood plasma using a recently developed bioassays based on the eel-specific FSH and LH receptors (Minegishi et al., 2012). Briefly, the bioassays consist of human embryonic kidney cells (HEK293 cells), which stably express the LH receptor of the European eel or the FSH receptor of the Japanese eel and contain a stably integrated luciferase reporter gene driven by a cAMP responsive-element. After incubation at 37 °C for five hours for cell stimulation, a luciferase assay was performed using Steadylite plus Reporter Gene Assay System (Perkin Elmer, Waltham, MA, USA). The luminescence signal was measured on a multilabel plate reader (Victor, PerkinElmer).

Blood plasma levels of testosterone were measured using a Testosterone ELISA kit (HUMAN Diagnostics Worldwide GmBH) following manufacturer's instructions.

2.5. Histological analysis

The testis samples were first dehydrated in a series of ethanol (70%–80%–90%–100%), followed by incubation in 100% Histo-Clear (National Diagnostics, Biozym TC B.V., The Netherlands) and a 1:2 mixture of Histo-Clear with paraffin (Paraclean, Klinipath B.V., The Netherlands), respectively. Afterwards, the tissue samples were embedded in paraffin. Sections (7 μm thick) were obtained using a microtome (Leica RM2165) and, after rehydration, nuclei and cytoplasm were stained using Mayer's haematoxylin and eosin counterstaining (H–E-staining). The stages of the testis were determined following Peñaranda et al. (2010).

2.6. Statistics

All data were tested for normal distribution by Kolmogorov–Smirnoff tests and failed. (Kolmogorov–Smirnoff; $p < 0.05$). Therefore, Mann–Whitney *U* non-parametric tests were used to analyze the results. Differences in biometry (EI, HSI, GSI, DTWI) and blood plasma levels of FSH, LH and T were tested between and within the groups (initial control, swimmers and resters) over the consecutive time points (0, 1.5, 3 and 6 months). Data from drop-outs were not included in the analysis. At $p < 0.05$ the statistical difference was considered significant. In all cases values are expressed as average ± standard error (SE).