

Aula P12 Prática Coração II

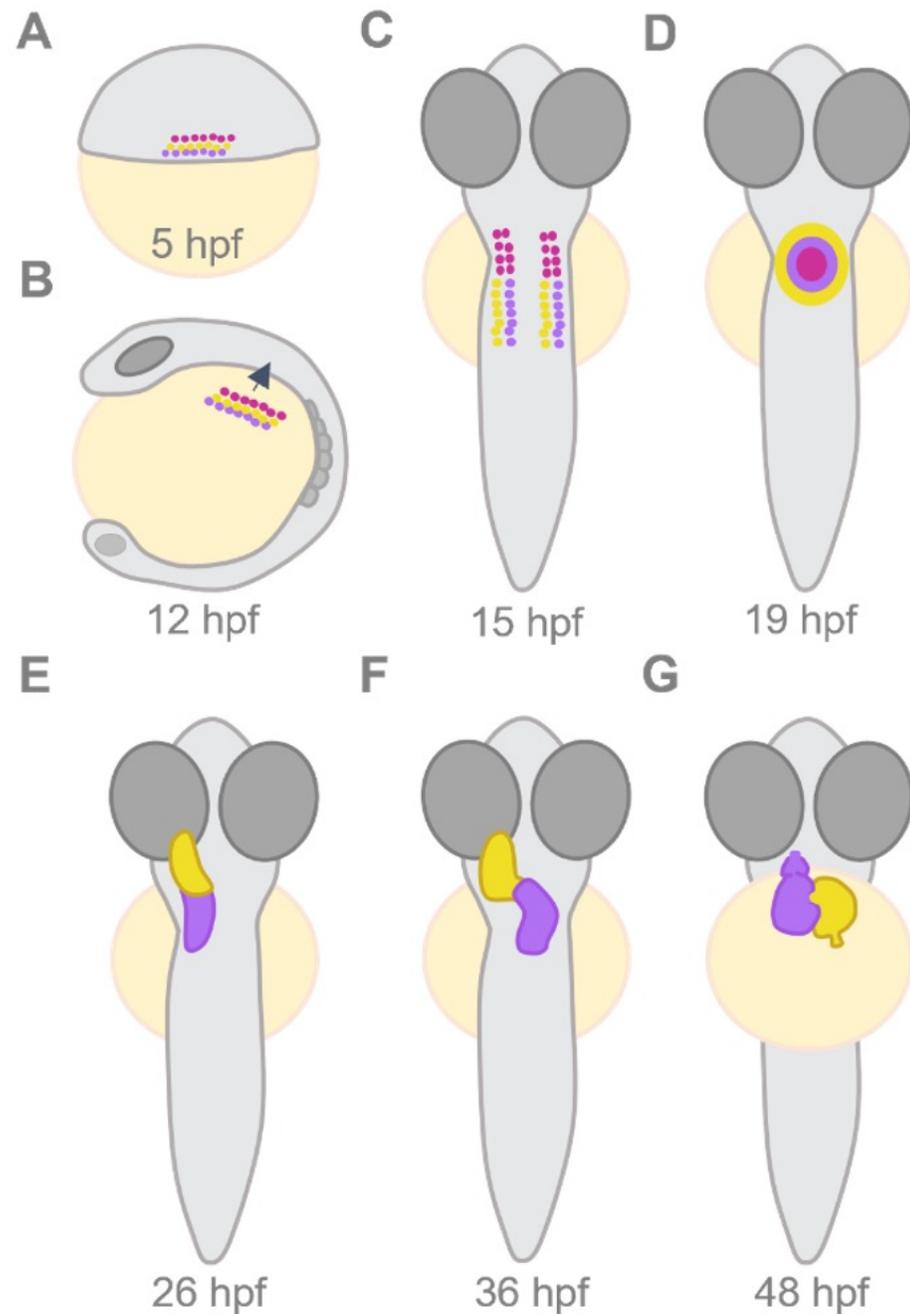


Figure 1.5 – Zebrafish heart morphogenesis. (A) Cardiac progenitor cells onset in the blastula lateral marginal zone at 5 hpf; purple, yellow and magenta represent the ventricular myocardial, atrial myocardial and endocardial progenitor cells. (B) At 12 hpf, myocardial and endocardial progenitors migrate to the anterior LPM, and (C) organize into bilateral symmetric cell populations at 15 hpf. (D) By 19 hpf, endocardial and myocardial precursors merge forming a shallow cone structure with the myocardial cells encapsulating the endocardial cells. (E,F) Cardiac jogging and looping occurs from 20 to 26 hpf, and from 30 hpf, respectively (dorsal view). (G) At 48 hpf, the zebrafish heart is a dextral S-shaped two-chambered structure with an anterior right-sided ventricle (purple-depicted), and a posterior left-sided atrium (yellow-depicted) (ventral view).

