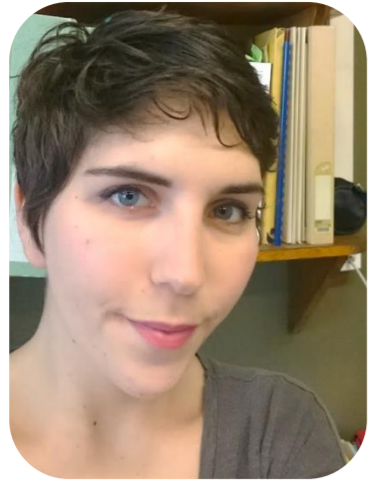


Testosterone enhances attractive sexual signals but no immunosuppression



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Background: Sexual selection

Female mate choice → Male sexual signals = **honest** and **reliable** signals of male quality [1]

The **immunocompetence Handicap Hypothesis (ICHH)** [2] could explain the signal honesty

↓

Testosterone

-

Immunity

+

Sexual signal expression

The ICHH has never been tested in a **multimodal communication system**.

Aim: Testing the ICHH in *Hyla arborea*



Testosterone?

Acoustic signals (vocalize performance)

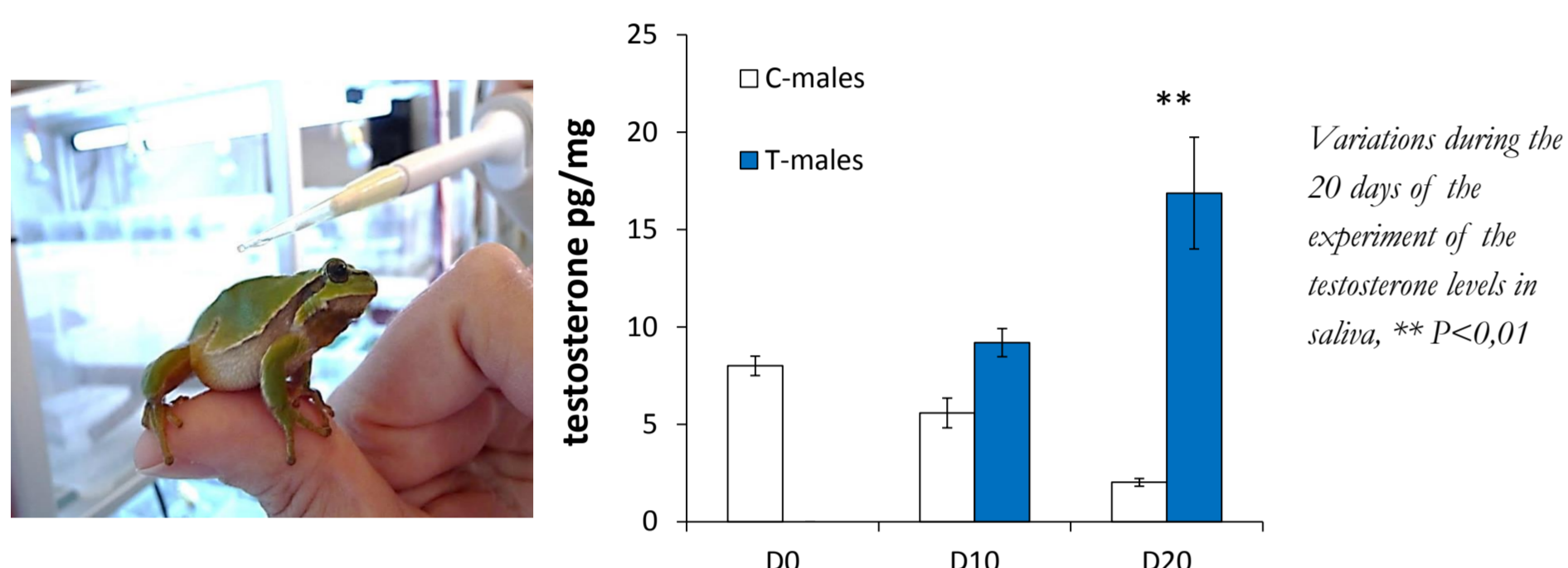
Visual signals (Vocal sac coloration)

Immune response capacity

- 1/ are acoustic signals testosterone-dependant?
- 2/ are visual signals testosterone-dependant?
- 3/ is testosterone immunosuppressive?

