Endocrine control of *Anguilla anguilla* glass eel dispersal: Effect of thyroid hormones on locomotor activity and rheotactic behavior

Eric Edeline\(^a\), Agnès Bardonnet\(^b\), Valérie Bolliet\(^b\), Sylvie Dufour\(^c\), Pierre Elie\(^a\)

\(^a\)Cemagref, Unité Ecosystèmes Estuariens et Poissons Migrateurs Amphihalins (EPBX), 50 avenue de Verdun, 33612 Cestas Cedex, France

\(^b\)INRA, Unité d’Hydrobiologie, Ecologie Comportementale des Poissons, 64 310 St Pée sur Nivelle, France

\(^c\)Muséum National d’Histoire Naturelle, USM 0401, UMR CNRS/MNHN/UPMC 5178 “Biologie des Organismes Marins et Ecosystèmes”, Bâtiment de Physiologie, 7 rue Cuvier, 75231 Paris Cedex 05, France

Received 27 October 2004; revised 1 February 2005; accepted 1 February 2005
Available online 17 March 2005

Abstract

Dispersal, one of the most important processes in population ecology, is an issue linking physiological and behavioral features. However, the endocrine control of animal dispersal remains poorly understood. Here, we tested whether and how thyroid hormones may influence dispersal in glass eels of *Anguilla anguilla*, by testing their influence on locomotor activity and rheotactic behavior. Glass eels were caught during their estuarine migration and treated by immersion in either a l-thyroxine (T\(_4\)) or a thiourea (TU) solution. As measured by radioimmunoassay, T\(_4\) and TU treatments induced, respectively, increased and decreased whole-body thyroid hormone levels relative to untreated controls. We tested a total of 960 glass eels distributed into control, and T\(_4\) and TU treatment groups, on their swimming behavior in experimental flume tanks equipped with upstream and downstream traps that allowed us to concurrently measure both the locomotor activity and the rheotactic behavior. Compared to controls, locomotor activity significantly increased among the hyperthyroid, T\(_4\)-treated eels, but significantly decreased among the hypothyroid, TU-treated eels. The results on rheotactic behavior suggested a more complex regulatory mechanism, since TU but not T\(_4\) treatment significantly affected rheotactic behavior. The influence of thyroid hormones on locomotor activity suggests a central role for these hormones in the regulation of mechanisms leading to the colonization of continental habitats by glass eels. Thyroid hormones are also implicated in the control of locomotor activity in mammals and migratory behavior in birds, suggesting that these hormones represent conserved, proximate mediators of dispersal in vertebrates.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Thryoid hormones; Dispersal; Migration; *Anguilla anguilla*; Locomotor activity; Rheotactic behavior

Introduction

Animal dispersal is of great ecological importance, shaping population structure, conditioning gene flow and influencing fitness (Clobert et al., 2001). Dispersal is affected by a range of factors including the organization of social systems, growth forms, seasonality, habitat and a host of other ecological and behavioral parameters. Hormones that may influence dispersal and migration include glucocorticoids and androgens (Dufty and Belthoff, 2001; Holekamp and Smale, 1998). However, the endo-
estuarine animals (Forward and Tankersely, 2001). Fishes ascend from the bottom and are carried by tidal currents during the flood tide. During slack water, they return to the bottom where they remain during the ebb tide. Movements are therefore achieved through saltatory steps. In fishes, the STST allows important energy savings and provides rapid downstream transport compared to constant counter-current swimming (Metcalfe et al., 1990; Weihs, 1978). When they reach the tidal limit, migrating glass eels have to face a constant downstream water current. At this point, they lose the circatidal rhythm and adopt a strict counter-current swimming (McCleave and Wippelhauser, 1987; Wippelhauser and McCleave, 1988). This behavioral shift, affecting both locomotor activity and rheotactic behavior, allows the colonization of river systems. Therefore, due to their life cycle, eels experience marine, estuarine and freshwater habitats. Throughout their migration, they have to cope with oceanic, tidal and river currents, using either flow-carried or active swimming. Hence, the eel is an especially good model to study fish dispersal.

