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# Evaluating Imaging Technologies for Non-invasive Sex Determination in Atlantic Cod

Final report



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## Report

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## **Preface**

In Atlantic cod farming, the ability to determine sex at early and intermediate growth stages is important for selective breeding, production planning, and broodstock management. However, existing methods such as dissection and ultrasound are either destructive or difficult to apply accurately to juvenile and pre-mature fish, which limits their use in commercial settings.

Developments in imaging technologies offer potential alternatives for sex determination. Hyperspectral imaging provides access to detailed spectral characteristics of biological tissues that are not accessible through conventional inspection, while enabling high-throughput assessment. Motivated by this potential, the assignment from FHF was to evaluate whether hyperspectral imaging, using the Maritech Eye system, could support sex determination in Atlantic cod under industrially relevant conditions.

This project provides an exploration and assessment of hyperspectral imaging and other imaging-based approaches, including fluorescence hyperspectral imaging and conventional RGB images, with the aim of identifying opportunities and current limitations to support future innovation in cod aquaculture.

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# 1 Summary

The project was motivated by the need for reliable and non-invasive methods to determine sex in farmed Atlantic cod, a capability that would support selective breeding, production planning, and improved broodstock management. Traditional approaches such as dissection and ultrasound are either destructive or difficult to apply accurately to juvenile and pre-mature fish, which limits their value in commercial settings. This project aimed to evaluate whether hyperspectral imaging, employing the Maritech Eye system, could detect biochemical or biophysical signals associated with sex that are not detectable through conventional inspection. Although hyperspectral imaging was the primary focus, other imaging based technologies including fluorescence hyperspectral imaging and deep learning using RGB images were also assessed to explore whether alternative modalities could support early and high throughput sex sorting in aquaculture.

To address the objective, several experiments were carried out on fish ranging from approximately 300 g to 3 kg, spanning sizes that align with industrially relevant classes from early growth stages to more mature individuals. Hyperspectral images were acquired from cod presented with different orientation (ventral and lateral), and sex was determined through dissection to ensure accurate ground truth labels in referencing. The study applied a range of data analytical methods, including exploratory spectral analysis, supervised classification models, and a tailored exploitation of the spectral signatures from excised gonads using linear discriminant analysis (LDA). Additional imaging modalities were also explored, including fluorescence hyperspectral imaging to detect potential sex related emission patterns and deep learning models applied to RGB images to assess whether externally observable visual information (such as morphology, skin pigmentation, or textural features) could be used for sex prediction. All methods were evaluated under controlled data partitioning and consistent validation procedures to ensure robust performance assessment.

The findings indicated that, although sex-specific spectral information could potentially be present in whole fish images, it was not sufficiently strong or separable from other tissues and spatial variability to be reliably linked to sex using supervised classifiers. In contrast, spectra obtained directly from the gonads showed a clear distinction between males and females, and the LDA projection derived from these spectra demonstrated promising transferability when applied to whole fish images with well-developed gonads. Fluorescence imaging did not reveal sex dependent signals, and deep learning networks trained on RGB images were unable to learn discriminative patterns for sex determination. Overall, the results indicate that while the LDA projection based on the gonad spectra shows potential for fish with developed gonads, none of the evaluated techniques currently offers a robust, noninvasive solution for early sex determination in Atlantic cod under industrial conditions.

## 1.1 Sammendrag

Prosjektet er motivert av behov for pålitelige og ikke-invasive metoder for å bestemme kjønn hos oppdrettet atlantisk torsk. Slik metode vil kunne støtte selektiv avl, produksjonsplanlegging og bedre forvaltning av stamfisk. Tradisjonelle metoder som disseksjon og ultralyd er enten destruktive eller vanskelig å anvende med tilstrekkelig presisjon på ung- og kjønns-umoden fisk, noe som begrenser nytteverdi i kommersielle sammenhenger. Målet med prosjektet var å undersøke om hyperspektral avbildning, ved bruk av Maritech Eye-systemet, kunne påvise biokjemiske eller biofysiske signaler knyttet til kjønn, som ikke lar seg avdekke gjennom konvensjonell inspeksjon. Selv om hyperspektral avbildning har vært hovedfokus, ble også andre bildebaserte teknologier vurdert, inkludert fluorescens-hyperspektral avbildning og dyp læring basert på RGB-bilder, for å utforske om alternative metoder kan bidra til tidlig og høykapasitets kjønnssortering i akvakultur.

For å nå målsettingen ble det gjennomført flere eksperimenter på fisk i størrelsesklasse fra om lag 300 gram til 3 kg. Dette dekker størrelser som er relevante for industriell produksjon fra tidlige vekstfaser til

mer modne individer. Hel torsk, presentert med ulike orienteringer (ventralt og lateralt), ble avbildet hyperspektralt, og deretter ble kjønn fastslått ved disseksjon for å sikre korrekte referansedata. I studien er det brukt et bredt spekter av dataanalytiske metoder, inkludert eksplorativ spektralanalyse, veiledet klassifikasjonsmodellering og tilpasset bruk av de spektrale signaturene fra gonader (gonader direkte avbildet) ved hjelp av Lineær DiskriminantAnalyse (LDA). Ytterligere bildemodaliteter ble også undersøkt, blant annet fluorescens-hyperspektral avbildning for å påvise eventuell kjønnsspesifikk lyssignatur, samt dyp læringsmodeller anvendt på RGB-bilder for å vurdere om eksternt observerbar visuell informasjon (som morfologi, hudpigmentering eller teksturelle trekk) kan brukes til kjønnsbestemmelse. Alle metodene ble evaluert med kontrollert datadeling og konsistente valideringsprosedyrer for å sikre robust vurdering av ytelse.

Resultatene viser at selv om kjønnsspesifikk spektralinformasjon potensielt kan finnes i bilder av hel fisk, så er det ikke tilstrekkelig tydelig eller forskjellig fra andre vevstyper og romlig variasjon til pålitelig å kunne knyttes til kjønn ved bruk av veiledet klassifisering. Derimot viste spektra hentet direkte fra gonadene en tydelig forskjell mellom hanner og hunner, og LDA-projeksjon basert på disse spektrene viser lovende overførbarhet når den ble anvendt på helfiskbilder av torsk med velutviklede gonader. Fluorescensavbildning avdekket ingen kjønnsavhengige signaler, og dyp læringsnettverk trent på RGB-bilder klarte ikke å lære diskriminerende mønstre for kjønnsbestemmelse. Samlet sett indikerer resultatene at selv om LDA-projeksjonen basert på gonadespektra viser potensial for å skille på hann- og hunn-fisk med utviklede gonader, gir ingen av de undersøkte teknikkene per i dag en robust løsning for tidlig- og ikke-invasiv kjønnsbestemmelse hos atlantisk torsk under industrielle forhold.

## 2 Objectives

### Main Objective

Evaluate whether hyperspectral imaging acquired using the Maritech Eye system can be used as a reliable and non-invasive method for determining sex in farmed Atlantic cod.

### Sub-objectives

- Conduct experiments spanning different fish size classes, from early growth stages to more mature individuals, providing hyperspectral images and corresponding accurate ground truth labels of sex determination.
- Assess the ability of hyperspectral imaging to reveal biochemical or biophysical signals associated with sex that are not detectable through conventional inspection.
- Develop, evaluate, and compare analytical methods and classification algorithms aimed at distinguishing males from females based on spectral information.

### 3 Project execution

#### 3.1 Materials and methods

##### 3.1.1 Instrumentation

The hyperspectral instrumentation used in this project was a Maritech Eye system (Maritech Systems AS, Molde, Norway), which is an industrial hyperspectral imaging platform. The system includes an interactance illumination unit, a hyperspectral camera, and a dedicated processing module for high-throughput data handling. The hyperspectral camera is a HySpex Baldur V-1024N (Norsk Elektro Optikk AS, Oslo, Norway). It operates as a pushbroom sensor and covers the visual and near infrared (VNIR) region from 485 to 960 nm. The camera provides a spectral resolution of 5.5 nm (88 spectral bands) and captures 1024 spatial pixels across the field of view.

The interactance illumination unit consists of two focused halogen light lines with a total electrical power of 900 watts. The light lines are separated by approximately 8 mm, and the focal plane of the hyperspectral camera is positioned between them. This configuration enables simultaneous acquisition of absorption and scattering information.

Compared with traditional diffuse reflectance configurations, the interactance illumination method allows the system to measure light interaction within subsurface regions of the sample. As a result, it provides access to internal optical properties rather than capturing only surface-level features.

##### 3.1.2 Experimental setup

The objective of the experiments in this project was to collect hyperspectral measurements from fish and to identify the sex of each individual. This allowed the creation of a dataset in which hyperspectral images are linked with confirmed sex information. During the early planning phase, ultrasound was considered as the preferred method for sex determination. However, practical testing showed that ultrasound was difficult to use reliably, particularly for smaller fish and for fish with undeveloped gonads. For this reason, sex determination in the project was carried out primarily through dissection.

The experimental procedure involved collecting hyperspectral images of each fish in several orientations. Data were recorded from the lateral side of the fish (Figure 1.B) and from the ventral side (Figure 1.C). For the lateral images, fish were placed on a conveyor belt during image acquisition. For ventral imaging, a specially designed metal support was used to hold the fish in an inverted position.