Material & methods:

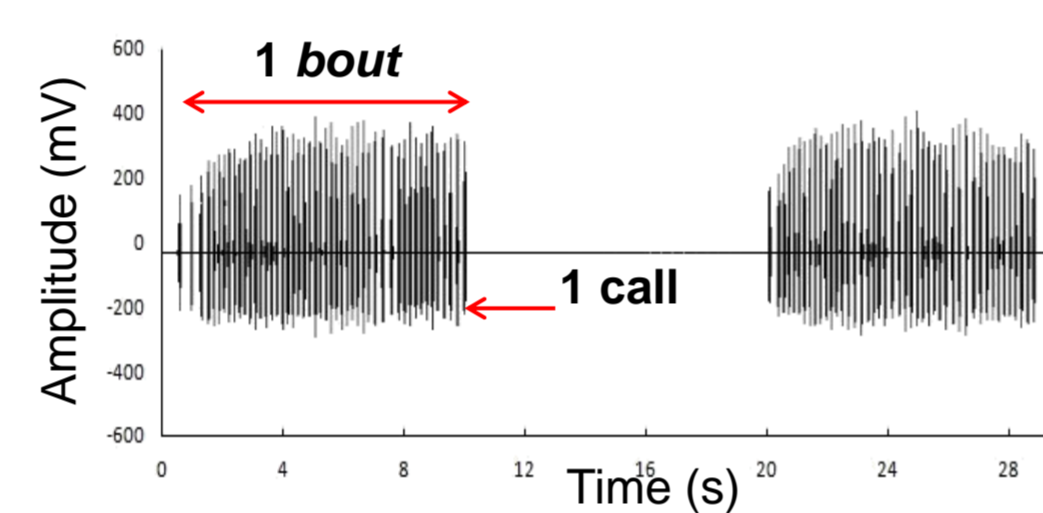
Testosterone supplementation: daily topical application (20 days). Measures of testosterone levels in saliva. N=48 T-males Vs N=48 C-males



4h call recording per tree frogs:

Acoustic parameters:

- within-bout call rate (in call/s)
- Bout duration (in s)
- Dominant frequency (in Hz)



Coloration measurements:

Vocal sac colour parameters:

- Chroma (more or less saturation)
- Brightness (more or less darker)

Using a spectrophotometer with a optic fibre light (SpectraSuite®). Analyses with AVICOL®

Frog manipulation during the colour measure of the vocal sac with the spectrophotometer.



Immunity test:

Injection of phytohaemagglutinin (PHA) in the leg. [3]