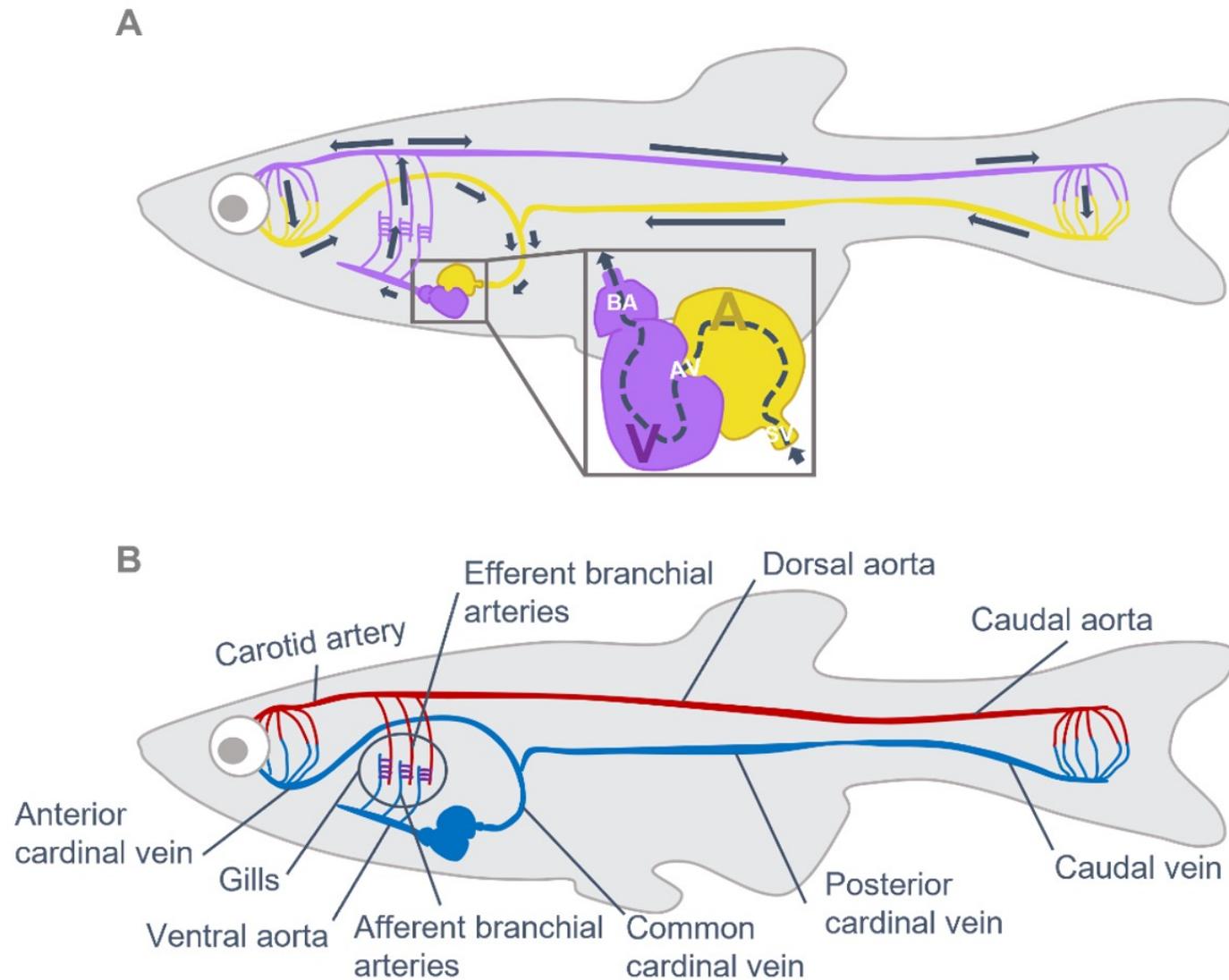