After the imaging session, each fish was weighed and dissected to determine its sex. Gonad weight was also measured and recorded. A schematic overview of the experimental setup is provided in Figure 1.A.

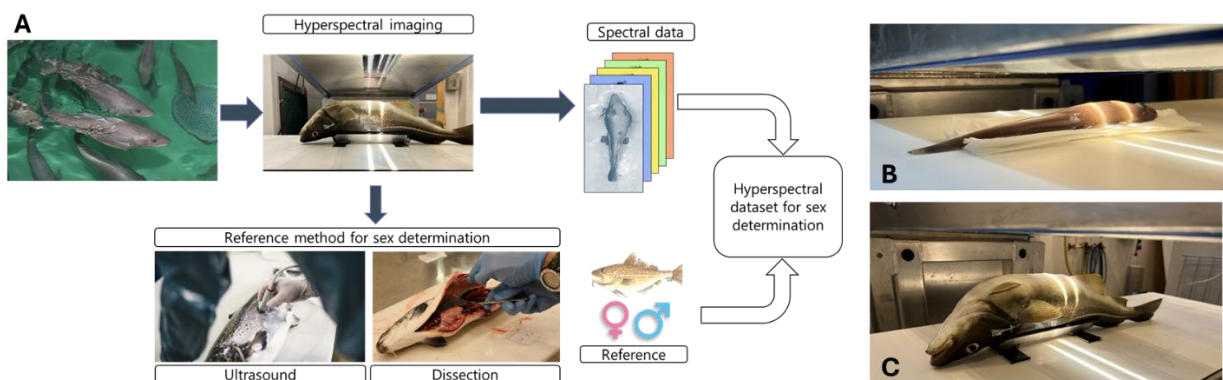


Figure 1 Workflow of the experimental design (A), with corresponding lateral (B) and ventral (C) image acquisitions

### 3.1.3 Summary of experiments

Four experiments were conducted to evaluate imaging-based approaches for sex determination in cod. A brief overview of the fish populations and methods used in each experiment is provided in the following section.

- **Experiment 1:** This experiment included 188 fish from Vesterålen Havbruk (98 females and 90 males). Fish weights ranged from 99 to 281 g, and no individuals showed visible gonad development. Hyperspectral images were collected from both the ventral and lateral sides. Ultrasound was evaluated but proved ineffective for sex determination at this small size. Sex determination was therefore performed by dissection. Due to the small size of the gonads, the procedure was challenging and required expert assessment of sex using magnification lenses.
- **Experiment 2:** This experiment included 136 fish from Kime Akva (73 females and 63 males). Fish weights ranged from 615 to 2327 g, with gonad weights between 1 and 78 g. Hyperspectral images were acquired from both ventral and lateral views. Some fish exhibited injuries, and others showed signs of degradation during the shipment. Ultrasound was again assessed for sex determination but achieved an accuracy of only 40% relative to dissection and was therefore not considered reliable, with reduced precision likely influenced by the observed fish degradation.
- **Experiment 3:** This experiment included 30 fish from the Nofima Cod Breeding Program (18 females and 12 males). Fish weights ranged from 1540 to 2986 g, and gonad weights ranged from 14 to 552 g. This was a small-scale study designed to obtain more precise information about gonad location in the ventral region and to collect spectral measurements directly from the gonads. Fluorescence images were also acquired. Sex determination was performed by dissection.
- **Experiment 4 (STRAMA):** STRAMA is a strategic institute program at Nofima (SIS). This experiment provided 998 fish (548 females and 450 males). Fish weights ranged from 500 to 2700 g, and gonad weights ranged from 1 to 423 g. Conventional RGB images were acquired for all individuals, and hyperspectral images of the ventral region were collected for a subset of 127 fish (75 females and 52 males). Sex determination was performed by dissection.

## 3.2 Identification of sex using hyperspectral imaging

### 3.2.1 Supervised classification based on the spectral data

The initial analyses conducted in this project aimed to determine whether the spectral information from the samples contained features that could be used to separate males from females. These analyses were performed using data from the first two experiments. The first step involved an exploratory evaluation of the spectral measurements by comparing the mean spectra of males and females as whole samples to identify differences in their spectral signatures. In the second step, a Partial Least Squares Discriminant Analysis (PLS DA) model with three latent components was fitted to the spectral data using sex as the response variable. PLS DA is a supervised method that constructs latent variables by maximizing the covariance between the predictors and the class variable. These latent variables are linear combinations of the original predictors that capture variation in the spectral data that is associated with sex. When representing the latent variable scores, each point corresponds to an individual sample, and any separation between data points from different sexes reflects spectral variation linked to the sex of the fish.

Following the exploratory data analysis, several supervised Machine Learning classification algorithms were used to assess whether reliable sex classification was possible. The models tested included Support Vector Machines (SVM), Random Forests (RF), and Neural Networks (NN), all of which are commonly used for classification tasks involving spectral data. Model performance was evaluated using accuracy and the F1 score, with the latter providing a more informative measure of overall model performance across the two classes. The classifiers were applied both to the mean spectrum of each individual fish and to spectra extracted from 20x20 pixels (superpixels), using both the lateral and ventral

images. The superpixel analysis provided finer spatial resolution and increased sensitivity to localized spectral features that may be lost when averaging the spectrum of the entire sample. For the supervised classification, for each experiment the dataset was divided into separate subsets: 70% of the individuals were used for training, 15% for validation to optimize hyperparameters, and 15% for testing to provide an unbiased evaluation of the final models.

### Experiment 1 (Target size 100 – 500 g)

The first experiment comprised 188 fish weighing between 99 and 281 g, including 90 males and 98 females. Exploratory analysis showed no meaningful differences between the mean spectra of males and females, and the PLS-DA score plots did not show separation between the sexes for either ventral or lateral views (Figure 2). Supervised classification based on both mean spectra and superpixels produced test accuracies close to 50% (Table 1), what is similar to random performance in a binary classification task. These findings indicate that the spectral information collected in this experiment does not contain features that allow discrimination between males and females. Since the fish showed no gonad development, internal tissues could not contribute to sex related spectral differences. Only skin related differences could, in principle, be detectable, yet no such variations were observed.

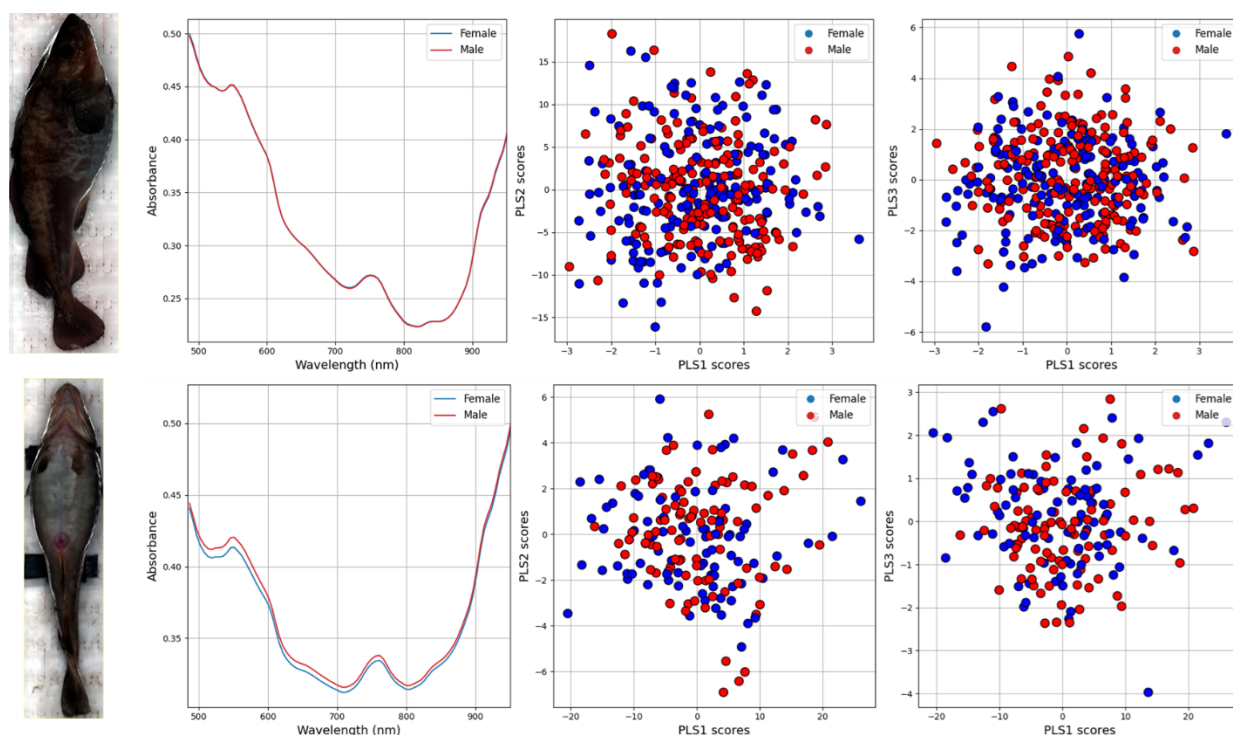


Figure 2 Exploratory data analysis of the spectral data from Experiment 1. First row corresponding to the spectral information extracted from the lateral side, and the second row corresponding to the spectral information from the ventral side.

