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Ichthyological note**

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## Morphological traits allow distinguishing their hybrids from the Northern pike, *Esox lucius*, and the Aquitanian pike, *Esox aquitanicus* (Actinopterygii, Esociformes)

by

Clara JEANROY (1) & Gaël P.J. DENYS\* (1, 2)

**Résumé.** – Distinction morphologique préliminaire des hybrides de *Esox lucius* et *Esox aquitanicus* (Actinopterygii, Esociformes).

Le brochet aquitain *Esox aquitanicus* Denys, Dettai, Persat, Hauteceœur & Keith, 2014 est une espèce endémique du Sud-Ouest de la France. Il est menacé par l'hybridation avec le brochet commun *Esox lucius* Linnaeus, 1758. Jusqu'à présent, seul l'outil moléculaire permettait la détermination des hybrides. Le but de cette étude est de trouver des caractères morphologiques pour caractériser les hybrides des deux espèces parentales à partir des trois critères distinguant l'*E. aquitanicus* de l'*E. lucius* : le patron de coloration du corps, le nombre d'écaillles sur la ligne latérale et la longueur du museau. Nos résultats ont démontré que les spécimens hybrides pouvaient être discriminés d'*E. lucius* par un museau plus court et un plus faible nombre d'écaillles sur la ligne latérale, et d'*E. aquitanicus* seulement par un plus grand nombre d'écaillles sur la ligne latérale. Le patron de coloration du corps des deux espèces est rencontré chez les hybrides. Ainsi, le meilleur caractère pour identifier les hybrides est le nombre d'écaillles sur la ligne latérale ; il est intermédiaire comparé aux espèces parentales (114 à 119 vs. 104 à 107 pour *E. aquitanicus* et (115)120 à 136(146) pour *E. lucius*). Il sera utile pour les gestionnaires en conservation du brochet aquitain. Néanmoins, les résultats de cette étude nécessitent d'être confirmés avec un plus grand échantillonnage en aquaculture.

**Key words.** – *Esox aquitanicus* – *Esox lucius* – Hybrids – Morphology.

Esocids, belonging to the *Esox* genus (Actinopterygii, Esociformes), are a famous freshwater teleost fish group with a circumpolar distribution of its 7 species: *Esox lucius* Linnaeus, 1758, *E. americanus* Gmelin, 1789 including 2 subspecies *E. a. americanus* and *E. a. vermiculatus* Lesueur, 1846, *E. niger* Lesueur, 1818, *E. masquinongy* Mitchill, 1824, *E. reichertii* Dybowski, 1869, *E. cisalpinus* Bianco & Delmastro, 2011, and *E. aquitanicus* Denys, Dettai, Persat, Hauteceœur & Keith, 2014 (Froese and Pauly, 2018). Hybridizations between these species are known and well-studied for North-American and Asiatic species with morphological comparisons (Crossmann and Buss, 1965; Crossmann and Meade, 1977). In Europe two new species have been recently described, i.e. *E. cisalpinus* in Italy and *E. aquitanicus* in the South-West of France. So far, only molecular data proved their hybridization with the Northern pike *E. lucius* (Denys *et al.*, 2014, 2018; Gandolfi *et al.*, 2017) and these hybrids have not yet been morphologically studied.

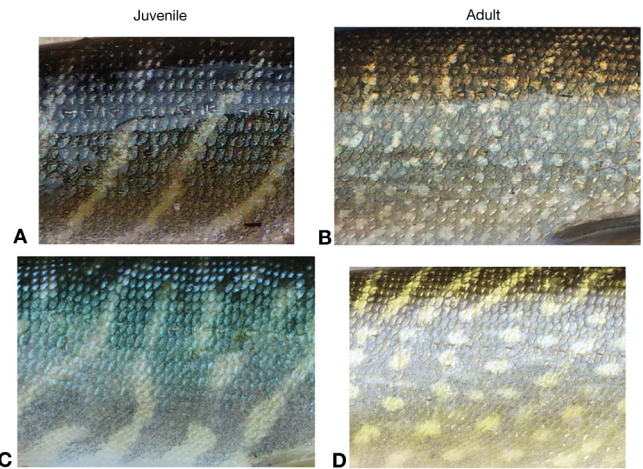


Figure 1. – Skin colour patterns characterizing *Esox aquitanicus* and *E. lucius* for juveniles and adult specimens; *E. aquitanicus*: marbled coat with 1-1.5 scale wide oblique vertical bands (A), little irregular white blotches (B); *E. lucius*: green coat with large yellowish oblique bands (C), regular ovoid dots (D).