During migrations, amphibialine fishes show a suite of physiological, morphological and behavioral modifications linked to endocrine alterations (Fontaine, 1975; Woodhead, 1975). However, the endocrine control of migratory behavior is still relatively unknown, especially in eels. The thyroid hormones (THs) L-thyroxine (T4) and 3,5,3′-triiodo-L-thyronine (T3) are phylogenetically conserved molecules that affect many aspects of development, growth and metabolism of vertebrates. In juvenile salmonids, THs play a central role in the regulation of smoltification that transforms river-dwelling parrs into migratory smolts that are adapted to the marine environment (McCormick et al., 1998). Moreover, during smolting, a surge of THs is proposed to trigger the downstream migratory behavior (Iwata, 1995; Katzman and Cech, 2001; Specker et al., 2000). In adult salmon caught during their spawning migration, THs are related to the river discharge rate, suggesting a further role in counter-current swimming ability (Youngson and Webb, 1992). Correlations between migratory behavior and plasma TH levels were observed in field studies of the Arctic char Salvelinus alpinus (Hogensen and Prunet, 1997) and the cod Gadus morhua (Comeau et al., 2000, 2001). In eels, THs also play a fundamental role in regulating the metamorphosis from the leptocephalus larval to the elver stage (Jegstrup and Rosenkilde, 2003; Ozaki et al., 2000; Vilter, 1946; Yamano et al., 1991). In subadult American eels Anguilla rostrata, elevated T4, but not T3, plasma levels are correlated with increased locomotor activity under natural conditions (Castonguay et al., 1990). In European glass eels A. anguilla, river-colonizers caught on a fish pass exhibit an increased thyroid status compared to estuarine migrants (Edeline et al., 2004). Moreover, compared to estuarine migrants, glass eels caught on the bottom of the estuary have decreased whole-body THs. Consistent with these observations, laboratory experiments show a stimulating effect of THs on locomotor activity in G. morhua, juvenile salmon Oncorhynchus spp. and goldfish Carassius auratus (Castonguay and Cyr, 1998; Hoar et al., 1952, 1955; Woodhead, 1970). However, there remain no studies of eels or any other elopomorph fish, one of the major groups of teleosts (see Nelson, 1994), of the role of THs in the behavioral changes occurring during the metamorphosis from the leptocephalus larval to the elver life history stages.

Eel stocks are currently collapsing (Briand et al., 2003; Dekker, 1998; Stone, 2003). Therefore, a greater knowledge of the behavioral biology of eels is essential to the improvement of conservation policies. Moreover, the study of the rather specialized life history stages of elopomorph fish may provide important insight into the endocrine mechanisms of vertebrate migration and dispersal. Here, we investigated the role of THs in controlling dispersal in glass eels of A. anguilla by assessing their influence on the intensity of locomotor activity and rheotactic behavior. To this end, glass eels caught during their estuarine migration were treated by immersion in either a T4 or thiourea (TU, an antithyroid drug) solution. Swimming activity and its orientation were concurrently monitored in flume tanks equipped with upstream and downstream traps. In addition, subsamples of control, TU-treated and T4-treated fishes were analyzed for whole-body TH content to assess the effects of the hormonal treatment on thyroid status.

Materials and methods

Animal manipulations were performed in compliance with the recommendations of the French ethical committee and under the supervision of authorized investigators.

Fish collection and maintenance

Glass eels were sampled with a pushed surface net at night and during flood tide on 22 March 2004 in the tidal freshwater zone of the Isle River, at Libourne, in southwestern France. The sampling site was located 100 km upstream of the mouth of the Gironde estuary. Water temperature was 11°C. Glass eels were transferred to the INRA station of St. Pée sur Nivelle in aerated tap water from which chlorine had been eliminated by aeration. Fishes were maintained in 5 l containers of aerated tap water. Ten percent of the water volume was renewed daily. Pilot experiments showed that T4 treatment sharply increased the aggressive behavior of glass eels (identified by skin injuries) when stocked at a high density of 35 individuals l–1. Therefore, during the present experiment, we acclimatized glass eels at a low density of 9.6 individuals l–1. Moreover, the animal’s welfare was improved by providing shelters (flat stones placed on the bottom of the holding containers). Under these conditions, we did not observe any skin injuries. Water temperature was regulated with an air conditioner at 11 ± 0.5°C. The room was maintained from
8:00 am to 18:00 pm under low light intensity (1.8 ± 0.11 lux) that corresponded to the natural photoperiod (10 L/14 D). This light intensity did not constitute a stress for glass eels and has been shown to not affect their levels of activity (Wippelhauser and McCleave, 1988).

**Hormonal treatments**

In teleost fishes, production of T₄, the main hormone secreted by the thyroid gland, is regulated by the hypothalamo–pituitary axis via thyroid stimulating hormone (Eales and Brown, 1993). T₄ is released into the circulation and deiodinated by T₄ outer ring deiodases (T₄ORD) into T₃ in peripheral tissues. Subsequently, T₃ is either deiodinated into 3,3’ diiodo-L-thyronine (T₂) by T₃ inner ring deiodases (T₃IRD) or excreted through urine or bile. The binding affinity of T₃ to TH receptors is approximately ten fold higher than T₄, as shown in hepatocytes of the rainbow trout Oncorhynchus mykiss and coho salmon Oncorhynchus kisutch (Bres and Eales, 1986; Darling et al., 1982).