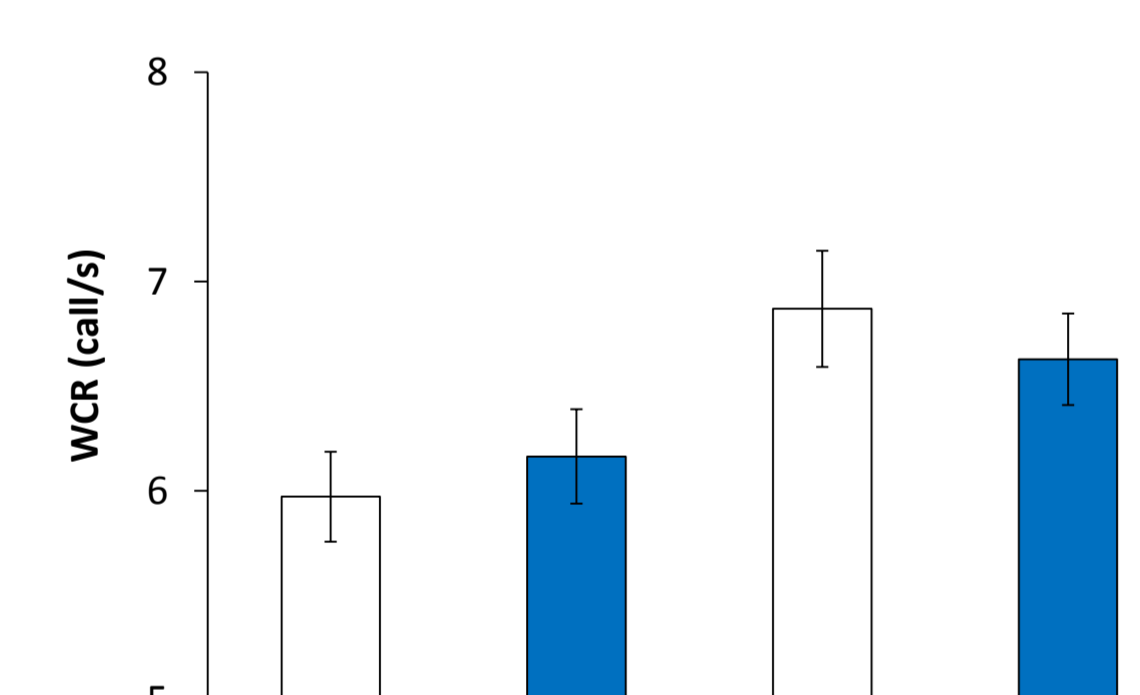


Measure of the leg thickness before and 18h after injection. Analyse of the leg swelling (mm)

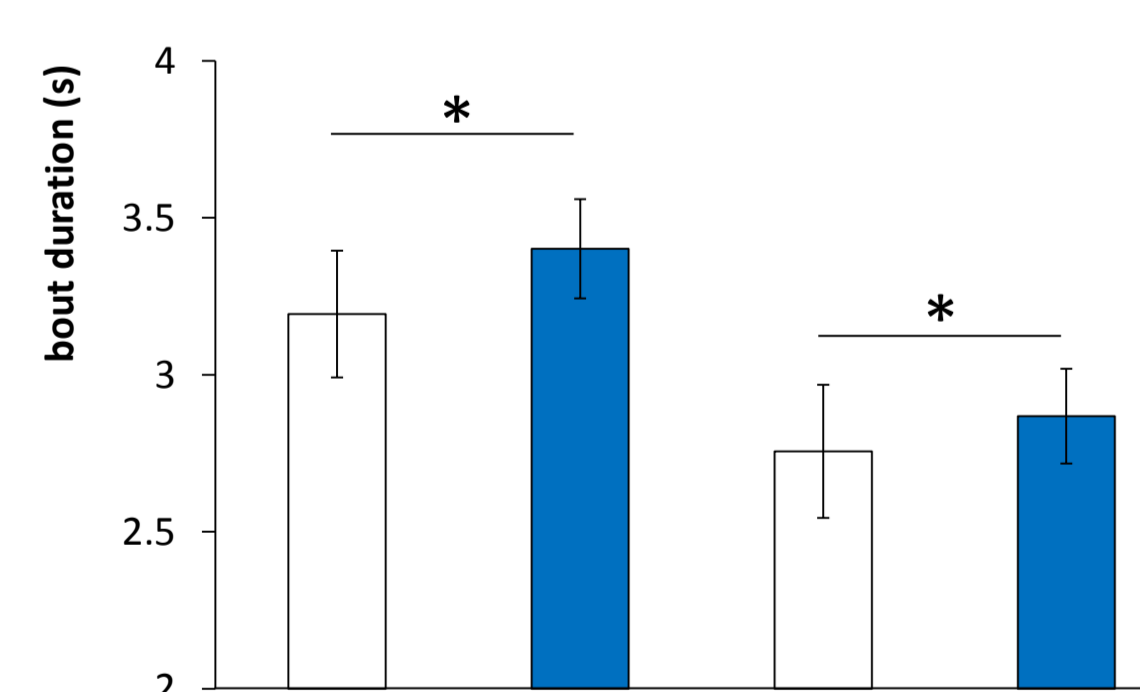
Results:

1/ Testosterone effects on acoustic signals

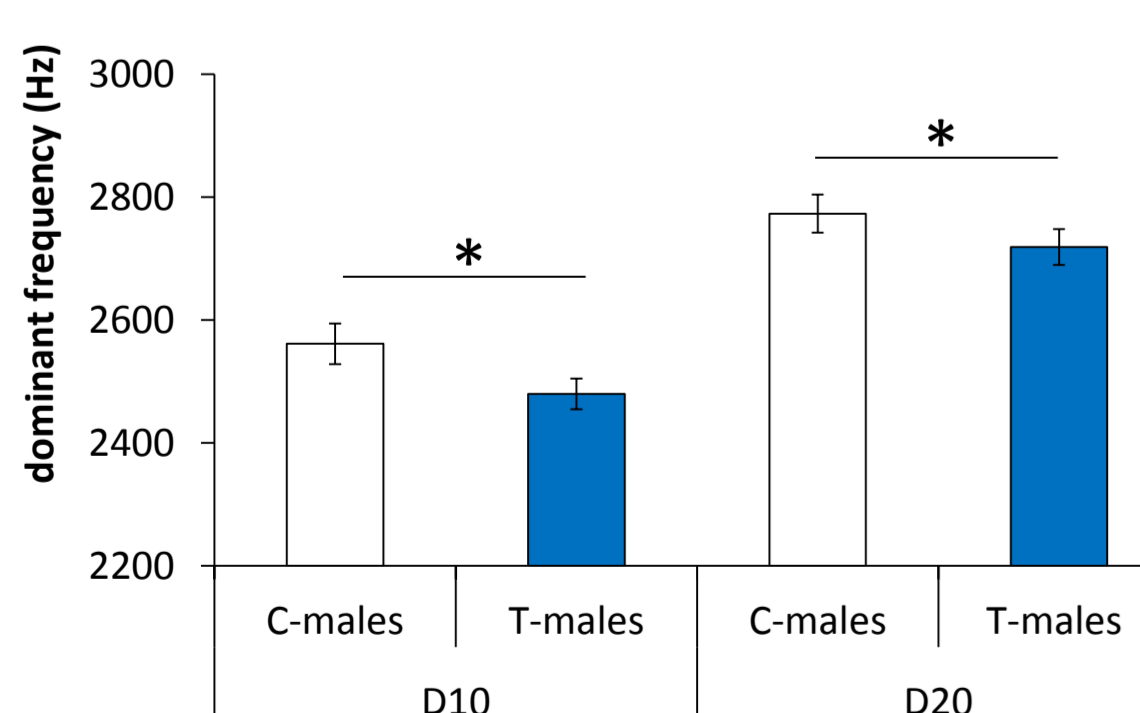
GEE analysis: $Call\ parameter \sim body\ mass + day + treatment + body\ mass \times treatment + day \times treatment$



- **Testosterone supplementation increases the bout duration** ($p < 0,05$) and **decrease the dominant frequency** ($p < 0,05$)



- More the male is bigger more its **dominant frequency is lower** ($p < 0,001$) and its **bout duration is longer** ($p < 0,001$)