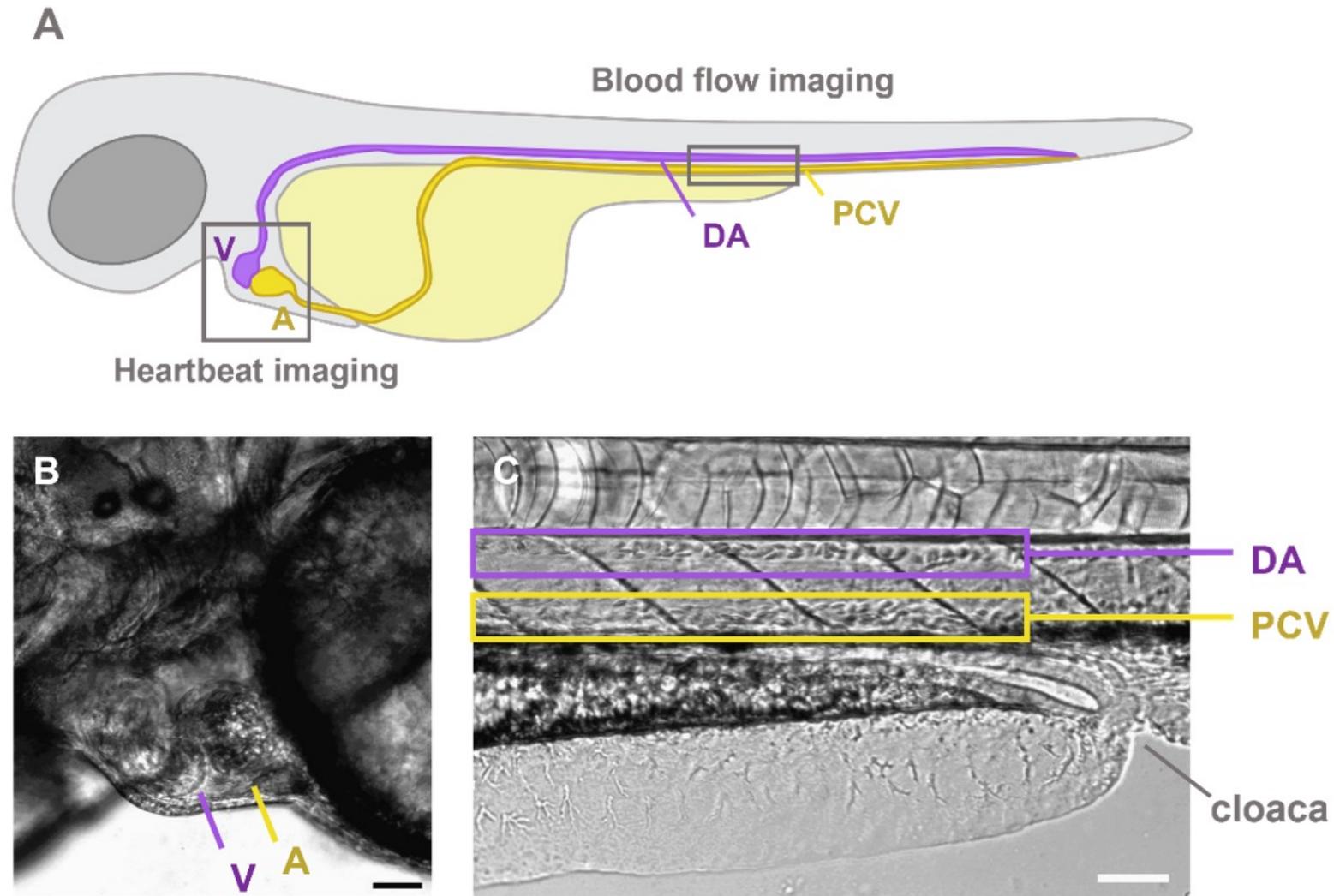