Administration of exogenous T₄ and TU by immersion is noninvasive and stress free (Higgs et al., 1982). This method is therefore especially well suited to examine the effects of hormonal manipulation on behavior. Therefore, we immersed glass eels in water into which we had previously added either T₄ to increase circulating TH levels or thiourea (TU) to decrease TH production. We used T₄ rather than T₃ in order to mimic, as close as possible, the natural secretion of the thyroid gland. TU prevents the iodination of the thyroglobulin in the thyroid gland (Davidson et al., 1979; Raby et al., 1990). Therefore, due to thyroglobulin stocks, TU treatments have to be applied for long periods of time to strongly deplete TH levels. Such a treatment would have involved a long period of captivity that may induce other behavioral alterations. Thus, we used a short term 7-day TU treatment. Thiourea also exerts an inhibitory action on T₄ORD activity over short periods (Frith and Eales, 1996) and was likely the primary mechanism by which TU influenced TH levels in the present experiment. Following standard concentrations used in prior studies of eels, we applied doses of 0.5 mg T₄ l⁻¹ and 500 mg TU l⁻¹ (Jegstrup and Rosenkilde, 2003; Pradet-Balade et al., 1997).

T₄–sodium salt (Sigma) was dissolved in 0.1 N sodium hydroxide (NaOH, 1.25 mg ml⁻¹), and TU (Sigma) was dissolved in distilled water (1.25 g ml⁻¹). Solutions were kept at 4°C, and 2 ml was added to water (5 l containers) to reach T₄ and TU concentrations of 0.5 and 500 mg l⁻¹, respectively. Both TU-treated and control groups received 2 ml 5 l⁻¹ water of 0.1 N NaOH (T₄ solvent). Concentrations of TU, T₄ and NaOH were maintained constant throughout the experiment by adding drugs daily to the 500 ml renewed water.

A total of 960 glass eels coming from a single catch were randomly distributed into 4 batches of 240 individuals. Each batch was randomly distributed in 3 treatment groups (T₄, TU, control; 80 glass eels per treatment) and treated for 7 days before testing their swimming behavior. The behavioral test required 24 h for a given trial (see below). Therefore, in order to keep hormonal treatment duration constant between batches, treatment started with a 24 h lag between each batch. For example, hormonal treatment began 36 h after capture for batch 1 and 108 h after capture for batch 4.

**Experimental flume tanks and behavioral tests**

Behavioral tests were performed using six experimental flume tanks (180 cm long, 30 cm wide, 17 cm in water depth); one of which is represented in Fig. 1. Upstream and downstream walls were pierced by a hole (4 cm in diameter) shut by a perforated plug (1 mm mesh), allowing water to circulate but preventing the escape of fish. Plugs were easily replaced by traps during the behavioral tests. Dechlorinated tap water, flowing through a pipe, was delivered to each flume tank with a constant discharge providing a flow velocity (mean ± SD) of 20 ± 2 cm s⁻¹ at the mouth of the upstream trap, 2 ± 2 cm s⁻¹ in mid channel and 2 ± 1 cm s⁻¹ at the mouth of the downstream trap. Therefore, flow velocity at the mouth of the upstream trap did not limit the swimming capacity of the eels (McCleave, 1980) but was still strong enough to select glass eels showing a pronounced upstream swimming behavior. In contrast, flow velocity at the mouth of the downstream trap was low, avoiding the accidental trapping of glass eels that did not clearly show a downstream swimming behavior. Moreover, in mid-channel, glass eels were able to either rest on the bottom by hiding under the stones or swim in the water column. Hence, the experimental device was designed to test the effect of hormonal treatment on the voluntary movements of glass eels. Trapped eels (upstream and downstream swimming) were considered to have exhibited locomotor activity consistent with dispersal behavior. In contrast, glass eels remaining in the channel, often hidden under the stones, were considered to have exhibited no locomotor activity. Following preliminary trials, eels were kept in the flume tanks without any water current for 22 h before being tested. This procedure avoided accidental catches due to exploratory behavior. Behavioral tests started at 14:00, when water flow was open. Traps were not immediately set in order to avoid the effect of the sudden flow (stress) on swimming behavior. Traps were set at 15:00 and trapping ran for 1 h. At 16:00, eels were removed from the traps and the flume tanks. After a 10 min rinse with clean water, new batches of glass eels were put into the flume tanks (40 glass eels per flume). To avoid any possible “flume tank effect”, we randomly assigned the tanks to a treatment for each trial, ensuring that a rotation between tank/treatment had been made. Moreover, behavioral tests were done by observers unaware of the treatments. For behavioral tests, two trials were performed per treatment group (40 glass eels in one flume tank per trial). All
together, a total of 8 behavioral trials were carried out for each hormonal treatment (T4, TU, control).

