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Short communication

State of the heart: Anatomical annotation and assessment of morphological cardiac variation in Atlantic salmon (*Salmo salar* L.)

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ABSTRACT

Farmed Atlantic salmon suffer from deviating cardiac morphology, likely compromising fish health and robustness. To mitigate the potentially devastating consequences of morphological deviations of the heart, the cause and consequences of these abnormalities need to be elucidated. This endeavour is, however, complicated by several obstacles. First, a detailed nomenclature of gross anatomical structures of the pyramidal fish heart is needed to develop standardized descriptions of morphological deviations. Second, standardized methods and scoring systems for quantitative and qualitative descriptions of cardiac anatomy are lacking. Lastly, the normal morphological development of the heart through the life cycle of wild specimens has not been described, making it challenging to identify deviating morphology at different life stages of farmed salmon. Here, we develop a nomenclature for the exterior of a pyramidal teleost heart, using the Atlantic salmon heart as a model. Further, we have unified and described the most common quantitative methods used for measuring heart morphology and developed a qualitative methodology to detect morphological abnormalities not identified by the quantitative methods. We describe the methodologies' applicability and assess the qualitative method's robustness. Using quantitative measures, heart morphology in the wild and farmed salmon is compared throughout their life cycles. Further, we show that the qualitative method is superior to the quantitative method in detecting traits more prevalent in deceased fish after exposure to stressful routine field treatments, illustrating the complementary nature of these two methods.

1. Introduction

The aquaculture industry and the scientific community are increasingly concerned with cardiovascular health of farmed Atlantic salmon (*Salmo salar* L.). In Norway alone, the mortality rate of farmed Atlantic salmon at sea is worrying, with mortality exceeding 20% (Sommerset et al., 2022). The underlying causes of mortality are not fully charted, however, mortality typically peaks during stressful interventions, such as delousing, transport and handling (Oliveira et al., 2021). A large fraction of recorded mortalities are attributed to heart diseases (Sommerset et al., 2022; Sommerset et al., 2022). Whereas infectious heart diseases such as cardiomyopathy syndrome (CMS) and heart and skeletal muscle inflammation (HSMI) have received considerable attention (Garseth et al., 2018; Sommerset et al., 2022; Wessel et al., 2015, 2020), non-infectious heart diseases have been overlooked. While molecular biological methods are readily available to detect infections, no specific tools for assessing non-infectious heart disease exists. Deviating heart morphology is an example of non-infectious heart disease with a potentially devastating impact on fish health and performance.

Farmed salmonids typically display heart morphology that differs dramatically from those of their wild counterparts (Kristensen et al., 2012; Leonard and McCormick, 2001; Poppe et al., 2003). Poppe et al.

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(2003) raised concerns, over two decades ago about severely rounded ventricles and misaligned bulbi in sea-farmed Atlantic salmon and rainbow trout (*Oncorhynchus mykiss*) in comparison to their wild counterparts. They speculated that such abnormal hearts could cause cardiac arrest under stressful events. Indeed, rounded ventricles have subsequently been associated with impaired cardiac and physical performance (Claireaux et al., 2005), and mortality due to CMS (Frisk et al., 2020). In addition, impaired cardiovascular function can have several other undesirable health effects as it will likely impair peripheral circulation and blood supply to vital organs. Given the potentially devastating consequences of deviation in cardiac morphology, it is paramount to identify risk factors associated with the development of these deviations and fully understand the consequences of these factors. Detailing this information is, however, complicated by several obstacles.

First, it is challenging to qualitatively describe cardiac deviations without a detailed nomenclature on the exterior of the Atlantic salmon heart. Current nomenclature is mainly limited to naming the different chambers of the heart (Anttila and Farrell, 2011).

Second, there is a limited standardisation methodology for assessing cardiac morphology in salmonids. Different methods have been used, and they are typically limited to a few measurements (ratios and angles quantifying e.g. ventricular roundness and alignment of bulbus arteriosus) (Brijs et al., 2020; Claireaux et al., 2005; Foddai et al., 2022; Frisk et al., 2020; Perry et al., 2020; Poppe et al., 2003). Even though such quantitative morphometric measurements represent a relatively objective method for analysing morphological heart deviations, it is not necessarily able to detect the vast variation observed. In cases of extreme cardiac deviations, the use of these methods can even be misleading, as commonly used quantitative measurements of heart morphology may appear perfectly normal in a severely deviating heart. For example, in cases of situs inversus where the heart is often severely deformed, the angle quantifying alignment of bulbus arteriosus often resembles angles obtained from wild salmon. Moreover, measuring a severely deformed heart using quantitative measurements may result in more or less extreme outliers masking the true morphological variation in that cohort. Alternatively, extremely deviating hearts are excluded from the data set, resulting in an underrepresentation of the extremities found.

Third, while deviating heart morphology is typically observed in larger fish at sea, insight into cardiac morphological development and remodelling throughout life has been lacking. The fish heart is highly plastic and able to adapt to changes in the environment (Gamperl and Farrell, 2004), and it is well-known that heart size and tissue composition change throughout the life cycle (Farrell et al., 1988; Gamperl and Farrell, 2004; Poupa et al., 1974). The question of whether this remodelling is accompanied by changes in heart shape has not to date been examined. Thus, mapping the morphological development of wild salmon throughout the life cycle is necessary to evaluate deviations from normal wild-type morphology at different life stages of farmed salmon.

In cases where morphological deviations are recorded qualitatively, for example, by producers or fish health personnel, site or farm specific scoring systems are often used (personal communication). To our knowledge, no common terminology or scoring system exists for qualitative assessment of heart morphology in salmonids. This represents a serious obstacle for communication and knowledge transfer within the industry as well as between the scientific community and stakeholders.

To offer a more precise description of the pyramidal fish heart, we here present an extensively updated nomenclature on the exterior of the Atlantic salmon heart. Next, we have used this nomenclature to standardise and improve the description of existing and novel quantitative morphometric measurements of the pyramidal fish heart. Further, the nomenclature was central in establishing the qualitative method based on common morphological traits observed in farmed Atlantic salmon. This type of qualitative method should enable detection of a large variation in cardiac morphology, including heart shape and phenotypic traits overlooked by the quantitative method.

Finally, we provide examples of the applicability of the two methods while simultaneously illustrating how the methods, in combination, detect a more extensive range of morphological variation. More precisely, we show that the quantitative method is useful when comparing distinct groups of fish, here exemplified by wild and farmed Atlantic salmon in different life stages. Further, we show that whereas the quantitative method fails to detect morphological traits associated with mortality, the qualitative method allows identification of morphological traits overrepresented in fish that died following a delousing procedure.