Table 1 Classification results obtained for Experiment 1

Side	Model	Train Accuracy	Train F1	Validation Accuracy	Validation F1	Test Accuracy	Test F1
Lateral	RF	0,98	0,98	0,63	0,62	0,50	0,50
	SVM	0,62	0,62	0,54	0,53	0,48	0,48
	GP	0,60	0,60	0,50	0,48	0,46	0,46
	NN	0,51	0,51	0,54	0,53	0,43	0,43
Ventral	RF	1,00	1,00	0,69	0,68	0,43	0,40
	SVM	0,58	0,56	0,55	0,51	0,50	0,46
	GP	0,58	0,57	0,55	0,51	0,54	0,50
	NN	0,89	0,89	0,55	0,55	0,36	0,34

### Experiment 2 (Target size 1.0 to 1.5 Kg)

The second experiment included 136 fish, consisting of 73 females and 63 males, with weights ranging from 615 to 2327 grams. As in Experiment 1, the exploratory data analysis did not reveal noticeable differences between male and female mean spectra, and the PLS-DA score plots showed no separation between the sexes (Figure 3). Supervised classification results were also similar, with test accuracies around 50 percent, indicating no predictive value. Classifiers trained on ventral data performed slightly better than those trained on lateral data, although the difference was small and not sufficient to support reliable sex determination. In this experiment, the fish exhibited moderate gonad development, which could potentially introduce subtle spectral effects. However, these influences were weak and confined to a small portion of the pixels. When spectra are averaged across the whole fish, these localized variations are obscured, leaving the algorithms unable to learn sex related spectral patterns.

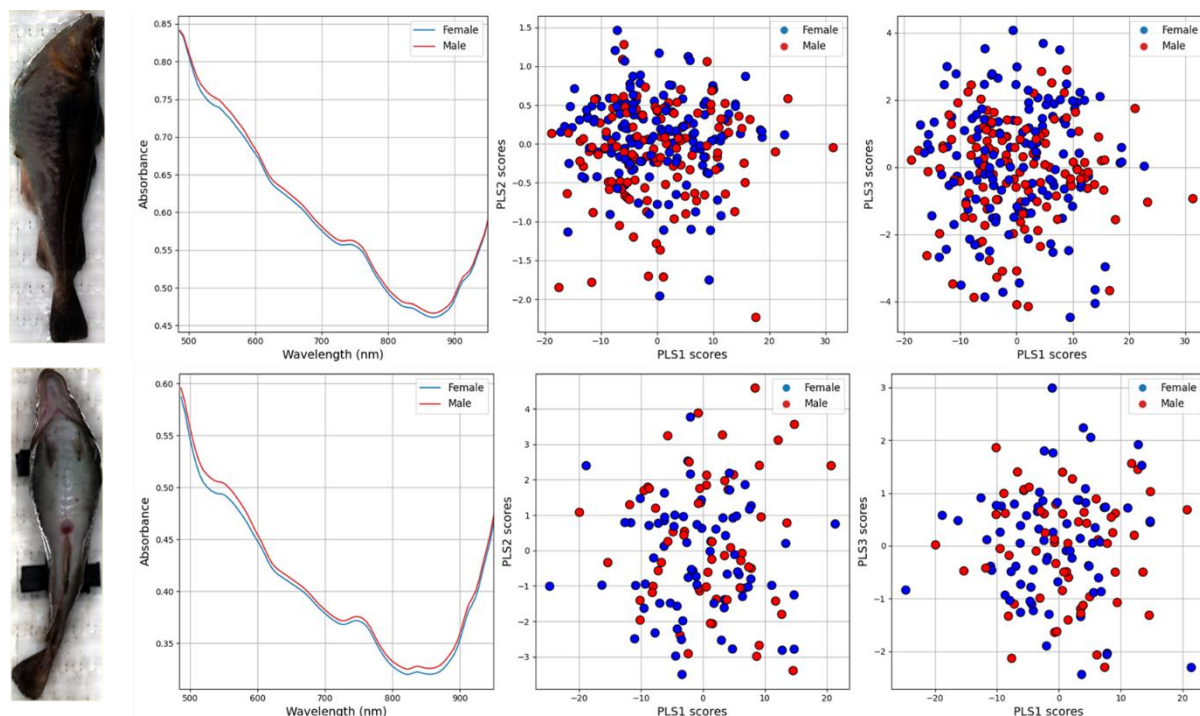


Figure 3 Exploratory data analysis of the spectral data from Experiment 2. First row corresponding to the spectral information extracted from the lateral side, and the second row corresponding to the spectral information from the ventral side.

Table 2 Classification results obtained for Experiment 2

Side	Model	Train Accuracy	Train F1	Validation Accuracy	Validation F1	Test Accuracy	Test F1
Lateral	RF	0,98	0,98	0,63	0,62	0,50	0,50
	SVM	0,62	0,62	0,54	0,53	0,48	0,48
	GP	0,60	0,60	0,50	0,48	0,46	0,46
	NN	0,51	0,51	0,54	0,53	0,43	0,43
Ventral	RF	0,98	0,98	0,61	0,60	0,55	0,54
	SVM	0,60	0,60	0,68	0,68	0,63	0,62
	GP	0,59	0,59	0,55	0,55	0,63	0,62
	NN	0,62	0,62	0,71	0,69	0,58	0,57

### 3.2.2 Identification of sex based on reference spectra from the gonads

#### Extraction of a Domain-Invariant Discriminant Axis from Gonad Spectra

Hyperspectral images of excised cod gonads were acquired in the visible–near infrared (VNIR) range, 407-996 nm (186 bands) using diffuse reflectance illumination. Raw image cubes were converted to reflectance using dark and white reference measurements and subsequently transformed to absorbance by applying a negative logarithm. For each gonad sample, pixels belonging to the annotated gonad region were extracted and spectrally preprocessed by mean-centering across wavelengths. A representative spectrum per gonad was computed as the 20% trimmed mean of all gonad pixels to reduce the influence of outliers and specular artefacts.

The resulting sample-by-wavelength matrix was interpolated onto the wavelength grid of the Maritech Eye, 487-956 nm (88 bands) to enable transfer of the learned discriminant axis to whole-fish images. This system is also based on interactance illumination, which gives increased light penetration into the interior of the sample compared to diffuse reflectance that mainly probes the sample surface. The gender was encoded categorically (female, male) based on direct observations following dissection, and a linear discriminant analysis (LDA) model was fitted to the interpolated gonad spectra.

Shrinkage regularization of the within-class covariance matrix was explored over several orders of magnitude (see Figure 4). Although performance exhibited a broad plateau across  $\gamma$  values, a shrinkage parameter of  $\gamma = 3 \times 10^{-3}$  was selected. This value produced a stable and spectrally smooth discriminant vector while maintaining maximal cross-dataset discrimination. Smaller  $\gamma$  values resulted in noisy, high-frequency coefficient structure consistent with covariance overfitting, whereas larger values increasingly suppressed biologically interpretable spectral features. The final discriminant vector was normalized to unit length.

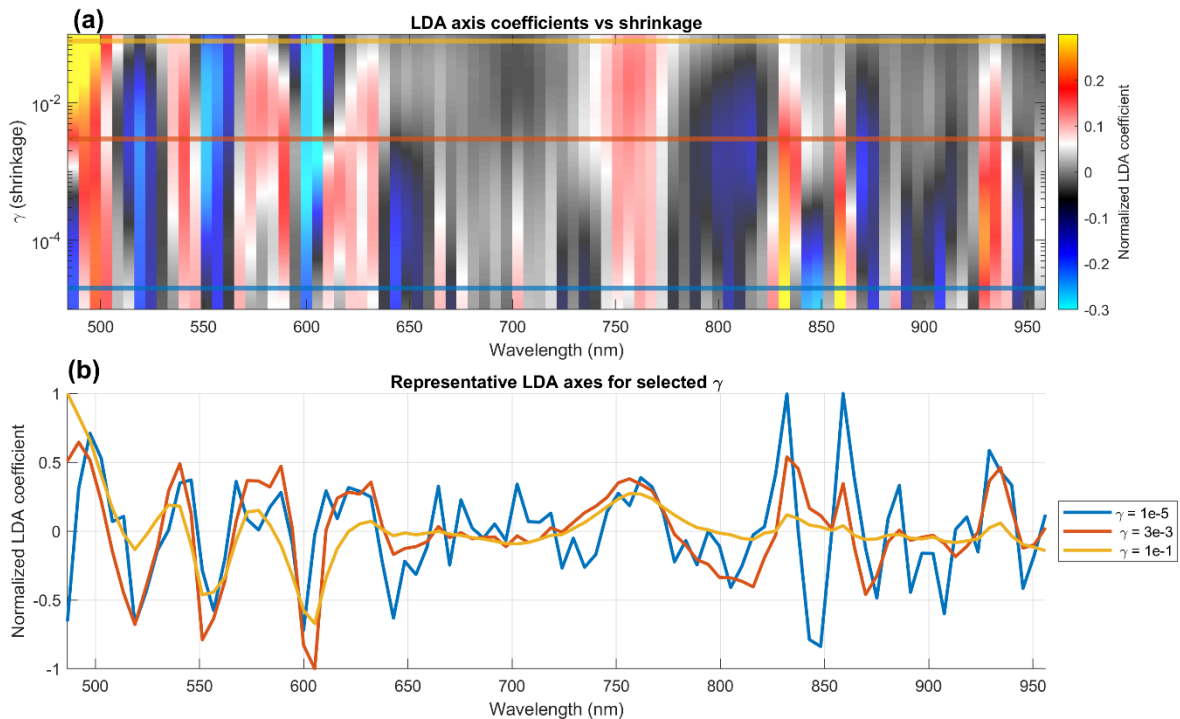


Figure 4 (a) illustration of the effect of shrinkage regularization on the LDA axis separating male and female gonad classes, horizontal lines mark the three representative curves displayed in (b) corresponding to specific  $\gamma$  values.