As emphasized above, we aimed to avoid an effect of stress on the swimming behavior of glass eels. Therefore, four flat stones were regularly arranged on the bottom of the flume tanks to provide shelters, and lateral tarpaulins hid to the fishes the movements of experimenters. Due to the low acclimatization density (9.6 glass eels l\(^{-1}\) during hormonal treatments and 0.4 glass eel l\(^{-1}\) during behavioral tests), no aggressive behavior was observed in any group (including T4-treated glass eels). The temperature during trials was identical to that of the stocking water and the light intensity was homogeneous among and inside the flume tanks. Photoperiod and light intensity were identical during the acclimatization period and the trials.

**Morphological analysis**

The glass eel stage is a late metamorphic stage between the translucent leaf-shaped leptocephalus larva and the fully pigmented elver. The extent of skin pigmentation is therefore indicative of the progress of metamorphosis. T\(_4\) and TU immersions are known to, respectively, enhance and reduce the development of pigment in glass eels over a 20 day treatment period (Jegstrup and Rosenkilde, 2003; Vilter, 1946). Furthermore, glass eels that stop feeding at metamorphosis are known to shrink and lose weight until feeding resumes (Elie et al., 1982). In order to detect potential developmental alterations due to the hormonal treatment and late glass eel metamorphosis, we monitored changes in body length, weight and pigmentation during the experiment. After being anesthetized with clove oil (10% diluted in ethanol, 4 ml l\(^{-1}\)), glass eels were analyzed for total length to the nearest mm and body mass to the nearest 10\(^{-3}\) g. Pigment stages were determined under a microscope and classified according to the extent of skin pigmentation over the head, tail and body regions through stages V\(_{A}\), V\(_{B}\), V\(_{IA_{0}}\), V\(_{IA_{1}}\), V\(_{IA_{2}}\), V\(_{IA_{3}}\) and V\(_{IA_{4}}\) to VIB following Elie et al. (1982). Stage VA is the earliest pigment stage, and stage VIB is the latest development pigment stage. A brief description of these pigment stages is given in the Table 1. For this morphological analysis, subsamples were made at capture (\(N = 69\)) from the whole batch and at the end of the experiment (\(N = 103\)) from batch 4 (see Table 2).

**Whole-body TH extractions**

After 7 days of treatment and before the behavioral test, subsamples of control, T\(_4\)-treated and TU-treated glass eels from batches 1, 2 and 3 (\(N = 5\) batch\(^{-1}\) treatment\(^{-1}\) = 45) were sampled from the containers for hormonal assays. After anesthesia with clove oil, glass eels were individually frozen in cryotubes which had been previously weighed to the nearest 10\(^{-3}\) g. The fresh mass of glass eels was then obtained without thawing by weighing the cryotube containing the glass eel and subtracting the mass of the tube.

The extraction procedure was previously described in Edeline et al. (2004). Briefly, extraction was performed in
Table 1

Brief description of the main features of each pigment stage of glass eels according to Elie et al. (1982) that was used for the morphological analysis in the present study.

<table>
<thead>
<tr>
<th>Pigment stage</th>
<th>Main features</th>
</tr>
</thead>
<tbody>
<tr>
<td>VA</td>
<td>The overall pigmentation is limited to the end of the caudal fin.</td>
</tr>
<tr>
<td>VB</td>
<td>Pigmentation appears on the head, and caudal pigmentation begins to extend on the back.</td>
</tr>
<tr>
<td>VIA0</td>
<td>Few pigments appear behind the head and dorsal pigmentation extends on the back.</td>
</tr>
<tr>
<td>VIA1</td>
<td>Head and tail dorsal pigments overlap, and lateral pigmentation begins to extend towards the head from the end of the tail.</td>
</tr>
<tr>
<td>VIA2</td>
<td>Lateral pigmentation does not exceed the dorsal fin. Moreover, ventral pigmentation develops from the tail towards the head.</td>
</tr>
<tr>
<td>VIA3</td>
<td>Lateral pigmentation exceeds the dorsal fin, but there is no ventral pigmentation between the anus and pectoral fins.</td>
</tr>
<tr>
<td>VIA4</td>
<td>Ventral pigmentation develops between pectoral fins and the anus.</td>
</tr>
<tr>
<td>VIB</td>
<td>Ventral pigmentation is compact, visibility of internal organs becomes reduced.</td>
</tr>
</tbody>
</table>

Skin pigmentation is related to the progress of metamorphosis from the translucent leptocephalus to the fully pigmented elver stages. For further details on eel metamorphosis, see Tabeta and Mochioka (2003).

cryotubes in 0.9 ml ice cold absolute ethanol containing 1 mM 5-Propyl-2-Thiouracil (Sigma) (EtOH–PTU). PTU was used to block endogenous desiodinase activity (Denver, 1993). Homogenization was carried out using an Ultra-Turrax homogenizer (Labo Moderne, Paris) and followed by sonication for 20 s with a Vibra Cell 72434 sonicator (Bioblock). The blades of the homogenizer were rinsed with 0.3 ml ice cold EtOH–PTU and the rinse was added to the homogenate. After centrifugation at 2950 × g for 20 min at 4°C, the supernatant was kept and the pellet was re-extracted in 0.3 ml ice cold EtOH–PTU by 20 s sonication. After a second centrifugation, both supernatants were pooled and centrifuged at 64 × g for 5 min at 4°C. The supernatant was vacuum dried at 37°C for 18 h in a Savant SVC 100 H Speed Vac. Samples were reconstituted by sonication in 800 μl of ice cold phosphate-buffered saline, pH 7.4, containing 1 mM PTU, and were analyzed for T3 and T4 content by radioimmunoassays (RIAs).