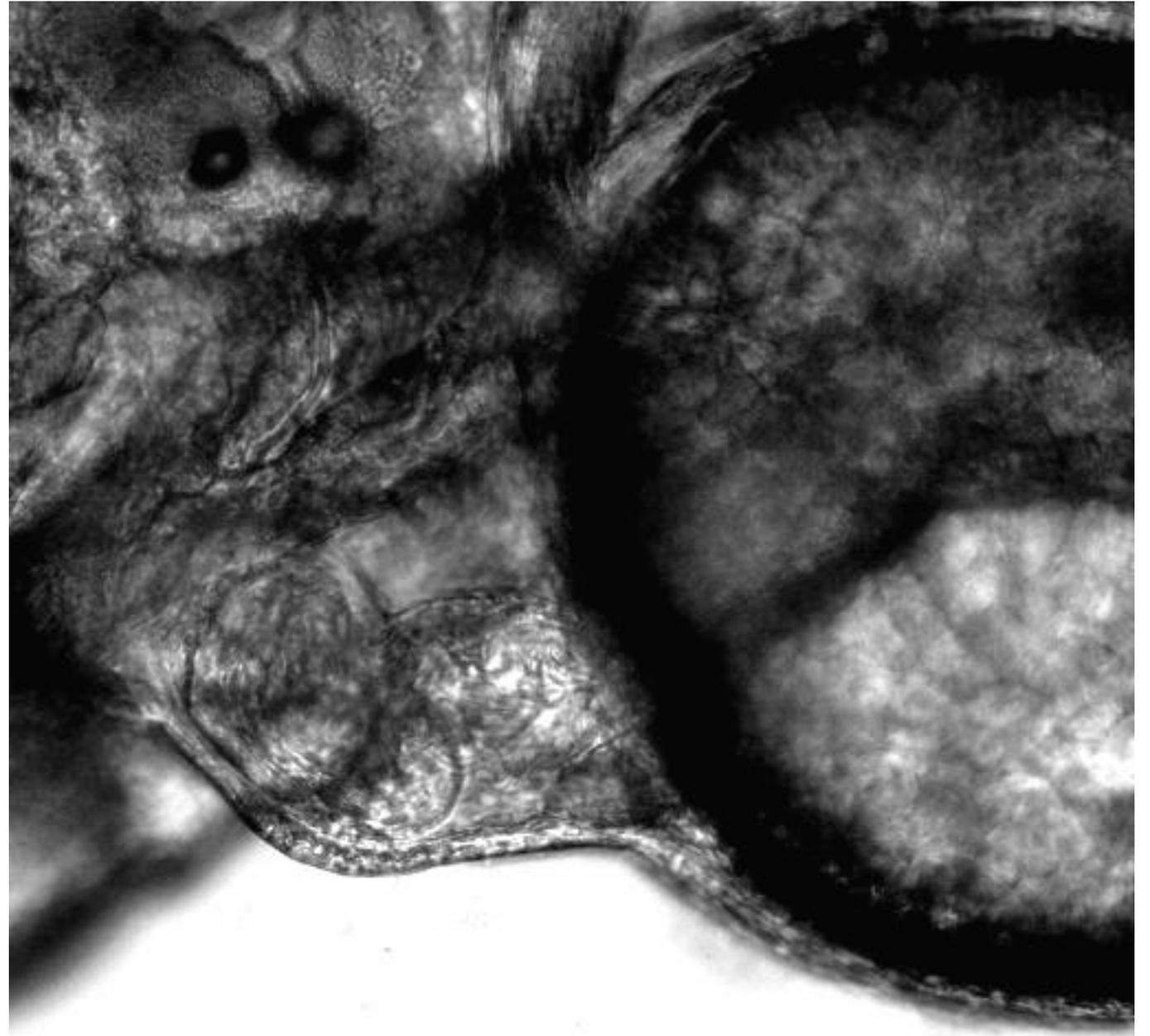