The Aquitanian pike *E. aquitanicus* is endemic from the Charente to the Adour drainages (Denys *et al.*, 2014). However, the pike has a high socioeconomic interest for commercial and recreational fishing (Raat, 1988; Mann, 1996). Since the 1950s, with the development of its aquaculture for restocking, the Northern pike *E. lucius* has been introduced in this area, causing its hybridization with *E. aquitanicus* (Denys *et al.*, 2014, 2018). Hybridization and introgression constitute real threats for an endemic species (e.g., Gozlan *et al.*, 2010; Cucherousset and Olden, 2011).

According to Denys *et al.* (2014), *E. aquitanicus* differs from *E. lucius* by a shorter snout (39.2 to 42.3% of the head length HL, vs. 39.8 to 52.1% HL), a lower number of lateral scales LL (101 to 121, vs. 117 to 148) and its colour pattern [a marbled coat with 1-1.5 scale wide oblique vertical bands (Fig. 1A), tending to be discontinuous, becoming little irregular white blotches in larger specimens (Fig. 1B) vs. green coat with large yellowish oblique bands (Fig. 1C), splitting in regular ovoid dots for adults (Fig. 1D)]. So far, no morphological character has been investigated to discriminate the Aquitanian pike from the hybrids. Unfortunately, angling and riverine managers do not have the molecular tools for identification, and only morphological criteria are within their reach.

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Table I. – Identification of our sampling specimens according to three markers: one mitochondrial (COI) and two nuclear (Plag2 and S7) genes.

Drainage (Stream)	Town	ID	Identification COI (Denys et al., 2014)	Identification Plag2 (Denys et al., 2014)	Identification S7 (Denys et al., 2018)	Identification	Standard length (SL) in mm
Adour	Estirac	BRO462	<i>Esox lucius</i>	<i>Esox lucius</i> × <i>E. aquitanicus</i>	<i>Esox lucius</i>	Hybrid	247
Charente (Boutonne)	Saint-Séverin-sur-Boutonne	BRO545	<i>Esox aquitanicus</i>	<i>Esox aquitanicus</i> × <i>E. lucius</i>		Hybrid	416
Lien (Charente drainage)	Condac	BRO505	<i>Esox lucius</i>	<i>Esox lucius</i>	<i>Esox lucius</i> × <i>E. aquitanicus</i>	Hybrid	402
Charente (Lien)	Condac	BRO506	<i>Esox lucius</i>	<i>Esox lucius</i> × <i>E. aquitanicus</i>		Hybrid	282
Lien (Charente drainage)	Condac	BRO509	<i>Esox lucius</i>	<i>Esox aquitanicus</i>	<i>Esox lucius</i> × <i>E. aquitanicus</i>	Hybrid	145
Eyre	Bélin-Bélie	BRO445	<i>Esox aquitanicus</i>	<i>Esox aquitanicus</i>	<i>Esox lucius</i> × <i>E. aquitanicus</i>	Hybrid	121
Adour (Estampon)	Saint-Gor	MNHN 2013-1246 (Holotype) BRO531	<i>Esox aquitanicus</i>			<i>Esox aquitanicus</i>	372
Adour (Geloux)	Garein	BRO534	<i>Esox aquitanicus</i>	<i>Esox aquitanicus</i>		<i>Esox aquitanicus</i>	135
Eyre	Bélin-Bélie	BRO441	<i>Esox aquitanicus</i>		<i>Esox aquitanicus</i>	<i>Esox aquitanicus</i>	119
Eyre	Bélin-Bélie	BRO443	<i>Esox aquitanicus</i>	<i>Esox aquitanicus</i>		<i>Esox aquitanicus</i>	101
Eyre	Bélin-Bélie	MNHN 2013-1245 BRO536	<i>Esox aquitanicus</i>	<i>Esox aquitanicus</i>	<i>Esox aquitanicus</i>	<i>Esox aquitanicus</i>	396
Eyre (Grande Leyre)	Sabres	MNHN 2013-0838 (Paratype) BRO538	<i>Esox aquitanicus</i>	<i>Esox aquitanicus</i>		<i>Esox aquitanicus</i>	231
Eyre (Grande Leyre)	Sabres	BRO541	<i>Esox aquitanicus</i>	<i>Esox aquitanicus</i>		<i>Esox aquitanicus</i>	346
		BMNH- 1853.11.12.114 (possible syntype)				<i>Esox lucius</i>	165
Garonne	Béruges	MNHT_ZOO_2006_0_31_01 BRO502	<i>Esox lucius</i>	<i>Esox lucius</i>		<i>Esox lucius</i>	170
Loire (Boivre)	Massigneu-de-Rives	BRO529	<i>Esox lucius</i>	<i>Esox lucius</i>		<i>Esox lucius</i>	429
Rhône	La Loye	BRO427	<i>Esox lucius</i>	<i>Esox lucius</i>		<i>Esox lucius</i>	250
Rhône (Clauge)	La Loye	BRO428	<i>Esox lucius</i>	<i>Esox lucius</i>		<i>Esox lucius</i>	278
Rhône (Clauge)	La Loye	BRO429	<i>Esox lucius</i>	<i>Esox lucius</i>	<i>Esox lucius</i>	<i>Esox lucius</i>	300
Rhône (Clauge)	La Loye	BRO431	<i>Esox lucius</i>	<i>Esox lucius</i>	<i>Esox lucius</i>	<i>Esox lucius</i>	256
Seine (Blaise)	Saint-Ange-et-Torçay	BRO525	<i>Esox lucius</i>	<i>Esox lucius</i>	<i>Esox lucius</i>	<i>Esox lucius</i>	402
Seine (Serein)	Pontigny	BRO464	<i>Esox lucius</i>	<i>Esox lucius</i>	<i>Esox lucius</i>	<i>Esox lucius</i>	159
Seine (Serein)	Pontigny	BRO466	<i>Esox lucius</i>	<i>Esox lucius</i>	<i>Esox lucius</i>	<i>Esox lucius</i>	138
Seine (Serein)	Pontigny	BRO470	<i>Esox lucius</i>	<i>Esox lucius</i>	<i>Esox lucius</i>	<i>Esox lucius</i>	145