Radioimmunoassay (RIA)

The RIA method followed that of Edeline et al. (2004), using RIA kits for total T4 and total T3 (Cis Bio International, Gif sur Yvette) with tubes coated with anti-T3 or -T4 antibodies and 125I radio-labeled T3 or T4. Sensitivity was 2.5 ng ml⁻¹ for T4 and 0.1 ng ml⁻¹ for T3. Intra-assay variation ranged between 4.1 and 6.6% for T4 and 3.7 and 6.5% for T3. Inter-assay variation, given by the manufacturer, was estimated to range between 6.5 and 10.1% for T3 and 4.6 and 14.3% for T4.

Briefly, after addition of extract or standard and radio-labeled hormone to antiserum coated tubes, tubes were incubated at 37°C for 2 h and decanted. The radioactive fraction bounded to the tube was counted in a gamma counter (Kontron Analytical MDA 312). The bounded radioactive fraction (B) was expressed as a percentage of the maximal bounded radioactive fraction (B0) which was obtained with no addition of cold TH in the antiserum coated tube. % B:B0 was inversely proportional to the amount of cold TH in samples, which was calculated from the standard dilution curve parameters. Individual whole-body T3 and T4 levels were measured in duplicate in both assays and expressed as ng hormone per g wet body mass.

Data analysis

All statistics were conducted with Systat 10. We tested how hormonal treatment affected locomotor activity and rheotaxis in glass eels using a logistic regression model (logit, dummy coding procedure), linking the probability for a behavior to be exhibited to one or more independent variable(s) with a logistic function. First, the effect of treatment on locomotor activity was modeled with a binomial logit, grouping the upstream and downstream swimming behaviors under a common swimming activity variable(s) with a logistic function. First, the effect of treatment on the rheotactic behavior was modeled with a multinomial logit, equivalent to two binomial logits processed simultaneously, comparing concurrently the probability to swim upstream to that to be sedentary. Second, the effect of treatment on the rheotactic behavior was modeled with a multinomial logit, equivalent to two binomial logits processed simultaneously, comparing concurrently the probability to swim upstream to that to be sedentary and the probability to swim downstream to that to be sedentary.

The differences of individual whole-body T3 and T4 levels, body lengths and weights between groups were

Table 2

Morphological characters of glass eels from batch 4 just after capture (day 0 of acclimatization) and at the end of the experiment (day 13 of acclimatization), including 7 days of hormonal treatment.

<table>
<thead>
<tr>
<th>Acclimatization period (days)</th>
<th>Hormonal treatment</th>
<th>Number of fish analyzed</th>
<th>Total length (mm) (mean ± SD)</th>
<th>Body mass (g) (mean ± SD)</th>
<th>Pigment stages (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>VB    VIA0 VIA1 VIA2 VIA3 VIA4 VIB</td>
</tr>
<tr>
<td>0 (at capture)</td>
<td>None</td>
<td>69</td>
<td>69.4 ± 4.8</td>
<td>0.294 ± 0.066</td>
<td>0 4 10.1 52.2 42.4 8.7 0 0</td>
</tr>
<tr>
<td>13</td>
<td>Control</td>
<td>31</td>
<td>69.1 ± 4.7</td>
<td>0.266 ± 0.063</td>
<td>0 0 22.6 45.2 32.3 0 0 0</td>
</tr>
<tr>
<td>13</td>
<td>T3 0.5 ppm</td>
<td>28</td>
<td>66.9 ± 4.5</td>
<td>0.235 ± 0.054</td>
<td>0 3.6 14.3 39.3 42.9 0 0 0</td>
</tr>
<tr>
<td>13</td>
<td>TU 500 ppm</td>
<td>44</td>
<td>68.2 ± 4.0</td>
<td>0.259 ± 0.054</td>
<td>0 36.4 34.1 27.3 2.3 0 0 0</td>
</tr>
</tbody>
</table>

Total length is to the nearest mm, body mass is to the nearest 10⁻³ g, and pigment stage is according to Elie et al. (1982).
tested with a Wilcoxon non-parametric test. For the analysis of pigment stages, differences between groups with a Pearson’s $\chi^2$ test, we pooled pigment stages VB and VIA0, and stages VIA2 and VIA4, to avoid pools of less than 5 individuals. A Pearson’s $\chi^2$ test was also used to compare the proportions of upstream and downstream swimmers for each treatment. Results are given as mean ± SD.