Figure 5 shows the classification performance of the LDA projection applied directly to the raw gonad hyperspectral images (using methodology described for whole-fish in section 0). Figure 5a presents the receiver operating characteristic (ROC) curve based on nested leave-one-out cross-validation (LOOCV), indicating excellent discrimination between male and female gonads, with area under curve (AUC) of 0.967 and a 95% confidence interval of [0.889, 1.044]. The optimal threshold for class assignment was 0.217, with only two misclassified samples. Figure 5b shows the corresponding boxplot of the LDA discriminant scores, highlighting a strong separation between classes, reflected in a Cohen's  $d$  of 2.592 and a Fisher discriminant ratio (FDR) of 3.360. Together, these plots demonstrate that the LDA projection yields robust class separation for hyperspectral images of extracted gonads.

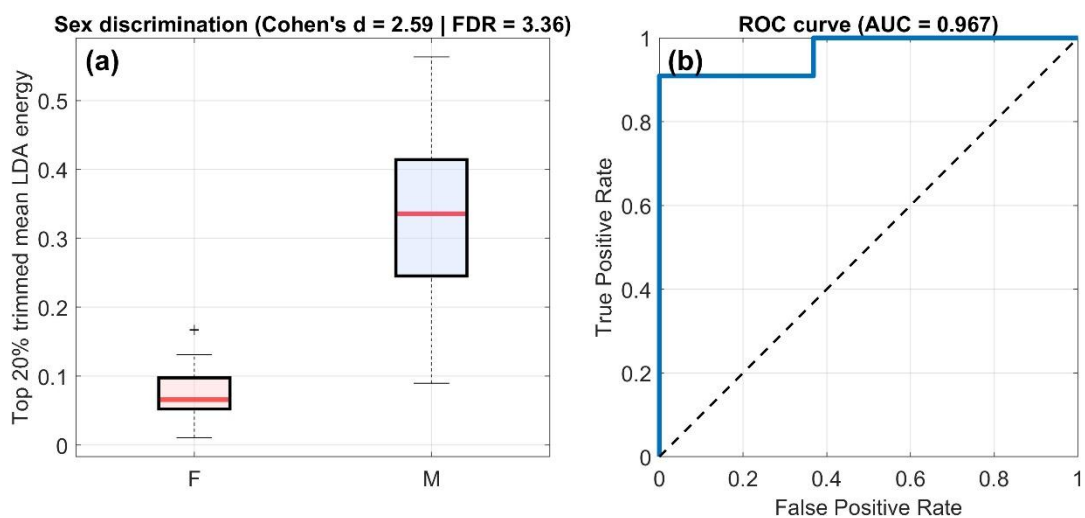
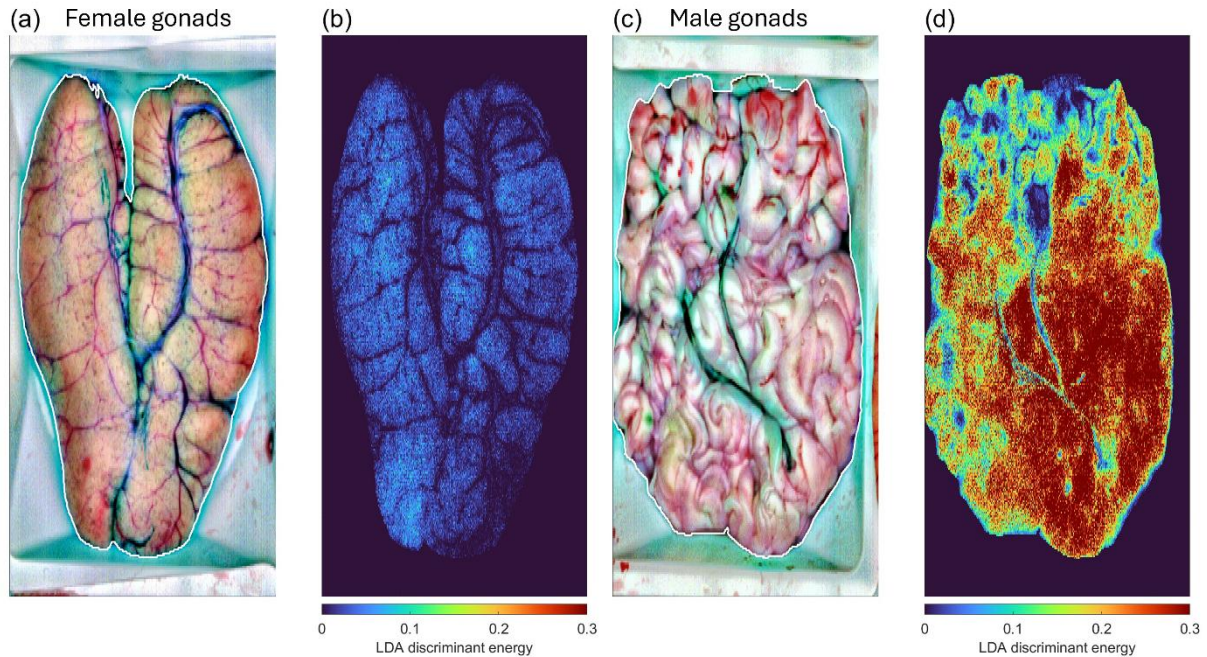


Figure 5 Classification of raw gonad hyperspectral images using LDA energy. (a) ROC curve from nested LOOCV shows excellent separation between male and female gonads (AUC = 0.967, 95% CI [0.889, 1.044]). (b) Boxplot of LDA scores illustrates strong class separation (Cohen's  $d = 2.592$ , FDR = 3.360).

While the LDA model was fitted to 20% trimmed mean spectra, i.e., one representative spectrum per sample, it is also interesting to examine the pixelwise predictions when the full hyperspectral images are projected on the LDA axis. Figure 6 shows an example of male and female gonads, where the male gonads are clearly associated with higher LDA discriminant energy. The spatial variation in the discriminant energy maps gives an indication of how the model responds to different tissues. Areas with higher blood concentration (e.g., capillaries), may be associated with lower discriminant energy, but we have not attempted to make a detailed biological interpretation of the spatial variation.



*Figure 6 Illustrative RGB false-colour hyperspectral images of (a) female and (c) male gonads and corresponding maps of pixelwise LDA discriminant energy. According to the fitted LDA axis, males are associated with higher discriminant energy.*

### Projection of Whole-Fish Images

To evaluate cross-domain generalization, the discriminant axis derived from gonads was applied to three independent whole-fish imaging experiments acquired under different conditions. Since some individuals had very small gonads, we focused the analysis on the subset with the largest gonads (as determined by gonad weight following dissection), while simultaneously ensuring a 50:50 split between males and females. The distribution of gonad weights across the selected subsets is shown in Figure 7 and we see that Experiment 2 contains smaller gonads, while Experiment 3 had some male individuals with moderately small gonads. The distribution of weights of the excluded samples is also shown in Figure 7.

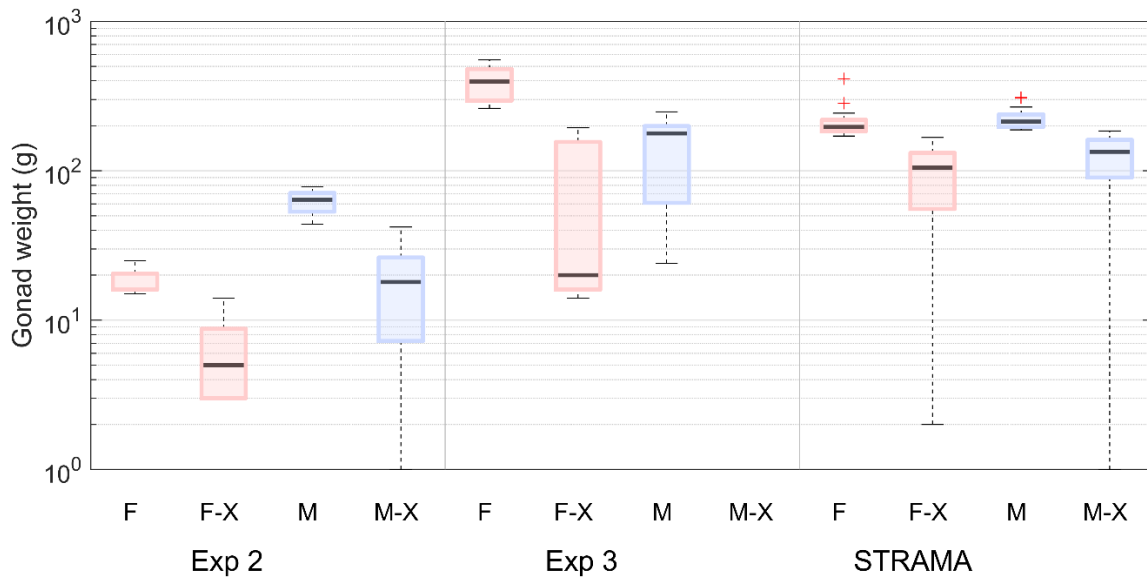


Figure 7 Comparison of sample subsets containing the highest gonad weights for each gender for the three independent experiments (Exp 2 - 22 samples, 11 M and 11 F, Exp 3 - 24 samples, 12 M and 12 F, STRAMA - 32 samples, 16 M and 16 F). The F-X and M-X groups are the samples with lower gonad weights that were excluded from further analysis, and for Exp 3 all male individuals were included.