Using specimens genetically identified, this study aims to find some morphomeristic features allowing the identification of hybrids between *E. lucius* and *E. aquitanicus*.

**MATERIAL AND METHODS**

For this study, we compared the morphology of a total of 25 specimens (Tab. I): 6 hybrids (Fig. 2), 7 *E. aquitanicus* (Appendix 1) and 12 *E. lucius* (Appendix 2). Among them, 23 specimens, molecularly identified by Denys *et al.* (2014, 2018), were photographed in the field with a Nikon D 3000 with a 50 mm lens. The sampling was completed with two specimens of *E. lucius*: the possible syntype (BMNH 1853.11.12.114) and a specimen of the Museum of Natural History of Toulouse (MNHT\_ZOO\_2006\_0\_31\_01) whose diagnosis allows an accurate identification (ovoid white dots on the flanks, SnL = 43% HL and 146 lateral scales) (Appendix 2). Photographs of these specimens were provided by the collection managers of the two institutes.

We focused on three diagnostic characters given by Denys *et*

*al.* (2014): the skin colour pattern (Fig. 1), the number of lateral scales (LL) and the snout length (SnL).

Due to the face length deformation in the photograph of the specimen BRO529, only the lateral line scales and the skin colour were taken into account.

The measurements were made on photos using the ImageJ software. For the snout length, measurements were taken from the tip of the upper jaw to the border of the eye (the closest point from the tip of the jaw). Correspondingly, the head length measurements were taken from the tip of the upper jaw to the furthest point of the operculum.

All three characters were compared following a morphomeristic approach and the differences within meristic characters (LL and SnL) were statistically tested. The differences between lateral scales numbers were tested using a Kruskal-Wallis rank sum test (pair-wise) and a Dunn’s test (multiple comparisons; R-package “dunn.test”; Dinno, 2016). Regarding the quantitative data (SnL), after confirming the normality of the 3 samples with a Shapiro-Wilk test (*E. aquitanicus* (p = 0.9342), *E. lucius* (p = 0.8062), and hybrids (p = 0.6441),  $\alpha = 0.05$ ) and their homoscedasticity (p = 0.05518,  $\alpha = 0.01$ ), we were able to do an ANOVA and a *post hoc* Tukey’s test in order to compare the means of each group. All statistic tests were done with the R package (R Core Team, 2018).