2. Materials and methods

2.1. Sampling and imaging of hearts

Hearts were collected from farmed and wild Atlantic salmon. For all fish, intact hearts (bulbus, ventricle and atrium) were dissected out and placed in PBS containing 50 mM KCl to stop the heart from beating before it was fixed in 70% ethanol or 4% buffered formaldehyde and stored at 4 °C. All hearts were photographed as follows.

Fixed hearts were submerged in water and mounted on a pushpin glued to the bottom of a custom-made transparent plastic box placed on top of a light plate (Slimlite LED,32 × 22.8 cm, Kaiser, Buchen, Germany). The ventrodorsal (VD, Fig. 2-I) and left lateral projections (LL, Fig. 2-II) of the heart were photographed from above using a Canon Powershot SX540 HS (Canon Inc., Tokyo, Japan) and used for the morphometric analysis.

2.2. Wild and Farmed salmon for comparison between life stages

Wild Atlantic salmon hearts were sampled from southern parts of Norway ((59°11′17.1" N 9°59′34.6″ E). Wild male (M) and female (F) fry and parr were sampled in Hagnesvassdraget, a westbound tributary to Numedalslågen (Kvelde, Norway) in August 2021. A total of 24 fry (1–7 g, mean 2,92 g) and 28 (M: 17, F: 10, NA: 1) parr (5–45 g, mean 15.64 g) were caught by electrofishing and euthanised by a lethal blow to the head. A total of 44 (F: 24, M: 17, NA: 3) downstream migrating wild Atlantic salmon smolts were caught in a Wolf trap located at the entrance of the surface fish passage at Rygene hydropower plant in the River Nidelva, Agder, Norway (58.41540° N, 8.74242° E). After capture, fish were euthanised by a lethal dose of buffered MS-222 at a concentration of 2 g/L until unresponsive and motionless (within approximately 30 s) and sampled as described above. Silver adult salmon fresh off saltwater and returning upstream were sampled by fishermen in June 2021 in Numedalslågen. A total of 22 (M: 5, F: 17) salmon were sampled (1.2–8.1 kg, mean, 4.07 kg). Upstream migrating spawning adults were caught in Numedalslågen and Hagnesvassdraget, Norway in November 2021. In total of 12 (M: 6, F: 6) hearts were sampled during broodstock control and cultivation of selected individuals at the Fosnes broodstock facility at Hvittingfoss December 2021.

Farmed fry, parr, smolt and silver adults were sampled from either Matre Research Station, Institute of Marine Research, Norway or different aquaculture facilities in Western and Mid-Norway. For silver adults, the duration of sea rearing varied across locations. Spawning adults were sampled from Benchmarck genetics Salten. A total of 15 fry, 11 parr, 92 smolts, 52 silver adults and 20 spawning adults were sampled and used in the analysis.

2.3. Dead and surviving farmed salmon

A total of 120 salmon from the strain Aqua Gen QTL InnOvaGAIN were collected from two different aquaculture locations during mechanical delousing: Kråkholmen (Flatanger, Norway) and Nordgjæslingan (Rørvik, Norway) in 2019. The fish were separated into two groups: diseased ($n = 60$) or survivor (n = 60). Diseased fish were collected with a "dead fish" collector, whereas survivors were captured directly from the sea cage following delousing with a large dipnet and euthanised by a blow to the head. Only 111 hearts were used in the analyses due to missing photographs for nine samples.

2.4. Nomenclature

To standardise the anatomical terminology of the pyramid fish heart, different anatomical landmarks were assigned anatomical names based on standard nomenclature as given in Illustrated Veterinary Anatomical Nomenclature by Schaller, 1992. This nomenclature encompassed different ridges, apexes, surfaces, junctions, and grooves that results from a pyramid-shaped ventricle forming the connection between an atrium and a bulbus arteriosus (Fig. 1).

2.5. Method for quantitative measurements

Fiji (Schindelin et al., 2012) was used to obtain measurements and angles. Further, the following measurements were taken:

For Ventricular height:width ratio (HW, Fig. 2-I, A: B), on VD surface (Fig. 2-I), ventricular height (Fig. 2-I, A) was obtained by measuring a line from the caudal ventricular apex (Fig. 1-I, 7) to the ventriculobulbar groove (Fig. 1-I, 14) according to Frisk et al. (2020). This measurement is modified from Poppe et al. (2003), where ventricle height was defined as the length between ventricular apex and ventricular base. The ventricular width (Fig. 2-I, B) was obtained by measuring a line from the right dorsal ventricular apex (Fig. 1-I, 9) to the left dorsal ventricular apex (Fig. 1-I, 10). The ratio was calculated by dividing ventricular height by ventricular width.

For Bulbus width:ventricular width ratio (BVW, Fig. 2-I, C: B), on VD surface (Fig. 2-I), bulbus width (Fig. 2-I, C) was obtained by measuring the bulbus on its widest. Ventricular width (Fig. 2-I, B) was obtained by measuring a line from the right dorsal ventricular apex (Fig. 1-I, 9) to the left dorsal ventricular apex (Fig. 1-I, 10). The ratio was calculated by dividing bulbus width by ventricular width.

For Alignment of bulbus arteriosus (ABA, Fig. 2-II, A) on LL surface (Fig. 2-II), the angle "alignment of bulbus arteriosus" (Fig. 2-II, A) was obtained by drawing a line from the caudal ventricular apex (Fig. 1-IV, 7) to the atriobulbar incision (Fig. 1-IV, 16). Then a line following the midsection of the bulbus to the lines cross. Angle A (Fig. 2-II, A) was then measured according to Poppe et al. (2003).

For Ventricular symmetry (VS, Fig. 2-II, B) on LL surface (Fig. 2-II), the angle was obtained by drawing a line from the caudal ventricular apex (Fig. 1-IV, 7) to the atriobulbar incision (Fig. 1-IV, 16). Then a line from the ventriculobulbar groove (Fig. 1-IV, 15) to the left dorsal ventricular apex (Fig. 1-IV, 10 (see Fig. S1. for references of placement)). Angle B (Fig. 2-II, B) was then measured according to Frisk et al. (2020).