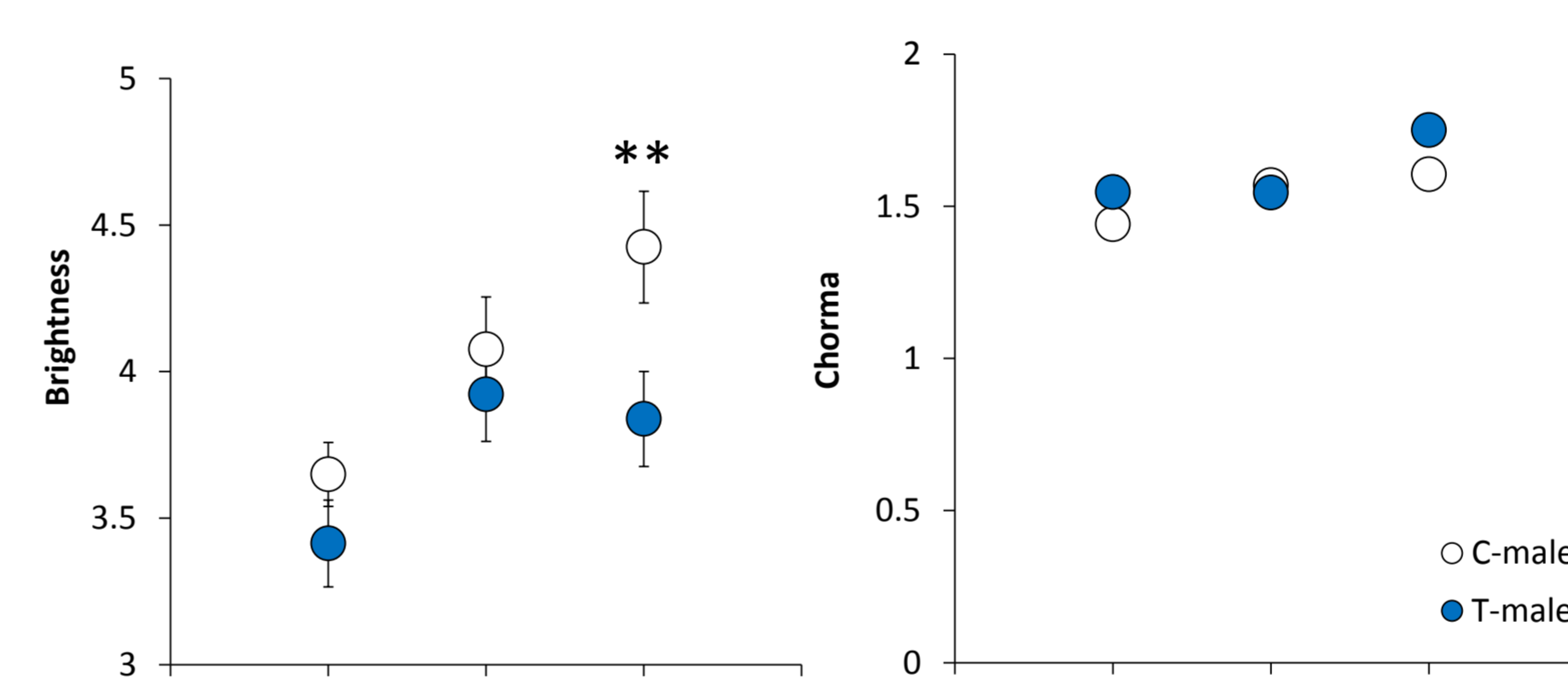


- There is a significant "Day effect" for all acoustic parameters ($p < 0,001$)

Variations during the 20 days of the experiment of the call parameters: WCR (within bout call rate) bout duration and dominant frequency, means +/- SEM, * P<0,05

2/ Testosterone effects on visual signals

GEE analysis: $colour\ parameter \sim body\ mass + day + treatment + body\ mass \times treatment + day \times treatment$