**RESULTS**

Three morphological traits were used to compare 25 specimens.

The first character tested was the skin colour pattern. On 6 hybrids, 4 individuals had the characteristic marbled pattern of *Esox aquitanicus*, and 2 individuals had the spotted pattern of *Esox lucius* (Tab. II). However, hybrids may have the skin colour pattern of both species, which does not allow their distinction from *E. lucius* or *E. aquitanicus*.

The second character tested was the lateral scale number. Our results show differences between the three groups (Tab. III), which is corroborated by the Kruskal-Wallis rank sum test ( $\chi^2 = 19.8019$ ,  $df = 2$ ,  $p < 0.001$ ). The difference in lateral scale numbers between *E. aquitanicus* (104 to 107) and *E. lucius* ((115)120 to 136(146)) is significant (p < 0.001,  $\alpha = 0.025$ ). The hybrid specimens have an intermediate range with 114 to 119 lateral scales. This difference is significant with *E. lucius* (p = 0.0113,  $\alpha = 0.025$ ). However, despite the absence of overlapping between the lateral scale numbers between *E. aquitanicus* and the hybrid specimens (104 to 107 vs. 114 to 119), the difference is not statistically significant (p = 0.0452,  $\alpha = 0.025$ ), likely due to the low number of samples (respectively 6 and 7 specimens).

Finally the snout length (SnL) was tested. Our results also show a difference between the three groups (Tab. IV), significantly corroborated by the ANOVA (p < 0.001,  $\alpha = 0.05$ ). The difference among the snout lengths between *E. aquitanicus* (34 to 38% HL) and *E. lucius* (37 to 45% HL) is significant (p < 0.001,  $\alpha = 0.05$ ).

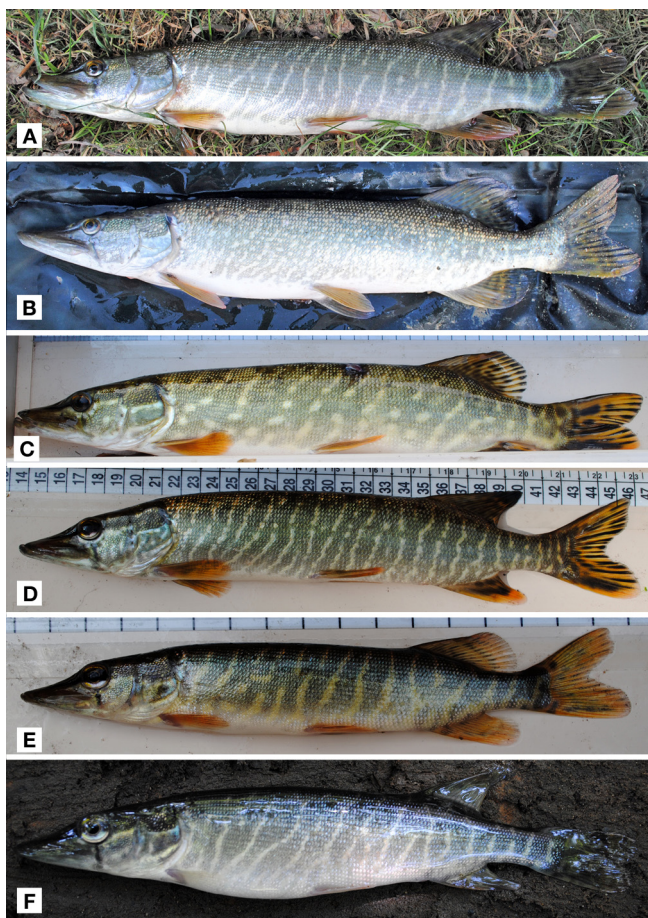


Figure 2. – Photos of pike specimens identified as hybrids of *Esox aquitanicus* and *E. lucius* according to Denys *et al.* (2014, 2018) (see Tab. I): Adour River at Estirac, 29 Aug. 2013, BRO462 247 mm SL (A); Boutonne stream (Charente drainage) at Saint-Séverin-sur-Boutonne, 10 Oct. 2013, BRO545 416 mm SL (B); Lien stream (Charente drainage) at Condac, 10 Sep. 2013, BRO505 402 mm SL (C), BRO506 282 mm SL (D) and BRO509 145 mm SL (E); Eyre River at Bélin-Béliez, 8 Jul. 2013, BRO445 121 mm SL (F). Credit photos: G. Denys / MNHN.