For Bulbus alignment (BA, Fig. 2-II, C) on LL surface (Fig. 2-II), the angle was obtained by drawing a line from the ventriculobulbar groove (Fig. 1-IV, 14) to the left dorsal ventricular apex (Fig. 1-IV, 10). Then a line from the ventriculobulbar groove (Fig. 1-IV, 14) to the atriobulbar incision (Fig. 1-IV, 16) following the ventriculobulbar junction (Fig. 1-IV, 17) was drawn. Angle C (Fig. 2-II, C) was then measured according to Frisk et al. (2020).

2.6. Method for qualitative measurement

The qualitative method was developed based on the author's extensive library of photographs of hearts from farmed Atlantic salmon collected in association with fish health surveillance and project samplings. Deviations from the wild Atlantic salmon heart morphology and other distinct morphological traits that appeared on several samples were included in the method, resulting in 38 common morphological traits (Table 1). One example of each trait is illustrated in Fig. 3, whereas the supplementary booklet provides several examples of each trait. In some example images, the atrium was removed if it covered parts of the bulbus or ventricle.

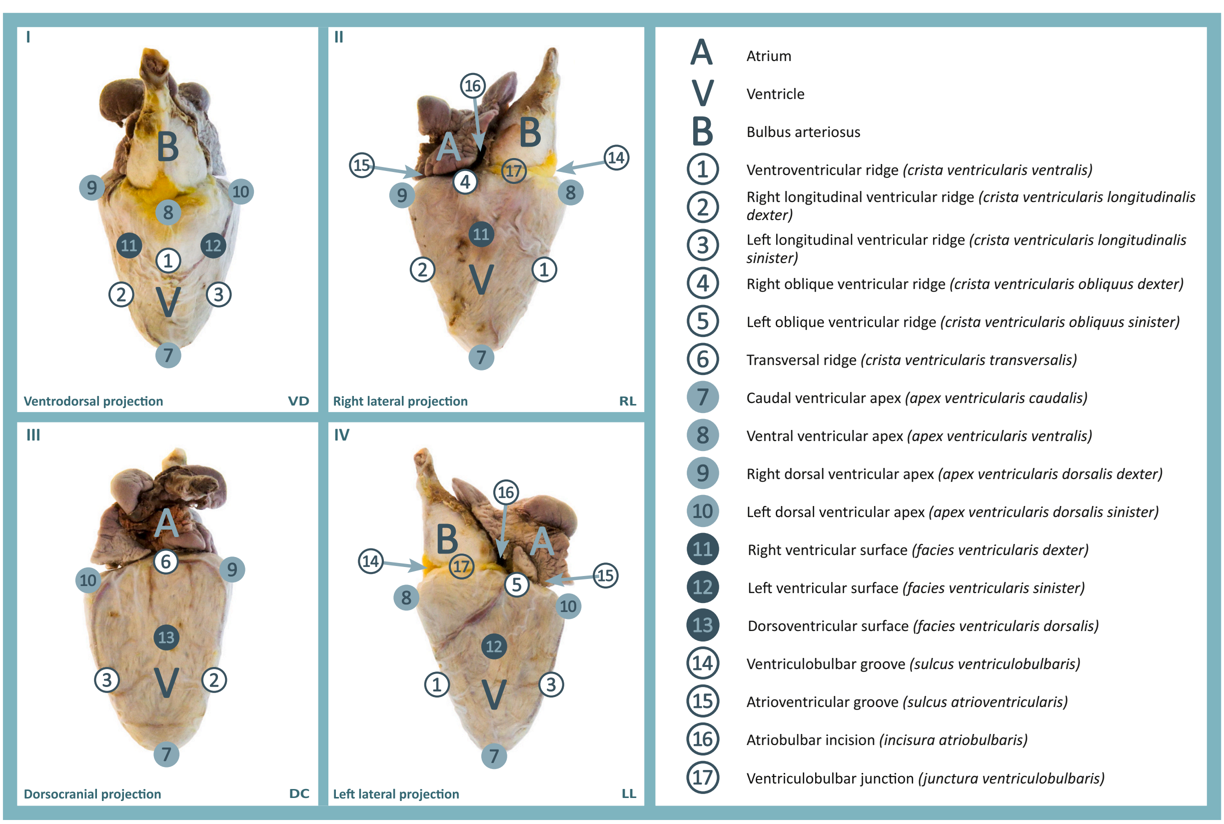

Fig. 1. Nomenclature of the Atlantic salmon heart displayed from I) ventrodorsal (VD), II) right lateral (RL), III) dorsocranial (DC) and IV) left lateral (LL) projections.