Results

Morphology

Detailed results are presented in Table 2. Over the acclimatization period, control glass eels did not shrink (Wilcoxon, $df = 1$, $\chi^2 = 0.497$, $P = 0.481$). However, there was a significant loss of weight due to the end of metamorphosis (Wilcoxon, $df = 1$, $\chi^2 = 4.481$, $P = 0.034$). The pigmentation of control fish significantly increased during the experiment (Pearson’s $\chi^2$ tests, $df = 3$, $\chi^2 = 19.178$, $P < 0.001$), indicating that the development of glass eels was normal. Compared to controls, neither T4- nor TU treatments had a significant effect on pigmentation (Pearson’s $\chi^2$ test, T4: $df = 3$, $\chi^2 = 2.213$, $P = 0.529$ and TU: $df = 2$, $\chi^2 = 1.747$, $P = 0.418$). Moreover, neither body length (Wilcoxon, T4: $df = 1$, $\chi^2 = 1.814$, $P = 0.178$ and TU: $df = 1$, $\chi^2 = 0.036$, $P = 0.849$) nor wet mass (Wilcoxon, T4: $df = 1$, $\chi^2 = 2.779$, $P = 0.095$ and TU: $df = 1$, $\chi^2 = 0.075$, $P = 0.784$) were significantly affected by the hormone treatments. Together, these results suggested that hormonal treatments did not induce any significant degree of stress.

Alteration of the thyroid status

Whole-body T4 and T3 contents of control fish were similar to those found in a previous study in glass eels of A. anguilla (Edeline et al., 2004). Whole-body T4 levels after 7 days of treatment were $11.5 ± 4.9$ ng g$^{-1}$ in controls, $104.7 ± 17.9$ ng g$^{-1}$ in T4-treated fish and $11.1 ± 1.8$ ng g$^{-1}$ in TU-treated fish (Fig. 2). T4 immersion induced, as predicted, a significant increase in whole-body T4 levels (Wilcoxon, $df = 1$, $\chi^2 = 21.774$, $P < 0.0005$). In contrast, due to the short treatment period, the effect of TU treatment on whole-body T4 levels was not significant (Wilcoxon, $df = 1$, $\chi^2 = 0.097$, $P = 0.756$).

Whole-body T3 concentrations after 7 days of treatment were $2.1 ± 0.4$ ng g$^{-1}$ in controls, $4.9 ± 1.4$ ng g$^{-1}$ in T4-treated fish and $1.5 ± 0.3$ ng g$^{-1}$ in TU-treated fish (Fig. 2). T4-treated glass eels had significantly higher whole-body T3 levels than controls (Wilcoxon, $df = 1$, $\chi^2 = 21.389$, $P < 0.0005$), indicating that exogenous T4 was physiologically deiodinated into T3. Moreover, whole-body T3 levels were significantly lower in TU-treated fish than in controls (Wilcoxon, $df = 1$, $\chi^2 = 11.995$, $P = 0.001$), indicative of the inhibitory action of TU on T4ORD activity.

Effect of thyroid status alteration on the locomotor activity

Average percentages of locomotor activity (upstream and downstream swimming/total number of glass eels ($n = 8$ trials per treatment; 40 glass eels per trial)) were $19.2 ± 7.3$% in controls, $31.9 ± 13.2$% in T4-treated fish and $12.8 ± 11.7$% in TU-treated fish (Fig. 3). When tested with the binomial logistic regression model ($n = 8$ trials per treatment; 40 glass eels per trial), locomotor activity was significantly higher in T4-treated fish ($\chi^2 = 17.9$, $P < 0.0005$) and significantly lower in TU-treated fish ($\chi^2 = 11.1$, $P = 0.001$), indicative of the inhibitory action of TU on T4ORD activity.

Effect of thyroid status alteration on the rheotactic behavior

For all the treatments, the proportion of downstream swimmers was significantly higher than that of upstream swimmers (Pearson’s $\chi^2$ test, $df = 1$, control: $\chi^2 = 14.254$, $P < 0.0005$, TU: $\chi^2 = 4.122$, $P = 0.042$, T4: $\chi^2 = 12.629$, $P < 0.0005$), likely reflecting that glass eels were exhibiting...
STST when caught. This suggested that the natural behavior of glass eels was not strongly affected by the acclimatization procedure. Average percentages of upstream and downstream swimmers for each treatment are presented in Fig. 4.