Table II. – Characterization of the coat coloration pattern of *Esox aquitanicus*, *E. lucius* and their hybrids according to four states: marbled coat with 1-1.5 scale wide oblique vertical bands (State a, Fig. 1A), little irregular white blotches (State b, Fig. 1B), green coat with large yellowish oblique bands (State c, Fig. 1C), regular ovoid dots (State d, Fig. 1D).

	N	State a	State b	State c	State d
Hybrids	6	3	1	1	1
<i>E. aquitanicus</i>	7	3	4		
<i>E. lucius</i>	12			10	2

Table III. – Lateral scale numbers of *Esox aquitanicus*, *E. lucius* and their hybrids.

LL	N	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124
Hybrids	6											3	1	1			1					
<i>E. aquitanicus</i>	7	1	2	2	2																	
<i>E. lucius</i>	12												1				1					

LL	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146
Hybrids																						
<i>E. aquitanicus</i>																						
<i>E. lucius</i>			1	1		1	1		1	2	1	1										1

Table IV. – Snout lengths of *E. aquitanicus*, *E. lucius* and their hybrids.

SnL (% HL)	N	34	35	36	37	38	39	40	41	42	43	44	45
Hybrids	6			1	1	4							
<i>E. aquitanicus</i>	7	1	1	2	2	1							
<i>E. lucius</i>	11				1	2		1	2	2	2		1

The hybrid specimens have shorter snouts than *E. lucius* (36 to 38% HL) ( $p = 0.0055$ ,  $\alpha = 0.05$ ); however, their snout length is not different from *E. aquitanicus* ( $p = 0.414$ ,  $\alpha = 0.05$ ).

## DISCUSSION

Our results suggest that hybrid specimens can be discriminated from *E. lucius* with the snout length and the lateral scale number and from *E. aquitanicus*, only with the lateral scale number; they need to be tested with a higher sampling.

The skin colour pattern was observed and our results showed that our hybrids may have the pattern of either *E. lucius* or *E. aquitanicus* (Tab. II), contrary to most of pike hybrids who have chimeric and diagnosable patterns (Crossmann and Buss, 1965; Crossmann and Meade, 1977). This might be explained by Fn+1 hybridizations between both species and introgression. Further studies in aquaculture hybridizing pureblood species and with more specimens are needed to characterize the skin colour pattern of the F1 hybrids. However, in fieldwork conditions, this character should not be used for hybrid identification.

Our hybrids have an intermediate range of lateral scale numbers between those of *E. aquitanicus* and *E. lucius* (114 to 119 vs. respectively 104 to 107 and (115)120 to 136(146); Tab. III). This characteristic is already known in many pikes hybrids (*E. reichertii* × *E. lucius*, *E. reichertii* × *E. a. americanus*, *E. masquinongy* × *E. lucius*, *E. lucius* × *E. niger* and *E. a. americanus* × *E. a. vermiculatus*). However, this character cannot be used as diagnostic for all other hybrids except for *E. reichertii* × *E. masquinongy* and *E. a. vermiculatus* × *E. a. americanus* who have, respectively, higher and lower lateral scale numbers than parental species (Crossmann and Buss, 1965; Crossmann and Meade, 1977). According to Denys *et al.* (2014), *E. aquitanicus* is characterized by 101 to 121 lateral scales. Our results demonstrated that this range included hybrid specimens, and non-hybrids seem actually to have less than 110 lateral scales (vs. about 111 to 121 for hybrids). The addition of more specimens should allow the clarification of the ranges. There is also a small overlap of lateral scale ranges between hybrids and *E. lucius*. However, this concerns only one specimen from the Seine catchment (BRO464). Therefore, the number of lateral scales number can be considered more as a clue than a diagnostic criterion.