Figure 1.6 – Zebrafish cardiovascular system. Simplified schematic representation of the zebrafish closed circulatory system, where: **(A)** Blood flow direction is indicated by arrows; purple represents the ventricle and outflow pathway, and yellow represents the atrium and inflow pathway. SV= sinus venosus, A= atrium, AV= atrioventricular canal, V= ventricle, BA= Bulbus arteriosus. **(B)** Blood oxygenation is indicated by colors; red represents oxygen-rich/arterial blood and blue represents oxygen-poor/venous blood.

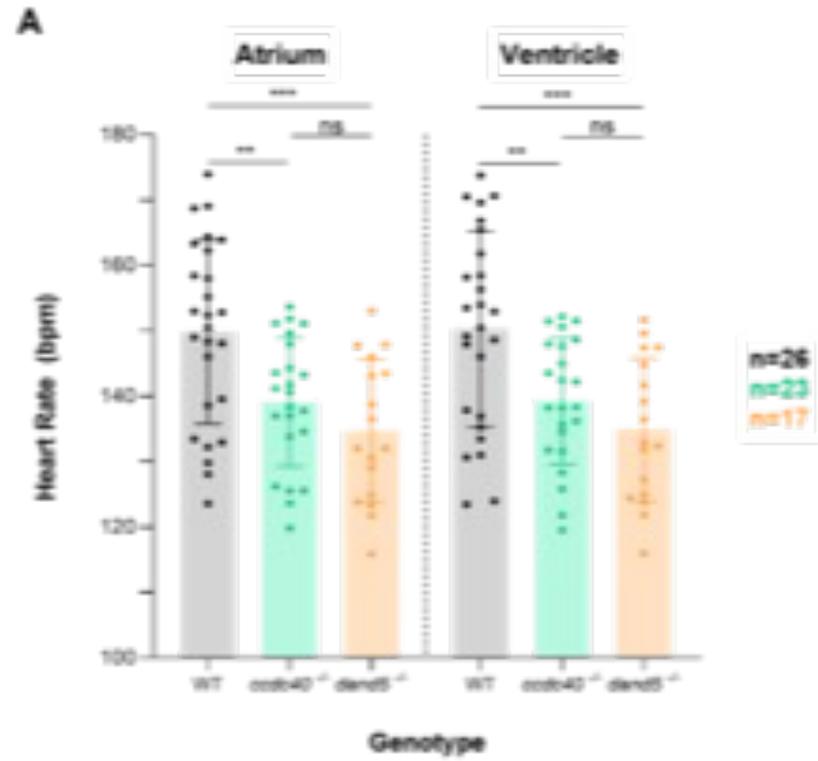


Zebrafish larvae at 3 dpf

Zebrafish larvae at 3 dpf

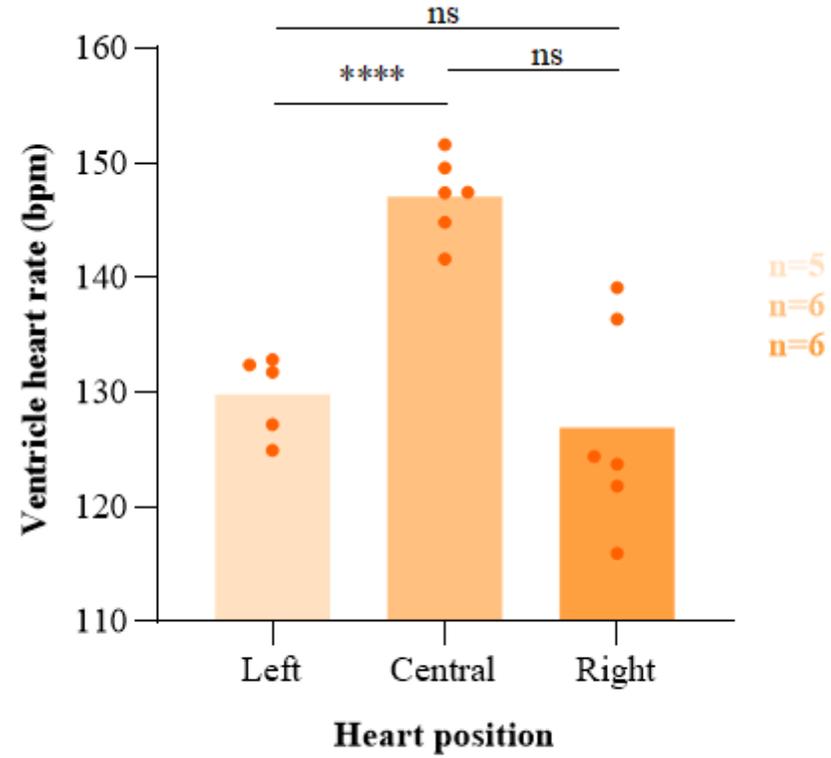
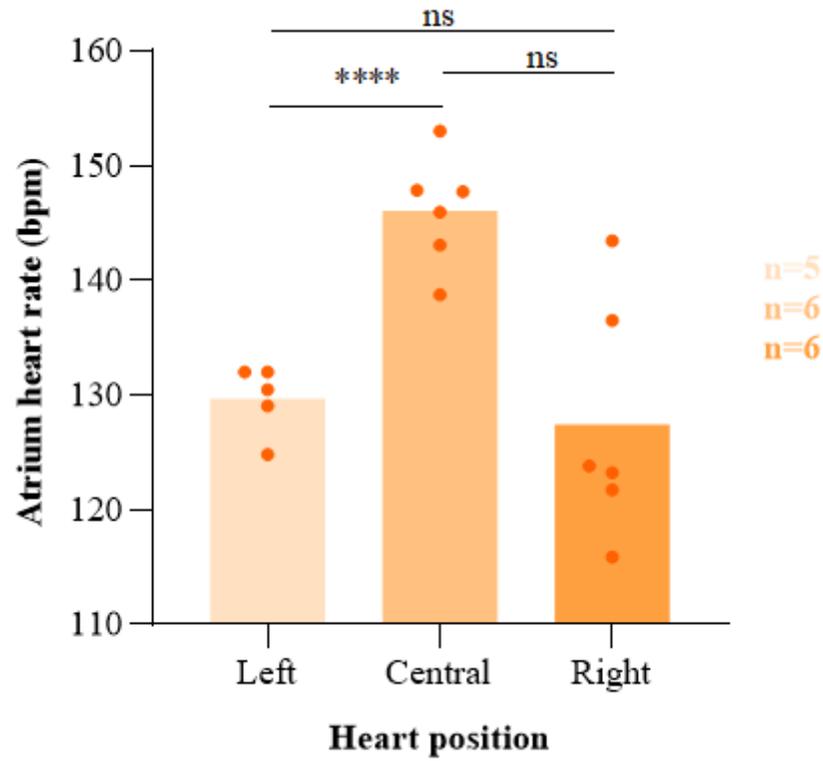


Zebrafish larvae heart rate at 3 dpf



Dand5mut

Zebrafish larvae heart rate at 3 dpf



Aula P12 – dias 24 e 29 de maio: coração II

Comparação da função cardíaca entre larvas selvagens e larvas com corações com a lateralidade alterada – mutantes *dand5* homozigotas.

Procedimento:



1. Vão filmar corações centralizados de mutantes para o gene *dand5* e comparar com larvas selvagens. As larvas têm 48 hpf e já se vêem bem os corações a bater.