When tested with a multinomial logistic regression model (n = 8 trials per treatment; 40 glass eels per trial), T4-treated fishes showed both significantly more upstream (logit, df = 4, t ratio = 2.719, P = 0.007) and downstream (logit, df = 4, t ratio = 2.647, P = 0.008) swimming activity than controls, suggesting that thyroid status did not affect rheotactic behavior.

In contrast, TU-treated glass eels showed a significantly lower downstream (logit, df = 4, t = -2.264, P = 0.024), but not upstream (logit, df = 4, t = -0.442, P = 0.659) swimming activity than control glass eels, indicating an effect of thyroid status on rheotactic behavior.

Discussion

The present study demonstrates that THs are involved in the regulation of eel locomotor activity. Indeed, T4 and TU treatments, respectively, increased and decreased glass eel locomotor activity. The results of this study support our previous field data showing that thyroid status is related to the migratory behavior of glass eels (Edeline et al., 2004). In our previous field studies of A. anguilla glass eels, individual whole body T4 and T3 levels ranged between 6.9 and 50 ngT4 g\(^{-1}\) and 0.57 and 3.2 ngT3 g\(^{-1}\), respectively (Edeline et al., 2004). Therefore, the TH levels experienced by glass eels after T4 or TU treatments in the present experiment were similar in magnitude to those found in a wild population of glass eels.

Our findings are consistent with previous laboratory experiments conducted in a wide range of teleosts including salmonids, cods and cyprinids (see Introduction) and show for the first time in an elopomorph fish, one of the major groups of teleosts (see Nelson, 1994), that thyroid status is involved in the control of locomotor activity. Other studies show that the onset of the migration is related to increased circulating TH levels in birds (Pathak and Chandola, 1982, 1984), while THs have been shown to be involved in the control of locomotor activity in mammals (Rastogi and Singhal, 1976, 1979). Together, these support the hypothesis that thyroid control of locomotor activity may be a conserved vertebrate trait that reflects a more general role for THs in the mediation of dispersal and migration among vertebrates.

The physiological mechanisms by which THs alter locomotion are not clear. In teleosts, THs activate the production of metabolic enzymes in brain, liver and skeletal muscle that lead to increased energy production and aerobic capacity (Tripathi and Verma, 2003; Varghese et al., 2001). THs are known to have general effects on cell metabolism through mitochondrial activation (Goglia et al., 2002; Lanni et al., 2001; Leary et al., 1996), independently of \(\beta\)-adrenergic stimulation (Bachman et al., 2004). Therefore,
THs probably affect fish activity and locomotion through an activation of cellular metabolic pathways.

THs have also been shown to affect a variety of target genes in brain (Anderson, 2001; Viguerie and Langin, 2003). In elopomorphs and other teleosts, TH receptors are present in the brain (Bres and Eales, 1988; Dasmahapatra et al., 1991; Kawakami et al., 2003a,b; Van Der Kraak and Eales, 1980) and THs have been shown to alter catecholaminergic activity (Chaube and Joy, 2003). Importantly, catecholamines have previously been implicated in the control of locomotion in several vertebrate groups, including fish ( Johannson et al., 2004; Jönsson et al., 2003; Le Bras, 1978) and mammals (Rastogi and Singhal, 1976, 1979). Although we did not specifically address catecholamine effects in the present study, this line of research offers opportunities for future work.

We report that T4 treatment significantly increased both upstream and downstream movements, suggesting that an increased thyroid status may promote migration, irrespective of the current direction. In contrast, TU treatment resulted in a significant decrease in downstream, but not upstream, swimming behavior. We predict that a stronger depletion of TH levels would have also decreased upstream swimming. This result suggests an influence of THs on rheotactic behavior and that in the wild, decreased TH levels could reduce STST. This trend is supported by our previous field data suggesting that glass eels settling in an estuary have significantly decreased whole-body TH levels compared to eels using STST (Edeline et al., 2004).

In juvenile salmonids, the TH surge during the parr–smolt transformation is associated with a suite of changes that occur during the downstream migration, including the acquisition of negative rheotaxis (Specker et al., 2000). During the present experiment, the hyperthyroidism and slight hypothyroidism induced, respectively, by the T4 and TU treatments affected glass eel rheotactic behavior in different ways, suggesting a complex regulatory mechanism. In the Singi fish Heteropneustes fossilis, protein synthesis in different brain regions was stimulated by different threshold doses of T4, suggesting region-specific TH sensitivity (Ghosh and Medda, 1982); similar results were obtained in mammals (Rastogi and Singhal, 1979). In the rainbow trout O. mykiss and sockeye salmon Oncorhynchus nerka, it has been shown that THs may differentially regulate brain desiodase activity (Plate et al., 2002). Various sensitivity thresholds to THs could explain our results, but further experiments are needed to more completely investigate the neuroendocrine control of rheotaxis. External orienting signals or “clues” (Harden-Jones, 1984), such as salinity and olfactory gradients, may also influence the rheotactic behavior of migrating glass eels (Tosi et al., 1988, 1989, 1990). For example, THs were shown to influence salinity preference in the stickleback Gasterosteus aculeatus and juvenile Pacific salmon Oncorhynchus spp. (Baggerman, 1960, 1962; Iwata, 1995).