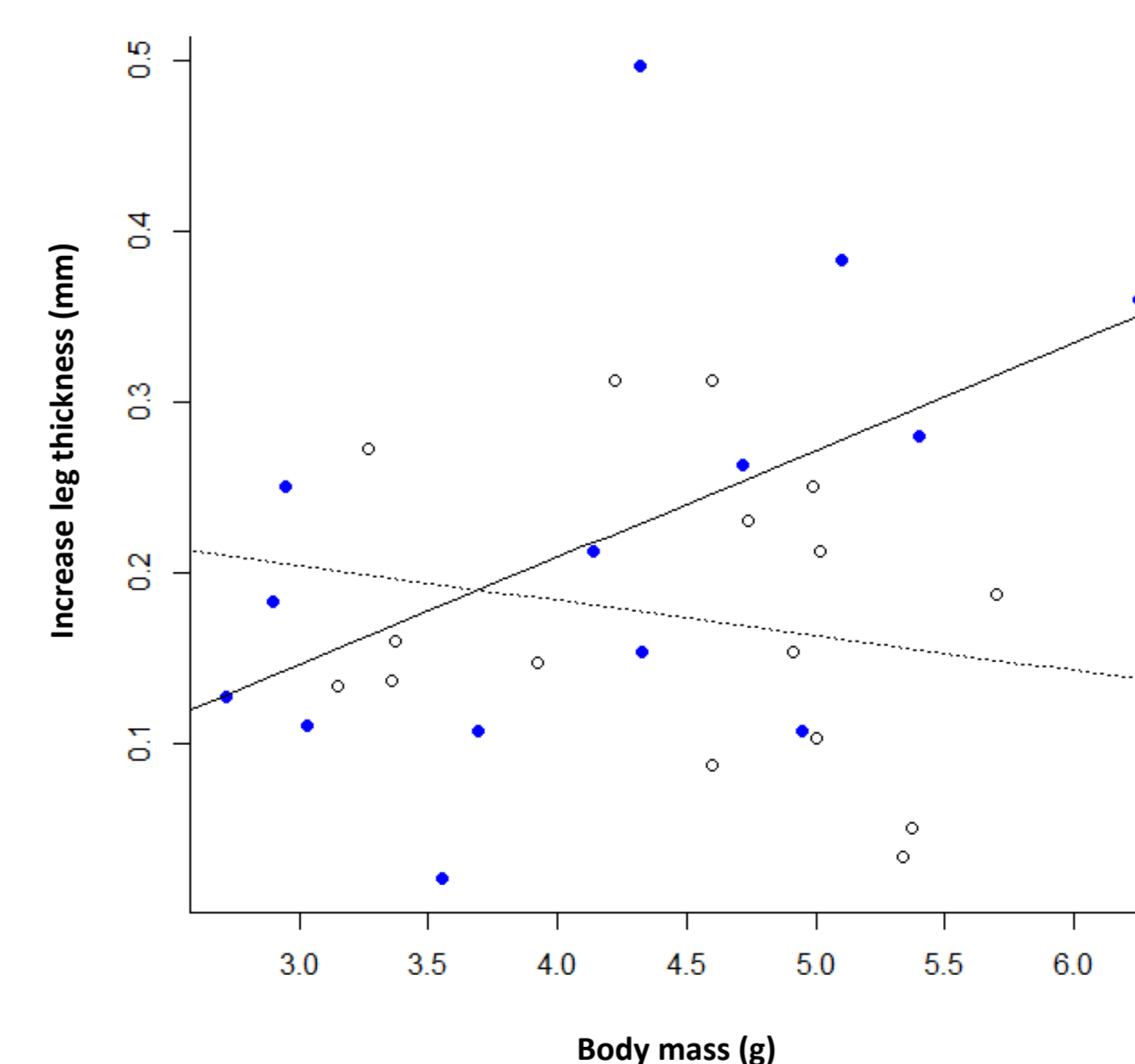


- **Testosterone supplementation** has no effect on chroma parameter but limits the leak of colour (brightening) in time. **After 20 days, T-males are darker than C-males** ($p < 0,01$).
- More the male is bigger more its vocal sac is **darker** ($p < 0,01$)
- There is a significant "Day effect" for all colour parameters ($p < 0,05$)

Variations during the 20 days of the experiment of the colour parameters: the brightness and the chroma, means +/- SEM, * P<0,01

3/ Testosterone effects on immune response

Linear mixed model: $immune\ response \sim body\ mass + treatment + body\ mass \times treatment$



- More the leg swelling is important more the immune response is important. **The immune response depends on the body mass of the male.**
- T-males with less body mass and C-males react in a similar manner.
- **Testosterone supplementation:** the strength of the swelling response **increased significantly for those T-males with a high body mass.** ($body\ mass \times treatment, p = 0,05$)

Effect of the interaction between treatment and body mass on leg thickness (in mm) after PHA injection (N=30). Prediction of the linear model for the significant interaction between the two explanatory variables are represented (full and dotted lines for T-males (blue circles) and C-males (white circle), respectively)

Conclusions:

The originality of this study relies on the **measurements of multimodal signals** in the context of ICHH.

As predicted by the ICHH, our study reveals that several components of **acoustic signal and visual signal are testosterone-dependant**. Nevertheless, our results did **not reveal an immunosuppression effect of the testosterone** and thus, we did **not validate the ICHH** as a proximate mechanism explaining signal honesty in *Hyla arborea*.

Surprisingly, the interaction between treatment and body mass on the immune response suggests an **immunostimulation in T-males greater than 4,5g**. The interaction of testosterone treatment and body mass effects **could be an indirect consequence of energy reallocation** from the immune system to other metabolic demands [4]

An integrative approach is now necessary to improve our understanding of the link between immunocompetence and sexual selection.

References:

- [1] Zahavi A, 1977. Cost of Honesty - (Further Remarks on Handicap Principle). J Theor Biol 67:603-605.
- [2] Folstad I, Karter AJ, 1992. Parasites, Bright Males, and the Immunocompetence Handicap. Am Nat 139:603-622.
- [3] Josserand R, Brepson L, Desprat JL, Lengagne T, Mondy N. 2015. A phytohaemagglutinin challenge test to assess immunocompetence of European tree frog *Hyla arborea*. *Amphibia-Reptilia*, DOI: 10.1163/15685381-00002983
- [4] Alonso-Alvarez C, Bertrand S, Sorci G, 2007. Energetic reserves, leptin and testosterone: a refinement of the immunocompetence handicap hypothesis. Biol Lett 3:271-274.
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