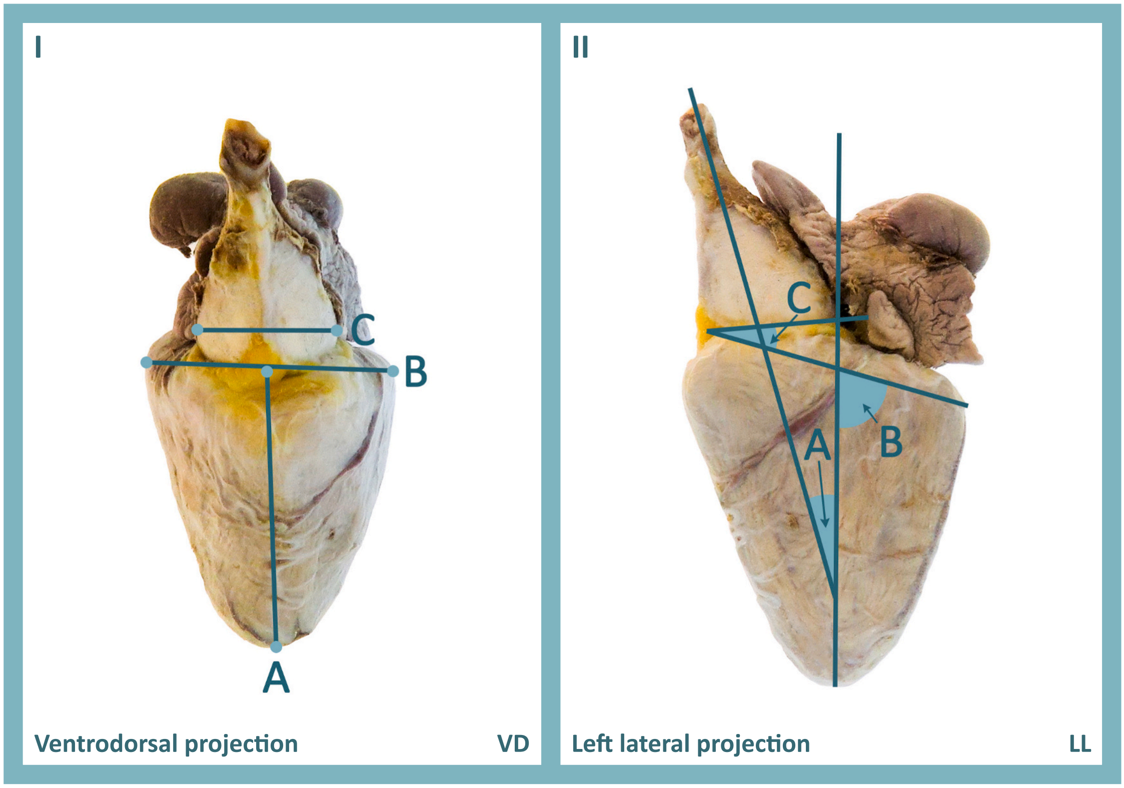

Fig. 2. Quantitative measurements of the Atlantic salmon heart for I) ventrodorsal (VD) and II) left lateral (LL) projections.

2.7. Application of the method: practical guidelines for use

Visualise the heart in the four projections (VD, LL, RL, DC, Fig. 1), either by manual inspection or photography. Go through Fig. 3 or the supplementary booklet and record each trait from the figure as either present (score 1) or non-present (score 0). One sample may possess multiple morphological traits and some traits are not specific to a projection and were marked with "AP" (any projection). The qualitative method focuses on morphological traits in the ventricle or bulbus arteriosus, however, other traits, like common fat deposit sites, were included.

2.8. Statistical analysis

RStudio software 4.3.0 (R Core Team, 2023) was used to conduct statistical analysis with the packages tidyverse, dplyr and FactoMineR (Lê et al., 2008; Wickham et al., 2019, 2023), and the package ggplot2, factoextra and corrplot was used to produce figure (Kassambara and Mundt, 2020; Wei and Simko, 2021; Wickham, 2016).

To ensure the validity of the subsequent analyses, the data were initially subjected to a Shapiro-Wilk test for normality and an F-test to examine the homogeneity of variances. In cases where data displayed a normal distribution and homogeneity of variance, a two-sample *t*-test was employed. When data did not adhere to the assumption of normality, the Wilcoxon rank sum exact test was used as a non-parametric alternative. Additionally, the Bonferroni correction was applied to adjust the *p*-values.

Two-sample t-test was used to analyse the mean between wild and farmed salmon for each life stage (Table S1). To control possible sex-specific differences in morphology, sexual dimorphism was investigated by a two-sample t-test in wild parr (Male $n = 17$, Female $n = 10$), smolt (Male $n = 17$, Female $n = 23$), and wild spawning adult (Male $n = 6$, Female $n = 6$). Fry and silver adults were excluded from the analysis due to the inability to determine sex or low sample size of males, respectively. Since these analyses did not reveal any sexual dimorphism (Table S2) sex was not included as a variable in subsequent statistical analyses.

Principal component analysis was performed to visualise the morphological variation between deceased and surviving farmed salmon after delousing. A cluster analysis plot was generated to illustrate the clustering of the two groups.

A correlation between five testers of the qualitative method was conducted to test the robustness of the method. A total of 111 hearts from two groups: dead and survivor, were used. The sample identification was blinded, and each heart scored 1 for a present trait and 0 for a non-present trait in Fig. 3. Multiple traits were allowed for each heart. Analysis was done by conducting a tetrachoric correlation.

A chi-square and Fisher's exact test were conducted on hearts scored with the qualitative method on the two groups: dead and survivor (Table S3). Traits similar to each other were pooled together before conducting the analysis.

To visually explore the relationships between different qualitative traits, a correlation heatmap was generated using the statistical software Python 3.7, specifically leveraging the Seaborn (Waskom, 2021) and Pandas (McKinney, 2010) libraries. From the initial dataset, traits with a frequency of score "1" exceeding 5% were selected for analysis. For these traits, Pearson correlation coefficients were computed between all possible pairs to assess their linear relationships.

3. Results and discussion

3.1. Heart morphology differs between wild and farmed salmon at all life stages

Hearts of wild and farmed salmon at different life stages were compared using the quantitative method (Fig. 2). Sexual dimorphism was investigated in wild female and male parr (M = 17, F = 10), smolt (M = 17, F = 23) and spawning adults (M = 6, F = 6) for every measurement. There was no significant difference between males and females for any measurements (Table S2).

Table 1
Description of qualitative morphological traits found in Atlantic salmon. The hashtag corresponds to the number in Fig. 3. The number in brackets corresponds to the nomenclature found in Fig. 1.