THs are also implicated in olfactory processes. In mammals, THs affect the maturation and the turnover of olfactory receptor neurons (Paternostro and Meisami, 1996a,b), while recent data in salmonids suggest that THs may be involved in the control of olfactory sensitivity during diadromous migrations (Lema and Nevitt, 2004; Plate et al., 2002). THs induce olfactory cell proliferation (Lema and Nevitt, 2004) and are likely involved in odor imprinting during the parr–smolt transformation (Dukes et al., 2004). In glass eels, olfaction also apparently plays a central role in orientation behavior, as indicated by their strong attraction towards green and earthy odors such as geosmin that are typical of inland waters (Sola, 1995; Sorensen, 1986; Tosi and Sola, 1993). Thus, by promoting the proliferation of olfactory neurons, THs could increase the sensitivity of migrating glass eels towards odorous clues. These mechanisms may constitute additional pathways by which THs influence rheotaxis, further supporting the view that the regulation of the rheotactic behavior involves complex interactions between external and internal stimuli. During the present experiment, we deliberately chose not to investigate the effects of salinity and olfactory clues on rheotaxis. This could explain why T4 treatment had a significant effect on locomotor activity but not on rheotactic behavior. Further studies should address the role of THs on responses to combined migratory clues (water current direction and salinity/terrestrial olfactory clues).

During the parr–smolt transformation, THs mediate physiological, morphological and behavioral changes that represent an adaptative specialization for downstream migration, seawater entry and marine residency (Boeuf, 1993; McCormick et al., 1998). Similarly, in glass eels, an adaptative role of THs in the colonization of continental habitats may be hypothesized. The metamorphosis from the leaf-shaped translucent leptocephalus to the eel-shaped fully pigmented elver may be considered an adaptive, morphological switch from oceanic drift to STST and river colonization. THs mediate this transformation (Jegstrup and Rosenkilde, 2003; Ozaki et al., 2000; Yamano et al., 1991), as well as the adaptation of the gut to osmoregulation in freshwater (Cicotti et al., 1993; Monaco et al., 1981; Vilter, 1946). Accordingly, Specker (1988) suggested that THs may play a fundamental role in preparing animals to exploit a new environment, especially through developmental changes in the gut. THs could also be involved in other physiological adaptations to continental waters colonization. In juvenile salmonids, T3 treatment induces increased muscle twitch rates, relaxation and maximum force, decreasing aerobic capacity and probably making smolts unable to maintain their position against the current (Katzman and Cech, 2001). This suggests that during smoltification, THs promote a transformation of muscle physiology from low intensity aerobic swimming towards the burst-like anaerobic swimming required for downstream migration. In glass eels, adapta-
tions of muscle physiology for the shift from oceanic drift to STST could also be regulated by THs. In addition, during the parr–smolt transformation, THs promote schooling behavior (Hutchison and Iwata, 1998; Iwata, 1995; McCormick et al., 1998). In the present experiments, we did not observe any schooling behavior in any treatment group at the very low density in our flume tanks. However, this does not exclude the possibility that THs may participate in the schooling behavior of migrating glass eels in the wild. Indeed, before the collapse of glass eel recruitments, huge shoals of migrants ascend rivers (Tesch, 2003).

In conclusion, our data show that THs are proximate mediators of transformations in glass eel swimming ability. THs stimulate locomotor activity, possibly through changes in energy metabolism, brain catecholamine synthesis and muscle physiology. From a broader perspective, THs could mediate a suite of morphological, physiological and behavioral adaptations allowing the colonization of continental habitats by glass eels. This includes a role for THs in the control of rheotactic behavior possibly by modulating sensitivity to a variety of environmental clues used for orientation. Hence, THs may play a central role in the expression of the glass eel migratory behavior during both the STST and the behavioral shift that occurs at the tidal limit. Together with other studies, the results also suggest that THs are conserved proximate mediators of dispersal among vertebrates.

Acknowledgments

We are indebted to Pr. Ian Mayer (University of Bergen) for precious critical comments and to Andrew Bass (Cornell university) for help with the manuscript. The authors thank Marc Jarry (INRA) and Laurent Beaulaton (Cemagref) for their help during data analysis. We are grateful to Philippe Camoin (Cemagref) for drawing Fig. 1. This study was partly supported by research grants from the GRISAM and Région Aquitaine.

References


Specker, J.L., 1988. Preredaptative role of thyroid hormones in larval and
juvenile salmon: growth, the gut and evolutionary considerations. Am. Zool. 28, 337–349.