Each hyperspectral cube was reflectance-corrected and converted to absorbance using the same preprocessing steps as for gonads. Fish pixels were segmented from the background, and cubes were spatially cropped to the bounding boxes surrounding the fish for processing speed and visualization convenience.

Per-pixel spectra within the fish mask were standardized using standard normal variate (SNV) normalization to mitigate illumination and scattering differences. This is an important step due to the strongly absorbing and scattering skin layer that obscures the gonad tissues beneath. The discriminant projection was then computed for each pixel as

$$z = (X_{\text{fish}}w)^2,$$

where  $w$  is the normalized gonad-derived LDA axis. Squaring the projection removes orientation dependence and yields a positive-definite discriminant energy, improving robustness to domain-dependent sign inversions. The experiments were conducted on different conveyor belts having different spectral characteristics and one experiment placed black fabric that was highly absorbing in the visible but highly reflective in infrared, so robustness to domain shift was a critical factor.

For each fish, a scalar score was computed as the trimmed mean of the upper  $\alpha = 20\%$  of pixel-level energies. This aggregation strategy emphasizes regions exhibiting strong gonad-like spectral characteristics while suppressing background tissue contributions. Performance was evaluated using nested leave-one-out cross-validation (LOOCV) within each experiment. Discrimination was quantified using the area under the receiver operating characteristic curve (AUC), Cohen's  $d$ , and the Fisher Discriminant Ratio (FDR).

## Results for whole fish gender prediction

Figure 8 summarizes the results of the LDA projection for Experiment 2. The AUC of 0.785 indicates that, for a randomly selected male and a randomly selected female, there is a 78.5% probability that the male receives a higher discriminant score than the female. It is also encouraging that the areas of high LDA discriminant energy that should be associated with the presence of male gonads typically occur in approximately the correct anatomical region of the fish (see Figure 8f). The modelling pipeline is spatially unaware since the spatial aggregation is a simple mean of the highest 20% pixel scores. The fact that the LDA discriminant energy peaks in the anatomical region where the gonads are located therefore increases our confidence that the model is able to resolve a direct optical signal related to the gonads that penetrates through the skin.

We see similarly strong results for experiment 3, with AUC of 0.889 and stronger class separation as indicated by Cohen's  $d$  and Fisher discriminant ratio (see Figure 9). These are the same fish whose extracted gonads were used to train the LDA model, so it is perhaps to be expected that the performance is best for these images. Although the same fish were used, these results still represent generalisation across domains from images of pure gonads under diffuse reflectance illumination taken by a different camera, to images of whole fish where gonads are obscured by skin, with interreflectance illumination and different spectral sampling. As a result, we still consider the performance on this set of images a strong indicator of model robustness to domain shift. In addition, the fish in Experiment 3 were fresher than those for Experiment 2, where some fish were significantly decayed and/or found to be rotten when opened, so we expect stronger and purer signals from the gonads in Experiment 3. The stronger performance for Experiment 3 could also be a result of the larger gonads in these fish (see Figure 7) leading to a stronger signal and higher probability of light penetrating the skin and interacting with the correct tissues.

We also applied the model to additional fish sampled in the STRAMA experiment, where we see a similarly strong ability to separate the genders, with AUC of 0.805 (see Figure 10). It is important to note that while the relative separation between genders was quite consistent across experiments, the optimal threshold score varies quite substantially (STRAMA experiment has generally much lower scores). This would be impractical in a production environment, where sorting should ideally proceed according to a robust, fixed threshold and is something that should be focused on in future studies. It was difficult to adequately resolve this issue within the current project since there were multiple factors that varied between the experiments, such as conveyor belt material, black fabric underlay and also differences in storage time and freshness of samples. These effects could contribute to baseline response shifts between experiments that are difficult to correct for explicitly without further controlled experiments, and may be less of an issue in an industrial environment where fish are always scanned in a fresh state and on the same substrate.

For the STRAMA experiment we also observed some anomalous pixel level predictions in association with specular reflections (see Figure 10). Specular reflection occurs when light reflects from a smooth or wet surface in a mirror-like manner rather than being diffusely scattered. In hyperspectral imaging, these bright pixels are dominated by the illumination spectrum rather than true tissue absorption. As a result, their spectra can deviate strongly from surrounding tissue and produce anomalous LDA energy values that reflect lighting geometry rather than biological differences. Refining illumination setup or avoiding excessively wet samples could both lead to reduced specular reflection and potentially improved model performance. Another alternative could be to selectively detect and mask out pixels affected by specular reflection from further analysis, but within the scope of this project we chose to keep the analysis pipeline as simple as possible.

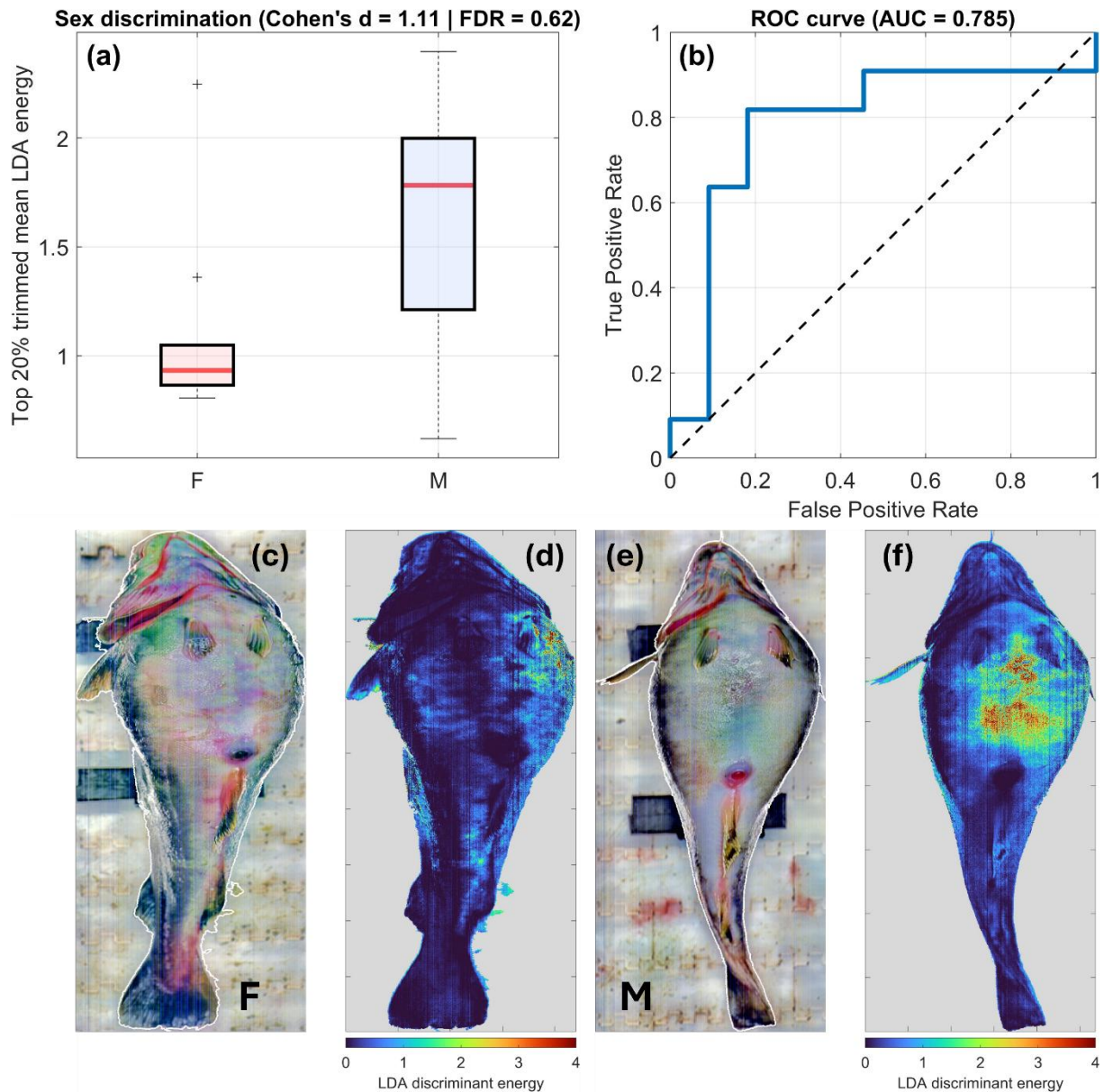


Figure 8 LDA projection applied to Experiment 2 (22 samples with highest gonad weight, 11 M and 11 F) hyperspectral images of whole fish imaged ventrally. (a) boxplot showing aggregated scores, (b) ROC curve summarising classification accuracy. (c) False colour RGB and (d) spatial projection map of a female individual, (e) and (f) show a corresponding example for a male individual.