#	Name	Description (Numbers in bracket refer to the nomenclature in Fig. 1).
2	Ventricle curved right	In VD projection; Inward curvature on ventricle in right ventricular surface (11). Caudal ventricular apex (7) shifted right may or may not occur.
3	Ventricle curved left	In VD projection; Inward curvature on ventricle in left ventricular surface (12). Caudal ventricular apex (7) shifted left may or may not occur.
4	Triangle ventricle	In VD projection; A triangle shape to the ventricle where the caudal ventricular apex appears in a clear corner. The length between caudal ventricular apex (7), right dorsal ventricular apex (9) and left dorsal ventricular apex (10) are approximately uniform.
5	Short ventricle height axis	In VD projection; The length from the ventriculobulbar groove (14) to the caudal ventricular apex (7) is distinctly shortened.
6	Ball-shaped ventricle	In VD projection; Ventricle appears round, caudal ventricular apex (7), right dorsal ventricular apex (9), and left dorsal ventricular apex (10) have less clear corners.
7	Tube-shaped ventricle	In VD projection; The ventricle appears elongated and narrow. Narrowing of left and right ventricular surface creating a tube-like shape.
8	Bulboventricular junction shifted right	In VD projection; Bulbus position on the ventricle shifted to the right side of the ventricle. The entire bulbus may be shifted or a ballooning only towards the right side of the ventricle may occur.
9	Bulboventricular junction shifted left	In VD projection; Bulbus position on the ventricle shifted to the left side of the ventricle. The entire bulbus may be shifted or a ballooning only towards the left side of the ventricle may occur.
10	Bulbus curved right	In VD projection; Bulbus curved right.
11	Bulbus curved left	In VD projection; Bulbus curved left.
12	Fat deposit on ventroventricular ridge	In VD projection; Fat deposit on ventroventricular ridge (1). Fat beyond the ventroventricular ridge - see trait 38.
14	Low left dorsal ventricular apex	In LL projection; Left dorsal ventricular apex (10) lowered beyond the ventral ventricular apex (8).
15	Extended left dorsal ventricular apex	In LL projection; Left dorsal ventricular apex (10) extended upwards, extended beyond the ventral ventricular apex (8).
16	Flat caudal ventricular apex	In LL projection; Caudal ventricular apex (7) appear flat.
17	Ventroventricular ridge curved inwards	In LL projection; Inward curvature of the ventroventricular ridge (1).
18	Narrowing of the ventricle at ventriculobulbar junction	In LL projection; The ventricle either appears pinched at atriobulbar incision (16) and between ventroventricular ridge (1) and ventral ventricular apex (8), resulting in a narrowing of the ventricle at ventriculobulbar junction (17). The axis from atriobulbar incision (16) to left dorsal ventricular apex (10) appears horizontal or tilted downwards towards left dorsal ventricular apex (10).
19	Bulbus positioned flat on left oblique ventricular ridge	In LL projection; Left oblique ventricular ridge (5) appears horizontal and bulbus is attached on top of ventricle in a similar manner as in wild salmon, differing from typical farmed salmon heart where the position of bulbus is skewed on ventricle. The three axis running from 7 to 8, 7–16 and 7–10 (nomenclature) are almost equally long.
20	Triangle ventricle	In LL projection; A triangle shape to the ventricle where the caudal ventricular apex appear in a clear corner. The length between caudal ventricular apex (7), right dorsal ventricular apex (9) and left dorsal ventricular apex (10) are approximately uniform.
21	Short ventricle	In LL projection; The length from the ventriculobulbar groove (17) to the caudal ventricular apex (7) is distinctly shortened.
22	Elongated and narrow ventricle	In LL projection; ventricle is elongated and narrow. Shortened length between left dorsal ventricular apex (10) and ventral ventricular apex (8).
23	Bell-shaped ventricle	In LL projection; The shape of the ventricle resembles a bell. Length from ventral ventricular apex (8) to caudal ventricular apex (7) is shortened or/and the left dorsal ventricular apex (10) lowered. Caudal ventricular apex (7) appears rounded.
24	Skewed bulbus	In LL projection; Bulbus sits in a obvious skewed position on the ventricle at the ventriculobulbar groove (14).
25	Large bulbus compared to ventricle	In LL projection; Bulbus appears large compared to the ventricle
26	Ballooning bulbus	In LL projection; Bulbus is ballooning, meaning that bulbus or parts of bulbus appear large and distended.
27	Constricted bulbus	In LL projection; Bulbus is clearly constricted, either by size, positioning or curvature.
28	Bulbus curved dorsally	In LL projection; Bulbus curved dorsally.
29	Bulbus curved ventrally	In LL projection; Bulbus curved ventrally.
30	S-shaped bulbus	In LL projection; Bulbus curved in a S-shaped formation.
32	Extended right dorsal ventricular apex	In RL projection; Right lateral ventricular apex (9) extended upwards beyond ventral ventricular apex (8).
33	Extremely skewed bulbus and high dorsal ventricular apex	In RL projection; Right lateral ventricular apex (9) extended upwards, and bulbus positioned extremely skewed on ventricle.
35	Fibrosis on dorsoventricular surface	In DC projection; fibrosis on dorsoventricular surface (13).
36	Extended right dorsal ventricular apex	In DC projection; Right lateral ventricular apex (9) extended upwards beyond left lateral ventricular apex (10), alternatively with retracted left lateral ventricular apex
37	Extensive atrium	In AP projection; Atrium appears extensive
38	Extensive fat	In AP projection; Extensive fat deposits in the epicardium. Multifocal or diffuse fat extending beyond the ventriculobulbar junction (17) and ventroventricular ridge (1).
39	Extremely deviating heart morphology	In AP projection; Heart morphology that appears extremely deviating and does not fall into the other categories.
40	Fat deposit on dorsal ventricular apex	In AP projection; Fat deposit on left dorsal ventricular apex (10) or right dorsal ventricular apex (9).
41	Fat deposit on caudal ventricular apex	In AP projection; Fat deposit on caudal ventricular apex (7).
42	Deviating coronary vessels	In AP projection; Coronary vessels that clearly deviate from normal structure.

The heart morphology of wild and farmed salmon at different life stages is illustrated in Fig. 4. The quantitative method revealed clear differences in heart morphology between these groups at all life stages (Fig. 5). Consistent with previous findings, farmed salmon ventricles were generally rounder than their wild counterparts, as indicated by smaller ventricular height-width ratios (HW, Fig. 5A). For example, Poppe et al. (2003) showed that farmed rainbow trout and Atlantic salmon had rounder ventricles than their wild counterparts. Whereas the Atlantic salmon specimens examined in the study of Poppe et al. (2003) consisted of wild spawning (0.5–6 kg) adults and silver adults (3–5 kg), our study also includes wild and farmed specimens at earlier life stages. In our material, including salmon at the fry, parr and smolt stages, HW was lower in farmed than in wild salmon at all life stages except for at the fry stage (Fig. 5A).

These results indicate that farmed Atlantic salmon seem to develop a rounder ventricle already at the parr stage, and the ventricle appears to become progressively rounder throughout life. In contrast, the more elongated ventricular phenotype observed in wild salmon appears to persist throughout life, and if anything, the ventricle tends to become more elongated with age.

Relative bulbus size was also different between wild and farmed salmon, where farmed smolt and spawning adults displayed a larger bulbus-ventricular width ratio (BVW) compared to their wild counterparts (Fig. 5B). Relative bulbus size is a novel quantitative trait not

Fig. 3. Qualitative method illustrated by A) images and B) sketches of morphological cardiac traits that can be identified from ventrodorsal (VD), left lateral (LL), right lateral (RL), dorsocranial (DC) or any (AP) projections (see Supplementary Fig. 2. and 3. For larger format).