Our results also showed that our hybrids have short snout lengths (36 to 38% HL; Tab. IV) in comparison to *E. lucius* (37

to 45% HL), but in the same range as *E. aquitanicus* (34 to 38% HL). Similar observations of hybrids having the same snout length as one of the parental species were already made for *E. reichertii* × *E. masquinongy*, *E. reichertii* × *E. lucius* and *E. niger* × *E. a. americanus* (Crossmann and Buss, 1965; Crossmann and Meade, 1977). Among other *Esox* hybrids, the snout lengths could be intermediate (*E. niger* × *E. a. vermiculatus*, *E. reichertii* × *E. niger*) but not significantly for *E. lucius* × *E. niger* and *E. reichertii* × *E. a. vermiculatus*) or higher than parental species (*E. masquinongy* × *E. lucius*, *E. lucius* × *E. niger* and *E. lucius* × *E. a. vermiculatus*) (Crossmann and Buss, 1965; Crossmann and Meade, 1977). However, this results need to be confirmed with a higher sampling. Our results also showed another range than Denys *et al.* (2014) (39.2 to 42.3% HL). This might be explained by many factors. With specimens measuring from 101 to 416 mm SL, allometric growth might have an impact on the results. However, Shamardina (1957) as well as Franklin and Smith (1960) revealed the proportions of the head and the jaw comparing to the body size are relatively constant for pikes bigger than 65 mm TL. The measurements protocol in this study (on photos) is different from the study of Denys *et al.* (2014) who used a caliper directly on collection specimens (with a 3D bias). Finally, a distortion of the picture due to the camera lens could also affect the measurements and the ratios. Thus, this character cannot be applied for hybrid identification on pictures obtained by managers or participative sciences because of the difficulty to standardize photo captions in the field in order to reduce picture distortion.

Finally, of the three characters tested in our study, only one allows us to identify the hybrids of *E. aquitanicus* and *E. lucius*: the lateral scale number. This meristic character is easy to observe and, contrary to morphometric ones (e.g., snout length), has the advantage of (1) never be affected by picture distortion due to the camera lens and (2) has a low operator bias. There is still a small overlap between hybrids and *E. lucius*, but using the combination of this character, the pattern coloration and the snout length, the risk of misidentification is reduced. Then, without any access to molecular data, the lateral scale number should be a good criterion for managers to distinguish hybrids and consequently better manage the distribution and the conservation of the threatened Aquitanian pike.

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**Appendix 1.** – Photos of pike specimens identified as *Esox aquitanicus* according to Denys *et al.* (2014, 2018) (see Tab. I): Estampon stream (Adour drainage) at Saint-Gor, 8 Oct. 2013, MNHN-2013-1246 BRO531 372 mm SL (Holotype (A)); Eyre River at Bélin-Béliez, 9 Oct. 2013, MNHN 2013-1245, BRO536 396 mm SL (Paratype (B)); Grande Leyre stream (Eyre drainage) at Sabre, 10 Oct. 2013, MNHN 2013-0838 BRO538 231 mm SL (C), BRO541 346 mm SL (D); Eyre River at Bélin-Béliez, 8 Jul. 2013, BRO441 119 mm SL (E), BRO443 101 mm SL (F); Geloux stream (Adour drainage) at Garein, 8 Oct. 2013, BRO534 135 mm SL (G). Credit photos: G. Denys / MNHN.





**Appendix 2.** – Photos of pike specimens identified as *Esox lucius* according to Denys *et al.* (2014, 2018) (see Tab. I): Possible syntype, BMNH-1853.11.12.114 165 mm SL (A), Credit photo: H. Taylor / NHM; Garonne Drainage, MNHT\_ZOO\_2006\_0\_31\_01 (B), Credit photo: MNHT; Boivre stream (Loire drainage) at Béruges, 6 Sep. 2013, BRO502 170 mm SL (C); Rhône River at Massigneu de Rives, 1 Oct. 2013, BRO529 429 mm SL (D). Clauge stream (Rhône drainage) at La Loye, 1 Jul. 2013, BRO427 250 mm SL (E), BRO428 278 mm SL (F), BRO429 300 mm SL (G), BRO431 256 mm SL (H); Blaise stream (Seine drainage) at Saint-Ange-et-Torçay, 24 Sep. 2013, BRO525 402 mm SL (I); Serein stream (Seine drainage) at Pontigny, 21 Aug. 2013, BRO464 159 mm SL (J), BRO466 138 mm SL (K), BRO470 145 mm SL (L). Credit photos: G. Denys / MNHN.