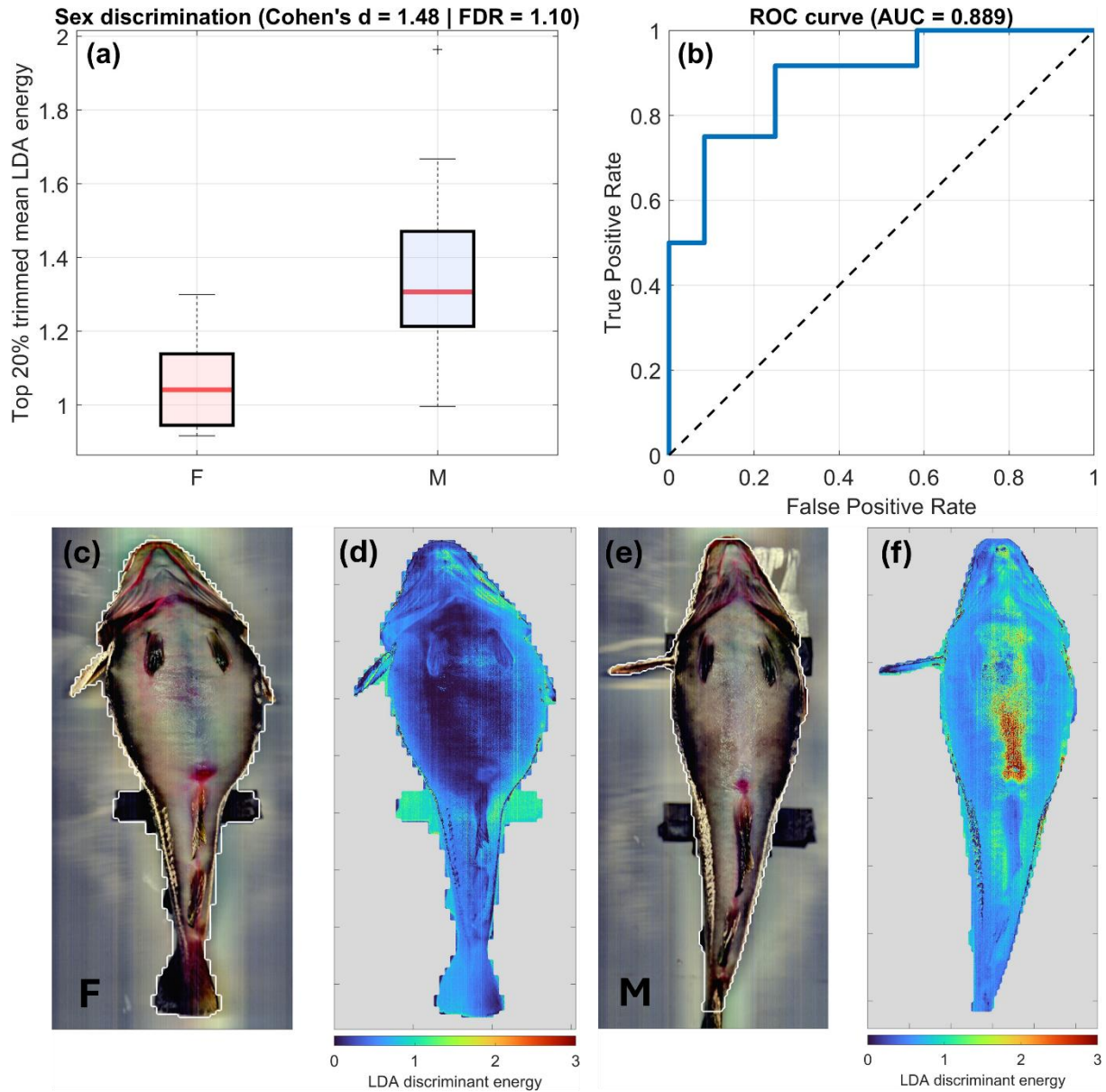


Figure 9 LDA projection applied to Experiment 3 (24 samples with highest gonad weight, 12 M and 12 F) hyperspectral images of whole fish imaged ventrally. (a) boxplot showing aggregated scores, (b) ROC curve summarising classification accuracy. (c) False colour RGB and (d) spatial projection map of a female individual, (e) and (f) show a corresponding example for a male individual.

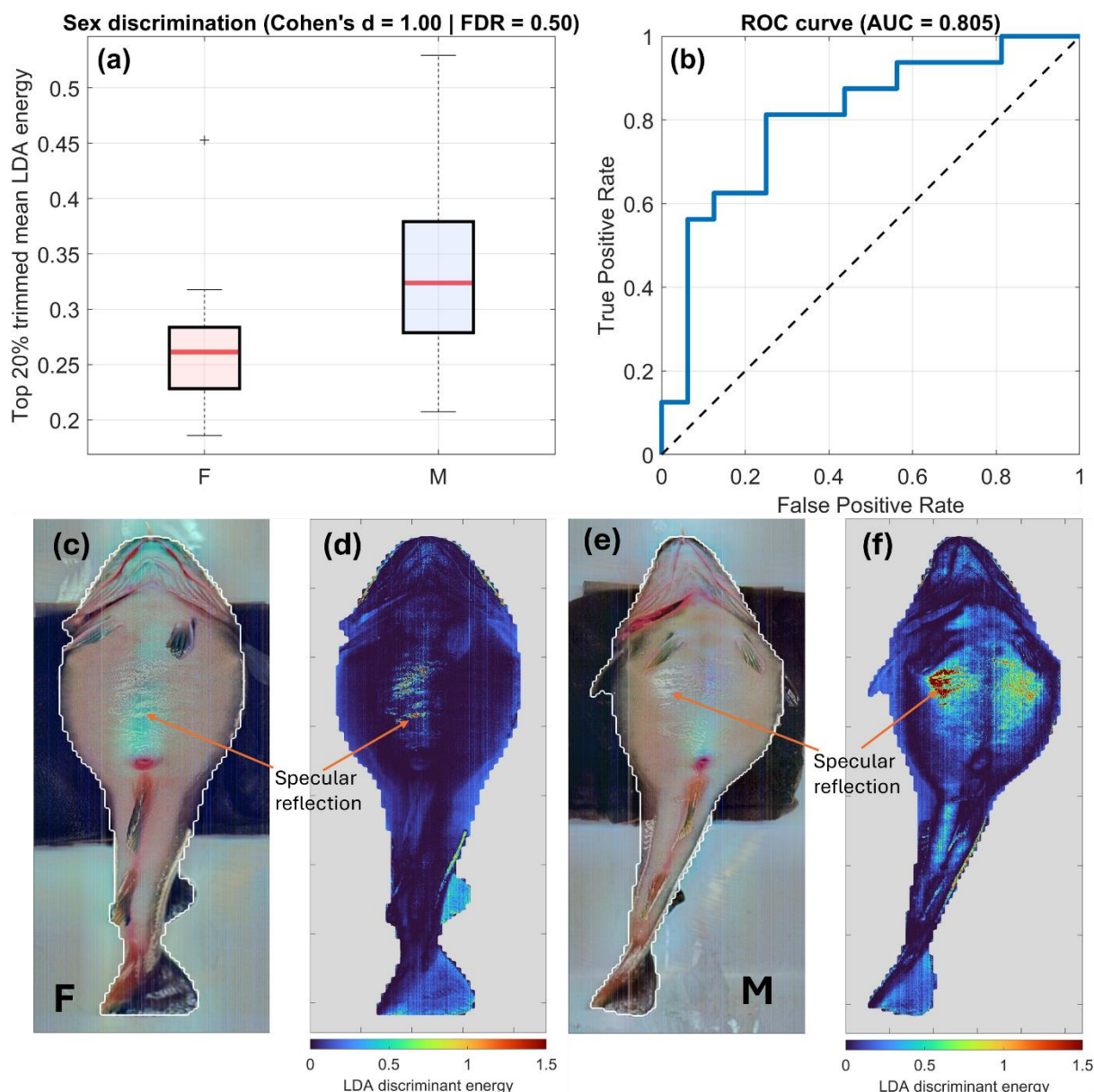


Figure 10 LDA projection applied to fish from STRAMA experiment (32 samples with highest gonad weight, 16 M and 16 F) hyperspectral images of whole fish imaged ventrally. (a) boxplot showing aggregated scores, (b) ROC curve summarising classification accuracy. (c) False colour RGB and (d) spatial projection map of a female individual, (e) and (f) show a corresponding example for a male individual. Areas of specular reflection are annotated since these appear to give anomalous results under LDA projection.