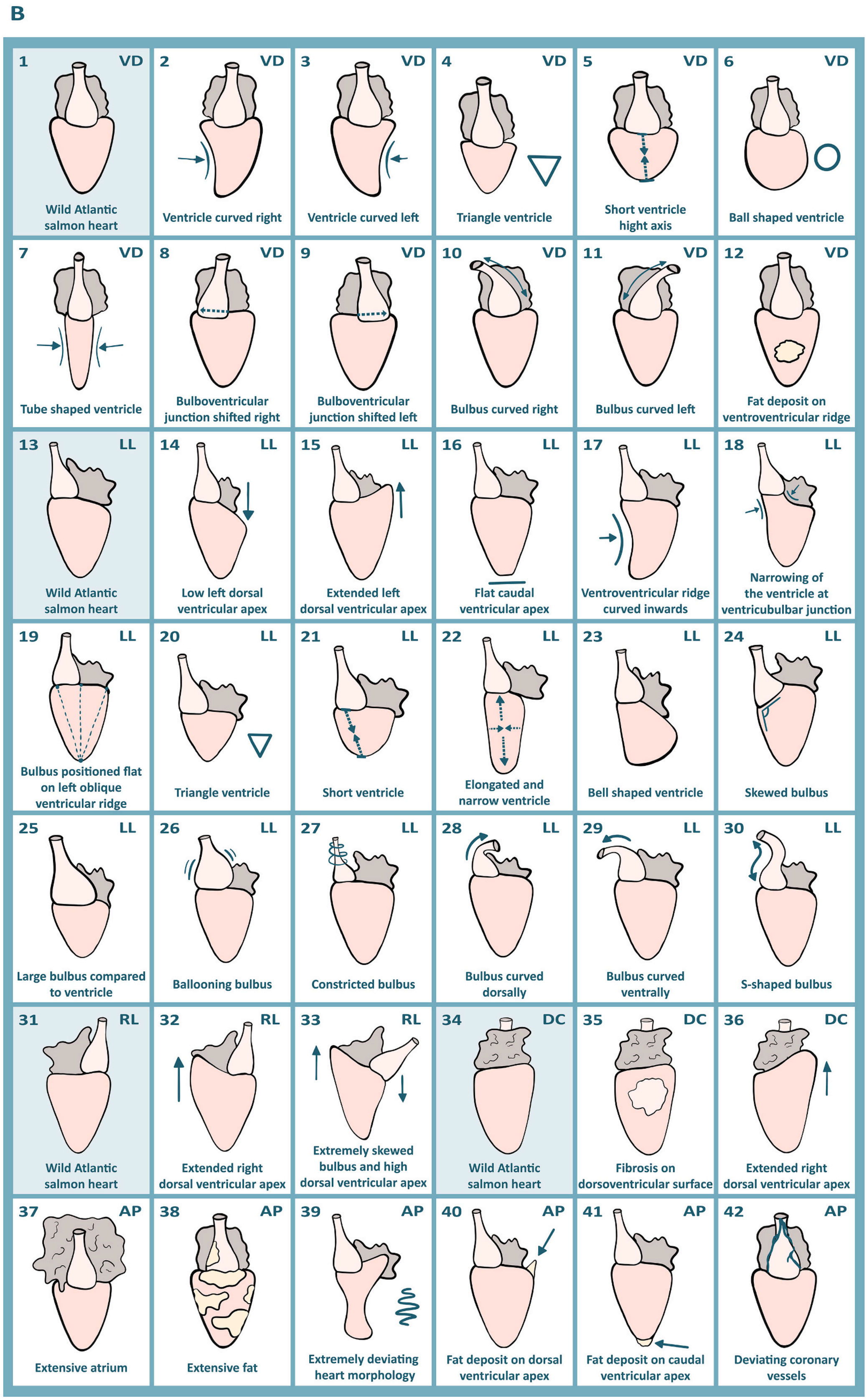

Fig. 3. (*continued*).

previously compared between wild and farmed salmon. The positioning of the bulbus has, however, previously been shown to differ between wild and farmed salmon. More precisely, Poppe et al. (2003) showed that farmed salmon have more misaligned bulbi, as indicated by an increased angle between the ventricular and bulbar axes (alignment of bulbus arteriosus, ABA). We observed the same phenomenon in our

