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Impact of individual morphological and physiological characteristics on the sexual maturation of the European eel (*Anguilla anguilla*).

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INTRODUCTION

Since the 1980's worldwide populations of eels declined steeply (Moriarty 1986, ICES 2002, Dekker 2003a). Several causes have been identified and studied, but mainly those related to the freshwater life phase. Marine causes for this decline have also been hypothesised such as deteriorating reproductive capacities of the adults (Feunteun 2002, Dekker 2003b). The European eel is believed to spawn in the Sargasso Sea (Schmidt 1922), approximately 6000 km from its continental habitat. It is only during the long-distance migration to the spawning grounds that the eel undergoes true sexual maturation (vitellogenesis and ovarian development), triggered by environmental factors encountered during this phase. However, the oceanic life phase of eels remains for the most part a mystery as no sexually maturing individuals have ever been caught or observed in the open sea.

At present, the only way to obtain sexually maturing eels, is to artificially induce sexual maturation in migratory individuals (silver eels) caught in freshwater (for review Dufour et al. 2003). However, accounts of eels (particularly true for *Anguilla anguilla*) not responding to gonadotropin injections are frequent and yet unexplained (Pedersen 2003, Pedersen 2004, Palstra et al. 2005). Overall, there is evidence that although eels receive the same treatment, they display tremendous variability in the way they sexually mature and in their reproductive capacities (Dufour unpublished data, this study). The objective of this study was to describe the variability in the response of eels in terms of gonadal yield to standardized gonadotropic treatment (CPE injections), and to relate this variability to individual characteristics. To do this, sexual maturation was induced in groups of eels from different locations in France. Their capacity to mature was evaluated throughout the experimental periods using external indicators, and compared to their initial physiological and morphological characteristics.

MATERIALS AND METHODS

In order to obtain a wide range of sizes of silver eels (i.e. migratory eels), individuals were collected at 5 locations in France, which corresponded to very different types of aquatic systems. Female eels were all caught during their downstream migration from November to December. Experiments were held at the Cemagref Aquaculture station (Bordeaux, France). The 3 experiments (2000, 2001, 2002) involved a total of 533 eels, among which 240 received a gonadotropic treatment (CPE) and 293 were controls.

At their arrival on site, the fish were measured externally and tagged.

Eels received one perivisceral injection a week of carp pituitary extract (CPE) at a dose

equivalent to 20 mg pituitary powder / kg body weight, according to the method previously developed (Fontaine et al., 1964; Dufour et al., 1993).

The following external measurements were taken prior to any hormone injection and every 2 weeks throughout the experimental period: total body length (Lb), pectoral fin length (Fl), and eye diameters (Dh, horizontal and Dv, vertical). Dm was calculated as the mean between Dh and Dv. Body diameter (Db) was measured with the calliper placed around the anterior part of the body at the dorsal fin level. Body weight (Wb) was recorded every week to calculate the condition factor. Relative condition (LeCren, 1951) was used to compare body weights:

$$Kn = \frac{Wb}{W'}.10^5$$

where Wb is the body weight and W' is the predicted length-specific mean weight for the sample. W' was here equal to $(Lb)^{3.21}$. The initial stage of eels relative to the silvering process was determined using Lb, Wb, Fl and Dm measured at their arrival on site according to the classification system described by Durif et al. (2005) and Durif & Elie (accepted).

Control and treated eels were sacrificed at regular intervals up to 24 weeks of treatment. Ovaries, liver and digestive tract were removed and weighed (Wg: gonad weight; Wl: liver weight, Wdt: digestive tract weight). Blood samples were taken and vitellogenin plasma levels were assayed. Otoliths were collected and age was determined according to the protocol described in Adam (1997).

In order to compare maturation response regardless of any size effect, all variables, except for vitellogenin, were standardized to body length. Variables (except for body mass) were first log-transformed then standardized following Claytor & MacCrimmon (1986) using the formula:

Var_std= Var – (M (Lt – Lt_mean))

Where Var_std is the corrected variable, Var is the original variable, M is the slope of the regression of the variable on total body length, and Lt_mean is the mean length of the eels in the sample. Henceforth, standardized variables are referred to as: BD_m for maximum body diameter, ED_m for maximum eye diameter, PF_m maximum pectoral fin length. Standardized initial measurements (measured before any hormone injection) are noted BD_i, ED_i, PF_i. And internal parameters are GW for gonad weight, LW for liver weight, and DTW for digestive tract weight. Vitellogenin was only log-transformed as it was not correlated to body length, and this variable is referred to as VTG. The Pearson correlations between standardized variables were calculated, and their significance determined using a Bonferroni test.

Maximum body diameter (BD_m) was highly correlated to gonad weight (R=0.80), and was therefore chosen to assess the maturation response of eels as well as the changes occurring during sexual maturation. Values of BD_m were plotted against the corresponding number of injections (*i.e.* number of injections an eel had received to reach its BD_m value). Exponential curves were fitted to each yearly set of data (least square method). Eels that displayed a BDm value lower than the predicted value (negative residual) were classified into the LMR group (low maturation response), and eels with a higher than predicted BD_m were assigned to the HMR group (high maturation response).

RESULTS

Gonads of most treated eels underwent a significant increase, as much as 50.3% of total body weight (mean value in initial controls was 1%). However, variability in individual responses to the treatment was very high. The coefficient of variation (CV=mean/standard deviation) calculated for GW of treated eels at each organ sampling date ranged between 4 and 59%.