### 3.3 Exploring the biofluorescence as a potential tool for determining the sex

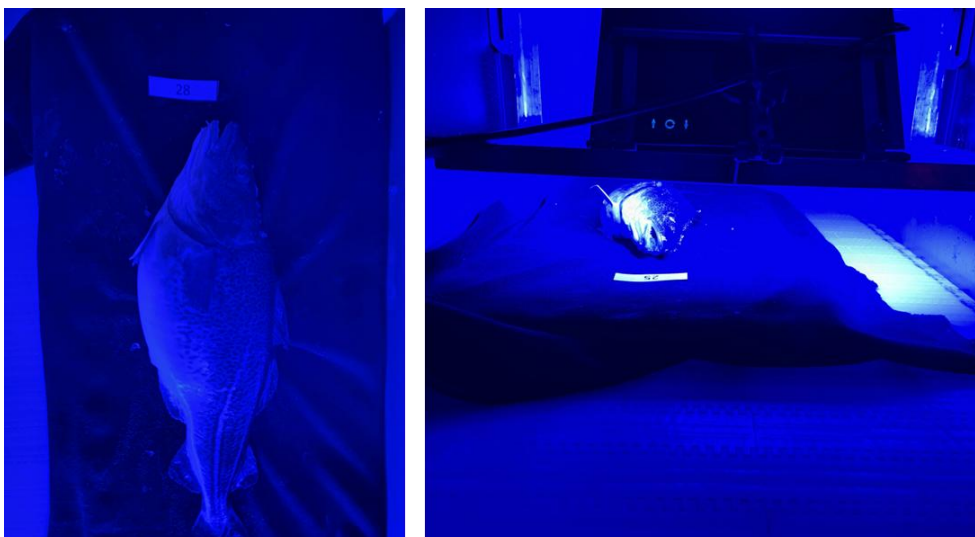
Biofluorescence is a phenomenon where living organisms absorb high-energy light, typically in the blue to ultraviolet range (around 300–450 nm), and reemit it at longer, lower-energy wavelengths, producing visible colors such as green (500–550 nm), orange (590–620 nm), and red (620–750 nm) (Sparks et al., 2014). Many marine fishes and invertebrates exhibit biofluorescence in species-specific patterns, which serve multiple roles in nature and have been documented to exhibit sexual dimorphisms in certain species (Carr et al., 2025; Cohen & Summers, 2022).

Recent studies have demonstrated that biofluorescence in marine species can be effectively documented using hyperspectral imaging technology. Previous work at Nofima has shown the potential of biofluorescence as a non-invasive indicator of stress in red king crab, green sea urchin, and lumpfish

(Juhasz-Dora, Lindberg, James, et al., 2024; Juhasz-Dora, Lindberg, Karlsen, et al., 2024). Building on these findings, we conducted a small-scale experiment to assess whether Atlantic cod exhibit measurable differences in biofluorescence between males and females.

The hyperspectral instrumentation used for this experiment was a custom imaging platform centered around a VNIR-1800 hyperspectral camera (Hypex, Oslo, Norway). The system consisted of the camera mounted above a conveyor-belt scanning unit and supported by a dedicated illumination setup optimized for fluorescence measurements. The VNIR-1800 operates across the 400-1000 nm spectral range with a spectral resolution of 5.5 nm (188 bands) and records 1800 spatial pixels across the field of view. Illumination was provided by an LED unit (G5 XR30 Pro Radion, Ecotech) configured to emit two different excitation wavelengths: Ultraviolet (central wavelength ~350 nm) and Royal Blue (central wavelength ~445 nm).

For this small-scale experiment, a total of 30 individuals (12 males and 18 females) were included, and hyperspectral images were captured using the instrumentation described above. Each fish was imaged first under the Royal Blue illumination, followed by imaging under the ultraviolet illumination. To ensure that only light from the LED sources was recorded, all external illumination in the room was turned off during image acquisition. The samples were placed in trays lined with black fabric to reduce reflections and limit background interference. An example of the measurements obtained under these conditions is shown in Figure 11.



*Figure 11 Representation of hyperspectral fluorescence images acquisition*

The hyperspectral data analysis consisted of performing a radiometric calibration to produce spectral radiance values from the raw spectral data acquired with the hyperspectral camera. After the calibration, a segmentation procedure was applied to the images to identify the areas of interest for the fluorescence analysis, specifically the surface of the fish. The spectral information was then cropped to remove the influence of the light source in the fluorescence measurements, since the excitation signal is much stronger than the emission signal recorded from the fish surface. For the Royal Blue illumination, the fluorescence analysis covered wavelengths between 500 and 800 nm, while for the ultraviolet illumination it covered wavelengths between 450 and 700 nm.

After this preprocessing, the spectral data from the surface of the fish were extracted and analyzed. The first step of the analysis focused on comparing the mean fluorescence intensity across the entire spectral range for the two excitation wavelengths. The mean and standard deviation of the recorded fluorescence values for both ranges are presented in Figure 12. The results show that no distinctive fluorescence

peaks can be identified for males or females for any of the spectral regions analyzed. No relevant differences were observed in the overall intensity of the fluorescence signal between the two sexes. Under ultraviolet excitation (Figure 12.A), the emission profiles of males and females were similar. Under Royal Blue excitation (Figure 12.B), females showed a slightly higher mean fluorescence intensity, but there was substantial overlap in the standard deviations of the spectra. This overlap indicates that the observed variation is not sex specific and cannot be used to distinguish males from females based on fluorescence measurements.

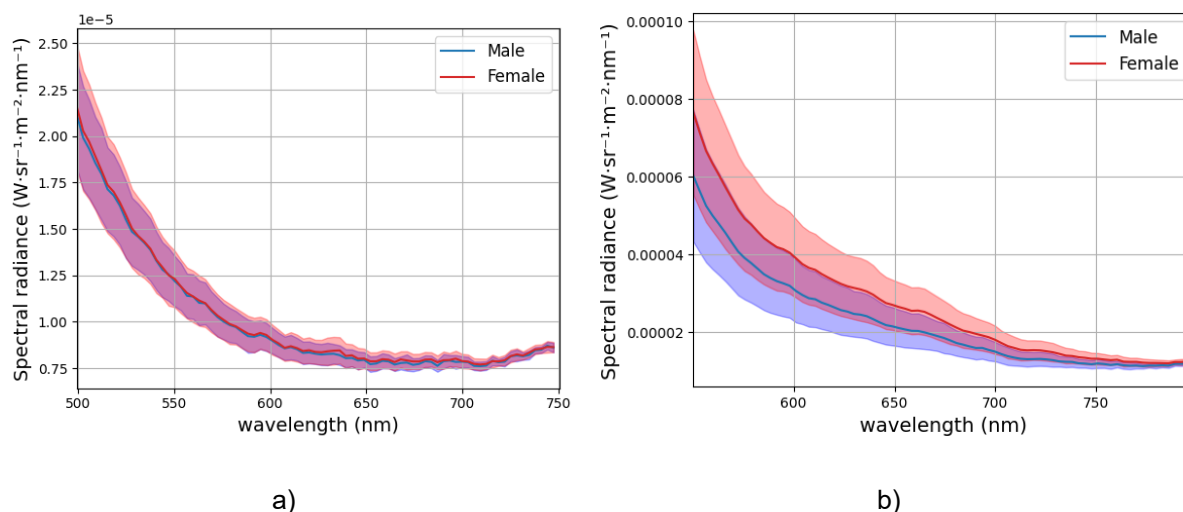


Figure 12 Mean fluorescence spectra measured for UV excitation (A) and Royal Blue excitation (B)

Additional data analysis was also performed to identify potential fluorescence features in specific regions of the fish. This type of analysis has been successfully applied in previous studies to identify distinctive fluorescence peaks in green sea urchins. However, the analysis conducted in this study did not reveal any characteristic fluorescence peaks in Atlantic cod.

The results from our sample and protocol indicate that Atlantic cod did not display evident sex specific fluorescence responses, and fluorescence-based sex identification was not feasible in this experiment. However, the partial separation of baseline fluorescence between males and females observed under Royal Blue excitation suggests a potential avenue for optimizing excitation and emission wavelength selection for sex discrimination. In this context, spatially resolved analysis of fluorescence patterns may further help identify localized features contributing to this separation. These aspects would need to be addressed in additional experiments and are therefore beyond the scope of this project.

### 3.4 Exploring artificial intelligence in RGB images for determining the sex

During an experiment conducted as part of the Cod Breeding Program in Nofima, conventional RGB images of approximately 1000 Atlantic cod were recorded along with several biological measurements, including sex. To investigate whether standard RGB images contain visual cues that could distinguish males from females, we performed a preliminary analysis.

Although the main objective of the project did not include sex classification based on conventional images, we conducted an exploratory screening using Convolutional Neural Network models that are widely used in computer vision research. The models tested included DenseNet, EfficientNet, ResNet, Inception and GoogleNet. These architectures are designed to learn morphological and structural features directly from images and have been proven effective for different computer vision tasks (Li et al., 2022; Zhao et al., 2024).