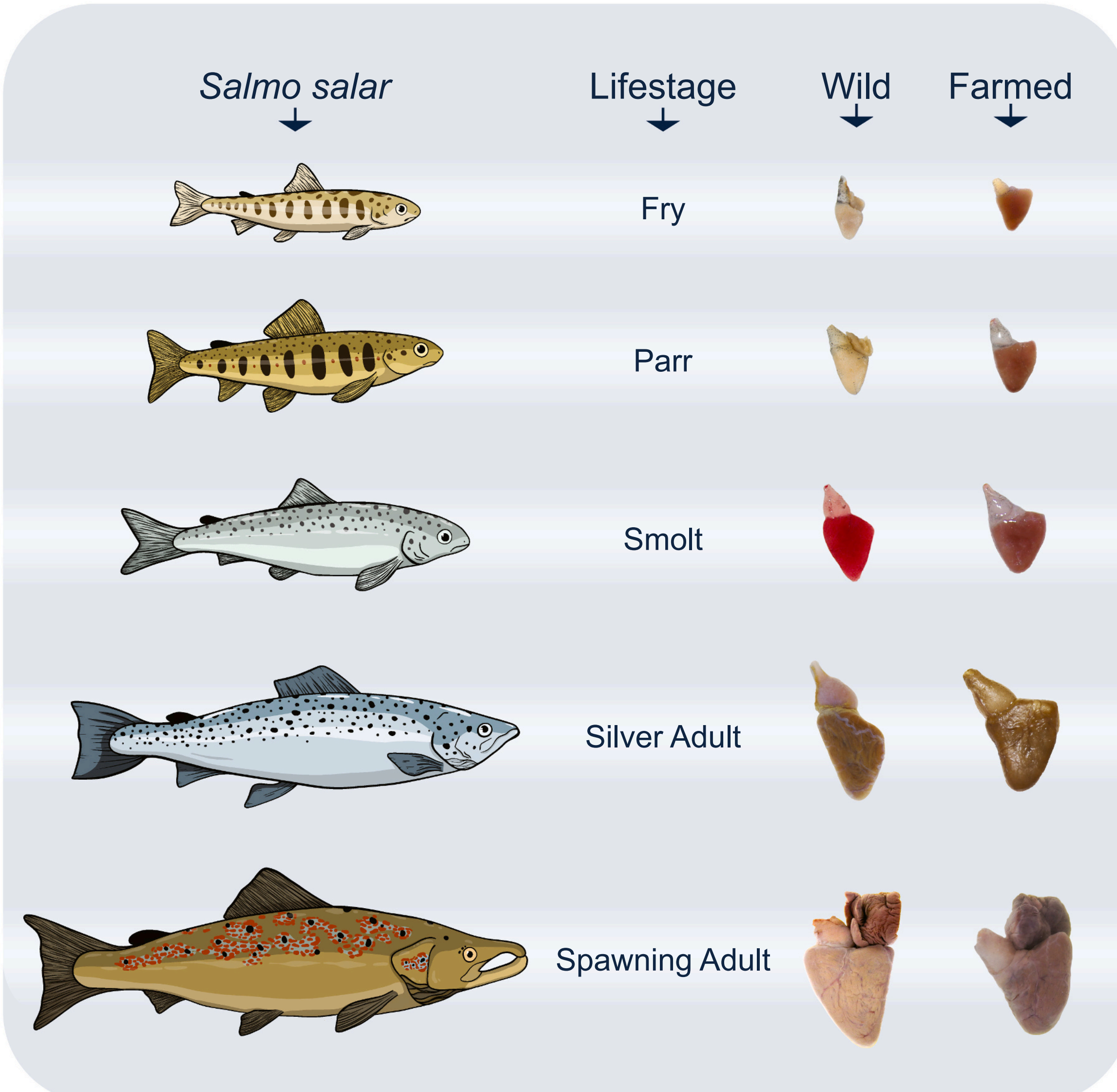

Fig. 4. Illustration of typical wild and farmed Atlantic salmon hearts at different life stages.

samples and add that this difference is apparent already at the fry stage (Fig. 5C).

Morphological traits related to symmetry of the ventricle have not been extensively studied in Atlantic salmon (Frisk et al., 2020; Kristensen et al., 2012). Using the quantitative measurement described here, a larger ventricular symmetry angle (VS) in farmed salmon suggests remodelling of the ventricle which manifests as an extension of the left dorsal ventricular apex. We observed that the ventricles from farmed salmon have a different symmetry (extension of left dorsal ventricular apex, Fig. 1. IV. 10) than those of wild salmon. This difference is apparent already at the fry stage and persists throughout life (Fig. 5D). Another quantitative measurement of bulbus alignment (BA) that incorporates bulbus position in relation to the left oblique ventricular ridge was introduced by Frisk et al. (2020). Through the life cycle, this angle was either larger (fry stage and silver adults), smaller (parr) or not different (smolt and spawning adult) in farmed compared to wild salmon (Fig. S4). Since this angle to a certain extent depends on both the VS and ABA, the relationship between these two angles and how they develop throughout the life cycle may explain this inconsistency. Of note, the developmental pattern of this measure is more difficult to interpret compared to the other quantitative measurements indicating that this angle is perhaps less useful when comparing morphological development in these groups. Overall, our observation on cardiac morphological development in farmed and wild salmon contribute to previous notions that cardiac morphology of farmed salmon is distinct from that of wild salmon (Poppe et al., 2003). We present a more comprehensive comparison of different life stages and discovered that morphological differences between the two groups appear already at an early life stage. Future studies on causes of morphological deviations in farmed salmon could therefore benefit from investigating early heart development and how environmental cues in early life contribute to morphological remodelling of the salmon heart. Compared to wild Atlantic salmon, hatchery-reared salmon are raised at elevated temperatures and often under continuous light to accelerate growth. Indeed, elevated temperature and accelerated growth in freshwater have been linked to the development of morphological deviations in farmed Atlantic salmon (Frisk et al., 2020) and rainbow trout (Brijs et al., 2020) alike. All these factors could potentially impact cardiac morphology. Further, the differences observed already at the fry stage indicate that morphological deviations of the heart may occur during early stages of salmon production, perhaps even during embryological development. Because it is well known that extreme cardiac abnormalities, including a missing septum transversum can result from elevated temperature during egg incubation (Nathanailides et al., 1995; Ørnsrud et al., 2004;

