Unveiling the underlying pathways of vitamin K requirements for fish gametogenesis.

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Description of the work carried out during the STSM

Sequencing of small RNAs from blood plasma will give insights on the mechanisms by which vitamin K (VK) might promote fish reproduction, particularly in males. Since Senegalese sole F1 broodstock could not be sacrified to collect main tissues of the reproductive axis, an alternative approach has been applied. Small RNAs from blood plasma samples isolated from Senegalese sole males (6 fed control diet and 6 fed a diet suplemented with 1250 mg Kg^{-1} of phyloquinone (VK_1)) were exttracted and purified. Additionally, to know how VK determine gamete quality, small RNAs from zebrafish embryos under or not an induced VK deficiency (by warfarin exposure) during embryonic development were also, since no spawns were obtained from Senegalese sole.

During this short mission at University of Nordland (Bodo, Norway) I learnt how to quantify small RNAs, how to prepare their libraries and to perform their sequencing on a MiSeq desktop next-generation sequencer. While in sole plasma samples only microRNA fractions (around 29 nt length; 156 nt length including adaptors and indexes) were found; in zebrafish embryo samples of micro- and piwi-RNAs (around 64 nt length; 191 nt length including adaptors and indexes) were identified (Fig. 1). A cluster of 6 samples was loaded in each flow cell (2 for sole and 2 for zebrafish samples) on MiSeq.

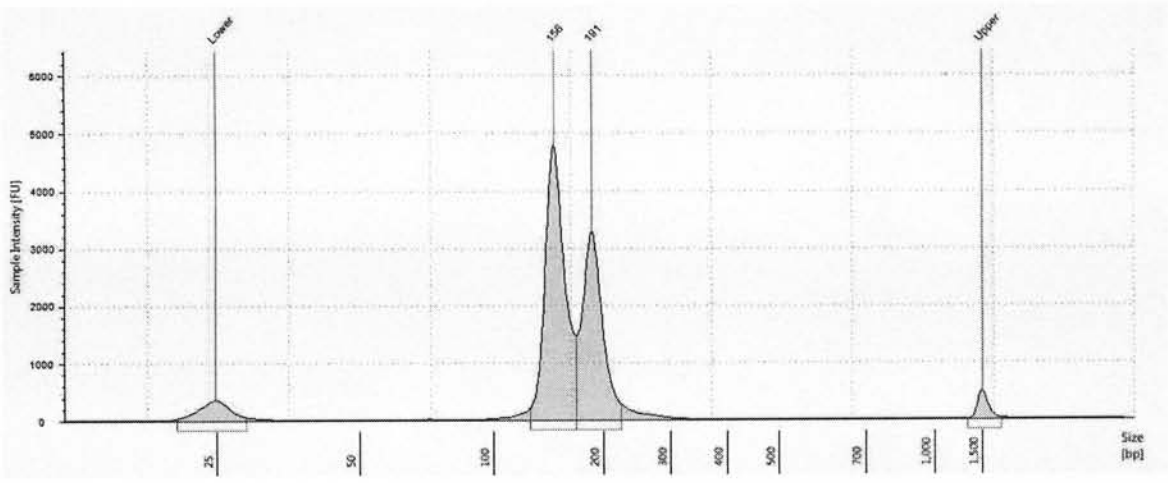

Figure 1. An example of small RNA fractions identified in a 2200 TapeStation. *Lower*, peak corresponding to smallest DNA ladder band; *156*, peak corresponding to microRNAs; *191*, peak corresponding to piwiRNAs; *Upper*, peak corresponding to biggest DNA ladder band.

31.6 and 29.1 million reads (75.34 and 74.49 % of good quality, respectively) from zebrafish samples, and 24.6 and 26.3 million reads (86.3 and 88.3 % of good quality, respectively) from sole samples were obtained.

Contact with researchers at host institution, namely those located at the Marine Station of Bukta (Fig. 2), has been most important to increase the researcher's network and the collaboration between host and beneficiary institutions.

Read mapping, annotation and differential gene expression is on-going at the beneficiary's institution in collaboration with the University of Nordland. The novel results anticipated will provide basic knowledge on how VK affects fish gametogenesis and early development (Working Group 3 of Aquagamete COST action).

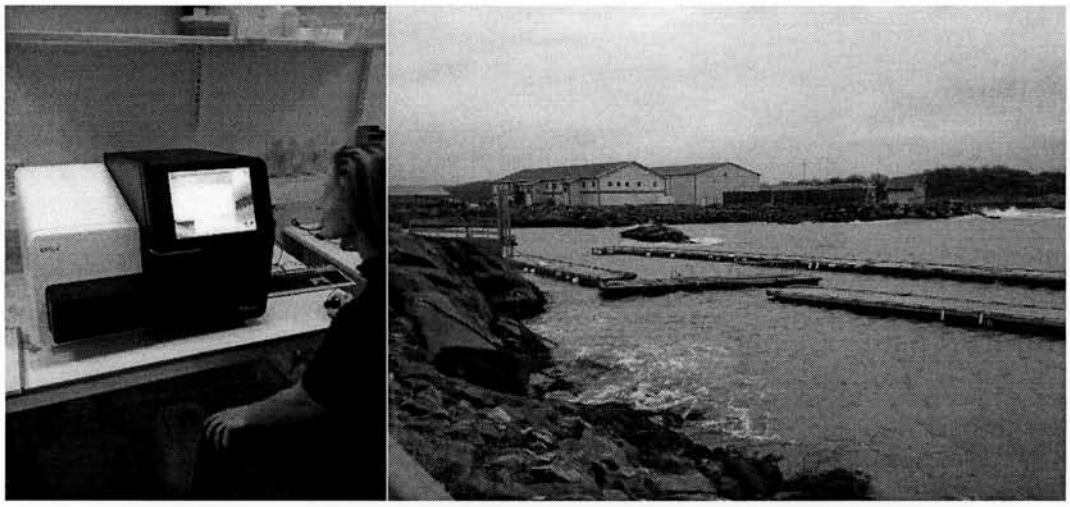

Figure 2. MiSeq desktop sequencer at the host institution and the technician in charge (Martina Kopp) on the left image and Marine Station of Bukta on the right image.

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