For each model, RGB images of individual Atlantic cod were used as input, and the task was to classify the sex of each specimen. Example images are shown in Figure 13. The dataset included 1066 images: 585 females and 481 males. The data were divided into training, validation and test sets according to the standard split of 70%, 15% and 15%. In general, each image corresponds to a unique individual, but a small number of fish have duplicate images captured under slightly different conditions. All images were included to increase the size of the dataset. Since the number of duplicates was limited, all duplicate images were assigned to the training set, ensuring that no individual appeared in more than one subset.

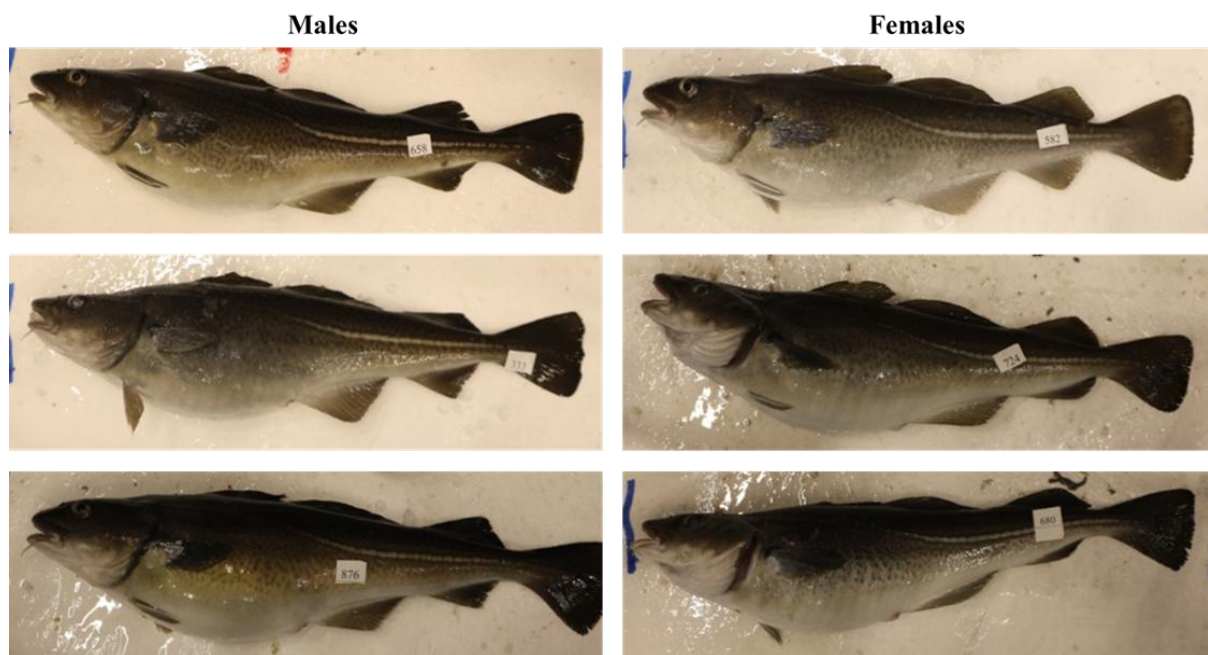


Figure 13 Example of RGB images used for CNN classification.

The results are summarized in Table 3. All models performed similarly and none achieved meaningful accuracy. Accuracies on the training set were between 0.55 and 0.58, with F1 scores in the same range. The validation and test sets showed comparable performance. It is important to highlight that even on the training set, where models typically achieve high performance due to repeated exposure to the same images, the models were unable to learn patterns that distinguish males from females. Their performance remained close to random guessing, which indicates that the RGB images do not contain clear or consistent visual differences between the sexes under the conditions in which they were collected. In practical terms, the external appearance of males and females appears too similar for a model to detect differences reliably.

Table 3 Results of CNN classification on RGB images for determining the sex of Atlantic Cod

Model	Train Accuracy	Train F1	Validation Accuracy	Validation F1	Test Accuracy	Test F1
DenseNet 201	0,60	0,56	0,62	0,58	0,63	0,58
EfficientNet (B0)	0,60	0,60	0,66	0,66	0,61	0,61
EfficientNet (B4)	0,55	0,55	0,56	0,56	0,55	0,55
GoogleNet	0,61	0,57	0,64	0,61	0,63	0,61
Inception V3	0,64	0,64	0,66	0,66	0,58	0,58
MobileNet V2	0,56	0,56	0,58	0,58	0,60	0,59
ResNet 152	0,65	0,65	0,70	0,70	0,61	0,61
ResNet 50	0,58	0,58	0,66	0,65	0,57	0,56

Several factors limit the usefulness of these results. Although the dataset contains over one thousand images, the models were unable to achieve high accuracy even on the training set. Deep convolutional neural networks typically achieve very high performance on the training data because they can easily memorize the examples presented during training. The fact that the training accuracy remained close to random guessing across all tested architectures therefore suggests that the limitation arises from the lack of consistent visual differences between male and female cod in the RGB images rather than from insufficient model capacity.

This interpretation is further supported by the fact that a wide range of CNN architectures were tested, including DenseNet, EfficientNet, ResNet, Inception and GoogleNet. Despite their different design principles and feature extraction strategies, all models converged to similar performance levels. This consistency indicates that the models were unable to identify reliable visual cues distinguishing males from females under the imaging conditions used in this experiment.

In practical terms, these results suggest that the external appearance of Atlantic cod in conventional RGB images does not contain sufficiently strong or consistent morphological signals for reliable sex classification using standard deep learning approaches. An alternative to deep learning classification is to focus on measurable morphological differences, such as fin or head dimensions, that might be captured in RGB images. Some studies have indicated that pelvic fin length may differ between males and females (Skjæraasen et al., 2006). Executing such an analysis would require detailed measurement protocols and a more comprehensive and systematically organized RGB dataset, which lay outside the boundaries of this project.

## 4 Findings, discussion, and conclusion

The main goal of this project was to evaluate whether hyperspectral imaging can be used to determine sex in Atlantic cod. Based on the data from the first two experiments, no clear sex-related differences were observed in the spectral signatures of males and females. Supervised classification algorithms were therefore unable to discriminate between the sexes, suggesting that any sex-specific spectral information present may have been confounded by contributions from other tissues and spatial heterogeneity. This approach has the main limitation of being unable to detect subtle differences in the spectra that could be associated with sex.

To explore a more direct approach, spectral information from the gonads was analyzed. LDA provided clear discrimination between male and female gonad spectra, and the resulting projection was applied to whole-fish images. This method showed the strongest potential for sex determination using spectral imaging and produced consistent results across experiments involving different fish sizes and imaging setups. However, good performance was limited to fish with well-developed gonads, which restricts its applicability for industrial needs where early sex sorting is required.

Although hyperspectral imaging was the primary focus, additional image-based modalities were also explored. Fluorescence imaging did not support robust sex discrimination in this project. However, the partial separation observed under Royal Blue excitation suggests that further optimization of acquisition and analysis parameters is a relevant direction for future studies. Similarly, conventional RGB imaging combined with CNN classifiers did not achieve reliable predictions. While neither approach proved effective within the constraints of the present dataset and protocol, fluorescence imaging presents opportunities for future investigation through optimized excitation and emission wavelength selection and spatially resolved analysis of fluorescence patterns. In contrast, conventional RGB imaging may offer potential through computer-vision-based methods targeting morphological traits, such as fin structure or other external features. Future advances in both approaches will require larger datasets and refined experimental methodologies.

Overall, the results of this project indicate that hyperspectral image analysis appears promising for sex identification but is limited to fish with well-developed gonads. As a result, this approach is not suitable for sex sorting in juvenile fish and therefore does not meet the requirements of the targeted industrial application. Although fluorescence imaging and conventional RGB imaging did not yield positive results in the present study, they may offer potential for improvement in sex identification using image-based technologies.

### **Contributions to improved sustainability**

The project explored noninvasive imaging technologies for sex determination in Atlantic cod. Earlier and more accurate identification of sex would enable better broodstock management, more efficient selective breeding, and improved production planning, all of which help reduce unnecessary resource use and support long term sustainability in cod farming.

## 5 Main findings (should be written in both Norwegian and English)

- The spectral information directly extracted from whole-fish hyperspectral images did not show clear differences between males and females, and supervised classification methods were unable to reliably separate the two groups. This suggests that, although some sex-specific spectral information may be present, it is not sufficiently strong to be directly exploited using supervised approaches. Spectral analysis of gonads showed clear separation between male and female gonads using LDA, and applying this projection to whole-fish images produced the most promising results across different experiments and fish sizes.
- The effectiveness of the gonad-based approach is restricted to fish with well-developed gonads, limiting its suitability for industrial scenarios where early sex sorting is required.
- Additional imaging approaches were evaluated, including fluorescence hyperspectral imaging and deep learning in conventional RGB images, but they did not yield reliable sex predictions. However, morphology-focused computer vision methods applied to larger RGB datasets may still offer potential for future research.

## 6 Deliverables

Deliverable	Description
D1	Kick-off meeting with the reference group to present the project plan.
D2	Minutes from reference group meeting
D3	Popular science article on Nofima's website
D4	Final Reference Group Meeting
D5	Final academic and administrative report

## 7 References

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