Significant correlations were calculated between several parameters (Table 1). Gonad weight (GW) increased with vitellogenin (VTG) and liver weight (LW), while the weight of the digestive tract (DTW) decreased. The GW was also significantly correlated with external descriptors that reflect energy stores: initial and maximum relative condition (Kn_m, Kn_i), as well as initial and maximum body diameter (BD_m, BD_i). The length of the pectoral fin did not increase during sexual maturation; therefore PF_m was not correlated with any parameter. However eye diameter increased with the number of hormone injections, and ED_m was indeed significantly correlated with GW, vitellogenin, and all associated parameters (LW, DTW, Kn_m, BD_m, Kn_i, and Bd_i). Initial length of the pectoral fin (PFi) was linked to relative condition, both initial and maximum.

Age was positively correlated with several descriptors linked to the fitness of eels: LW, BD_m, Kn_m, and Kn_i. The oldest eels also displayed the highest levels of vitellogenin.

At the beginning of the experiment, eels were at various silvering stages (from stage FII to stage FV, according to the classification established by Durif et al. 2005). None of the eels that were at the yellow stage (stage FII: sexually differentiated eels but with very limited gonad development) matured, and their body diameter (BD) decreased from the start. The majority (60%) of pre-silver eels (stage FIII: slightly developed gonads but no regression of digestive tract), displayed low maturation response. In the same way, 65% of stage FIV eels (silver eels at the beginning of migration) were in the LMR group. Finally, 56% of FV eels

(last silvering stage) belonged to the HMR group. These eels displayed the best maturation capacity, however 44% still showed low maturation response.

To remove the stage effect, only FV eels were used in further comparisons. Eels belonging to the HMR group had a higher initial condition factor (Kn_i) and body diameter (BD_i) than eels in the LMR group.

Overall, results showed that larger eels produced more gonads. Two eels were in the 400 mm class (body length between 400 and 500 mm), and they hardly matured as Wg only reached 9g after 12 injections (Wg=2.5g for control eels of the same size class). For eels in the 500 mm class, Wg increased linearly until the 17^{th} injection and underwent a 7-fold increase. Gonad development followed an exponential trend starting with 600 mm eels (12-fold increase after 17 injections). Gonad weights of the 700 and 800 mm eels had increased by a factor of 21 after 17 injections. Therefore, the highest gonad/length ratio corresponded to eels in the 700 mm size class.

CONCLUSION

ICES (1999) stated that the European eel stock is outside safe biological limits and recommended to set escapement targets. This study is the first to try and quantify the "quality" of future spawners. We have described large individual variability in the gonadal yield of female European eels. Our findings result in a set of recommendations that can help to predict the reproductive potential of eels according to their biometrical characteristics. This is a crucial aspect in the management of eel populations, but it may also be of interest for aquaculture purposes. Future spawners with a high reproductive potential are eels that have completed their silvering metamorphosis (stage FV), and whose body length are over 700 mm. For these size classes we can calculate Fulton's condition factor, using $W' = (Lb)^3$; the condition factor of high reproductive potential spawners would then have to be higher than 0.2. Evaluation of the reproductive potential, based on these criteria would be easy to implement on silver eels from selected locations. We recommend that these simple external measurements, namely body length and weight, eye diameters, and pectoral fin length, be applied to population dynamics studies to evaluate the proportion of future spawners with a high reproductive potential. This could help target and protect habitats that favor such eels. As older and larger eels (over 700 mm in length) are generally found upstream of watersheds, it is essential that these upstream populations be sustained and that efforts be maintained to improve upstream and downstream passage of eels at dams and hydroelectric facilities.

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Table 1. Pearson correlation matrix calculated on morphogical and physiological descriptors of treated eels. Significant correlation coefficients are in bold (Bonferroni, $p<0.05$). GW: standardized gonad weight, VTG: log (vitellogenin), LW: standardized liver weight, DTW: standardized digestive tract weight, Kn: relative condition factor, ED: standardized eye diameter, PF: standardized pectoral fin length, BD: standardized body diameter, (m: max, i: initial)

	GW	VTG	LW	DTW	Kn_m	ED_m	PF_m	BD_m	Kn_i	ED_i	PF_i	BD_i	Age
GW	1.00												
VTG	**0.58**	1.00											
LW	**0.65**	**0.43**	1.00										
DTW	**-0.60**	-0.34	-0.23	1.00									
Kn_m	**0.59**	**0.38**	**0.61**	-0.23	1.00								
ED_m	**0.66**	**0.50**	**0.46**	**-0.49**	**0.57**	1.00							
PF_m	0.10	0.11	0.03	0.02	0.28	0.23	1.00						
BD_m	**0.80**	**0.41**	**0.71**	**-0.41**	**0.87**	**0.63**	0.19	1.00					
Kn_i	**0.43**	**0.39**	**0.55**	-0.02	**0.81**	**0.45**	0.29	**0.58**	1.00				
ED_i	0.08	0.10	0.14	0.1	0.12	**0.46**	0.21	0.17	0.13	1.00			
PF_i	0.10	0.13	0.08	0.11	**0.35**	0.19	**0.77**	0.23	**0.40**	0.23	1.00		
BD_i	**0.43**	0.32	**0.50**	-0.11	**0.69**	**0.55**	0.05	**0.60**	**0.68**	0.14	0.18	1.00	
Age	0.36	**0.37**	**0.42**	0.09	**0.43**	0.24	-0.08	**0.43**	**0.39**	-0.03	0.11	0.30	1.00