 2. Vão fazer filmes de 15 segundos e gravar em formato mp4 no mesmo folder.
 3. Instalar FIJI (Windows).
 4. Criar uma pasta "FIJI" na raiz do sistema operativo, ou seja C:\FIJI\
 5. Fazer download do Fiji em <https://fiji.sc/>
 6. Abrir o fiji-win64.zip e arrastar a pasta "Fiji.app" para dentro da pasta criada, ou seja, C:\FIJI\Fiji.app\
 7. Correr o executável "ImageJ-win64.exe", e aceitar o pedido para fazer updates
 8. No menu do FIJI clicar em HELP e UPDATE... Clicar em "Manage Update Sites" colocar um "check" em "FFMPEG", Apply and Close, Apply Changes, fechar o programa e voltar a correr o executável.
- NOTA: NÃO MAXIMIZAR A JANELA DO FIJI. Corre tudo em janelas flutuantes.
9. Arrastar o ficheiro "bpm_from_kymograph_ver2.ijm" para dentro da janela principal do Fiji, clicar "Run"
 10. Seguir as instruções da macro ANTES de clicar OK.
 11. Fazer o mesmo para todos os filmes 10 WT e 10 *dand5* mutants para a macro calcular os BPM (batimentos por minuto).
 12. Fazer a média dos BPM para cada condição.
 13. Há diferenças?