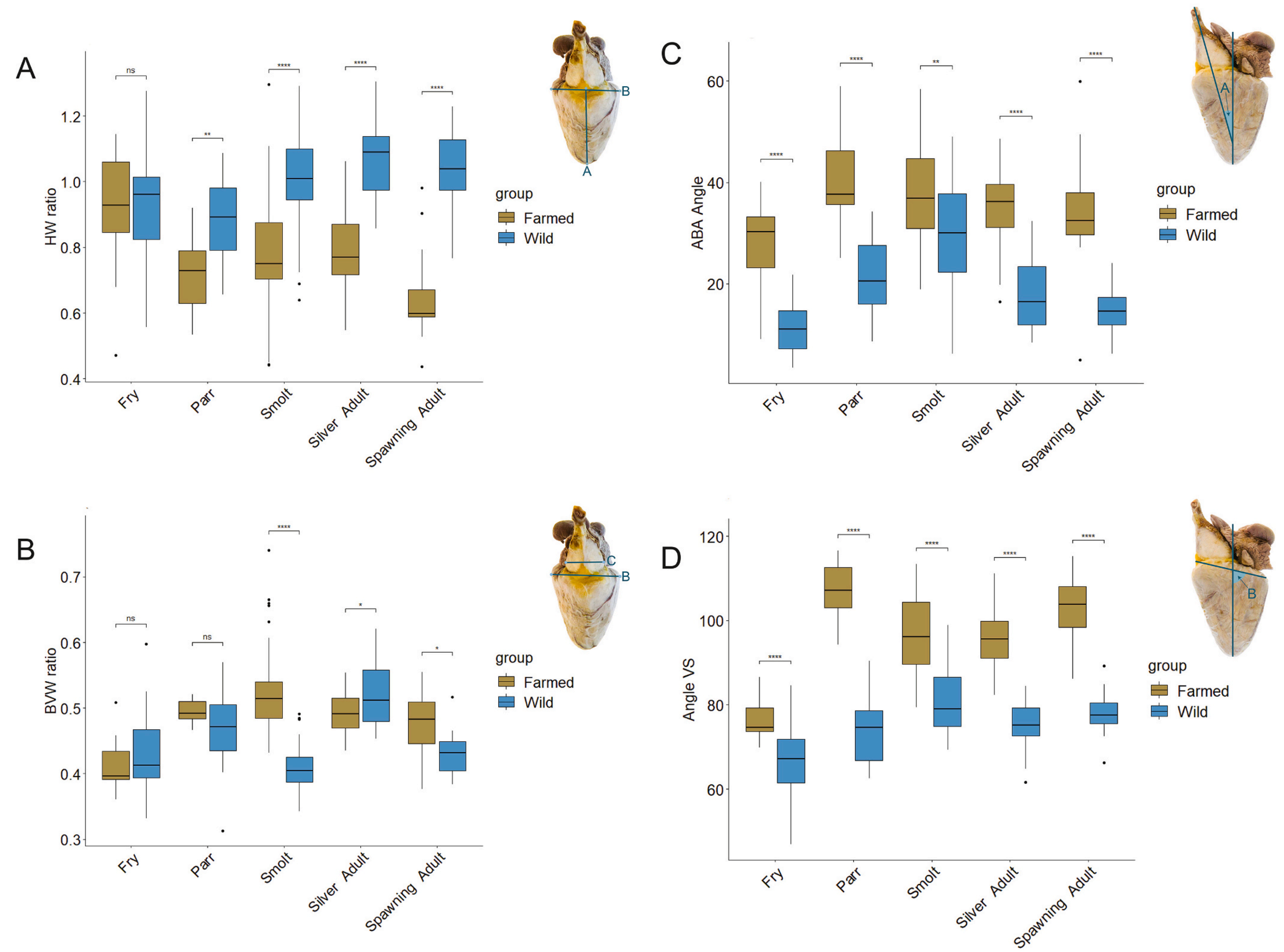

Fig. 5. A boxplot illustrating differences in A) Ventricular height:width (HW) ratio, B) Bulbus width:ventricle width (BVW) ratio, C) alignment of bulbus arteriosus (ABA) angle and D) Ventricular symmetry (VS) angle between wild and farmed Atlantic salmon at different life stages. Statistical differences at different life stages were tested using two sample *t*-test with Bonferroni correction for multiple testing. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

Takle et al., 2005), studies examining the effects of embryonic temperature on more moderate morphological deviations are needed.

Lastly, these results also illustrate the applicability of the quantitative method and highlight the importance of including morphometrics that are more complex than measurements of heart size (e.g. cardiosomatic index), commonly used in the field. This method may be particularly favourable when distinguishing between groups, such as wild and farmed fish, that experience vastly different lives. Of note, the presented results are based on a limited material from a restricted geographical area and should be interpreted with some care. For instance, we cannot exclude that heart morphology in wild salmon vary across different geographical locations.

3.2. Morphological differences between mortalities and survivors following delousing

Concerns that cardiac morphological deviations contribute to mortality during stressful operations were raised decades ago (Poppe et al., 2003) but very little empirical evidence exists to support these speculations. Here, we used quantitative and qualitative methods to assess morphological differences in the hearts of fish that died or survived following four mechanical delousing events at three different sea locations. This was done to 1) identify morphological traits associated with mortality, and to 2) test the applicability of the two methods in risk assessment. It is important to note that the cause of death was unknown in this material. Thus, the true difference in heart morphology between survivors and individuals dying from cardiac failure are likely larger than detected here.

Using the quantitative method on the pooled material, there were no significant differences in any of the quantitative traits measured between the groups (HW: $t = 0{,}81$, df $= 108$, $p = 0{,}42$; BVW: t $= 0{,}62$, df $= 108$, p $= 0{,}54$; ABA: $t = 1{,}34$, df $= 108$, p $= 0{,}18$; VS: t $= 0{,}93$, df $= 108$, p $= 0{,}36$; BA: t $= 0{,}42$, df $= 108$, p $= 0{,}68$.). In addition, a cluster plot of the PCA illustrates that the two groups largely overlap (Fig. 6) with no distinguished clusters. If plotted separately, it is however noteworthy that hearts from the different locations cluster somewhat differently (Fig. S11), illustrating that cardiac morphology may differ from location to location. Moreover, when comparing mortalities and survivors following separate delousing events (1–4), HW was lower in mortalities compared to survivors following event no. 3 (Fig. S5.) and VS was higher in survivors compared to mortalities following event no. 2 (Fig. S8). Of note, a clinical CMS outbreak was confirmed at event no. 3, and the material from this event has been used in a previous publication linking cardiac morphology to CMS susceptibility (Frisk et al., 2020). Our combined data do not support that rounded ventricles are associated with mortality in general but rather with CMS-related mortality.

Based on comparisons of quantitative traits on the pooled material, we suspect the quantitative method may have limited applicability in identifying morphology associated with mortality in supposedly similar cohorts. We can, however, not exclude that the quantitative method can be used to detect morphological traits associated with mortality on a larger scale. One reason for that is the limited sample size in our material. In addition, the cause of death was unknown and may have been unrelated to heart health. However, if this method requires a large sample size to detect traits associated with mortality, it may have limited applicability as a risk assessment tool.

In addition, the quantitative method does not necessarily reflect the true variation in heart morphology in a given material and as a result we tested this material using the qualitative method (Fig. 3).

Closely related traits were pooled before analysis. Firstly, trait no. 17 (ventroventricular ridge curved inwards) was overrepresented in mortalities on the pooled material (Fig. 7. A) and in event no. 4 (Table S3). In these hearts, the entire or parts of the ventricle is curved, which likely impacts blood flow from the caudal ventricular apex to the bulbus. Functional studies are necessary, however, to confirm this hypothesis.

When combining traits no. 18 (narrowing of the ventricle at ventriculobulbar groove) and 19 (bulbus positioned flat on LOV ridge), both related to bulbus positioning and ventricular characteristics at the ventroventricular junction, these traits were overrepresented in mortalities in the pooled material (Fig. 7. B), as well as in events no. 2 and no. 4 (Table S3). It was observed that in two events (2 and 4), the trait no. 24 (Skewed bulbus) was overrepresented in survivors compared to mortalities (Table S3). This finding was also the case for the pooled material (Fig. 7. C).

One feature that differentiates hearts scored with trait no. 24 from those scored with traits 18 and 19 is the orientation of the axis extending from the atriobulbar incision (Fig. 1. IV. 16) to the left dorsal ventricular apex (Fig. 1. IV. 7). In hearts with trait 24, this axis tilts upward, whereas, in hearts with traits 18 and 19, it's either horizontal or tilted downward. These findings suggest that a ventricular structure with a horizontal or downward-tilting axis might be correlated with a higher risk of mortality. On the other hand, an upward-tilting axis may reflect adaptive remodelling of the ventricle which could decreases mortality risk. It's worth noting that hearts possessing trait no. 24 frequently exhibit a large VS angle when measured quantitatively, in contrast to hearts with traits no. 18 or 19 which often present with a smaller VS

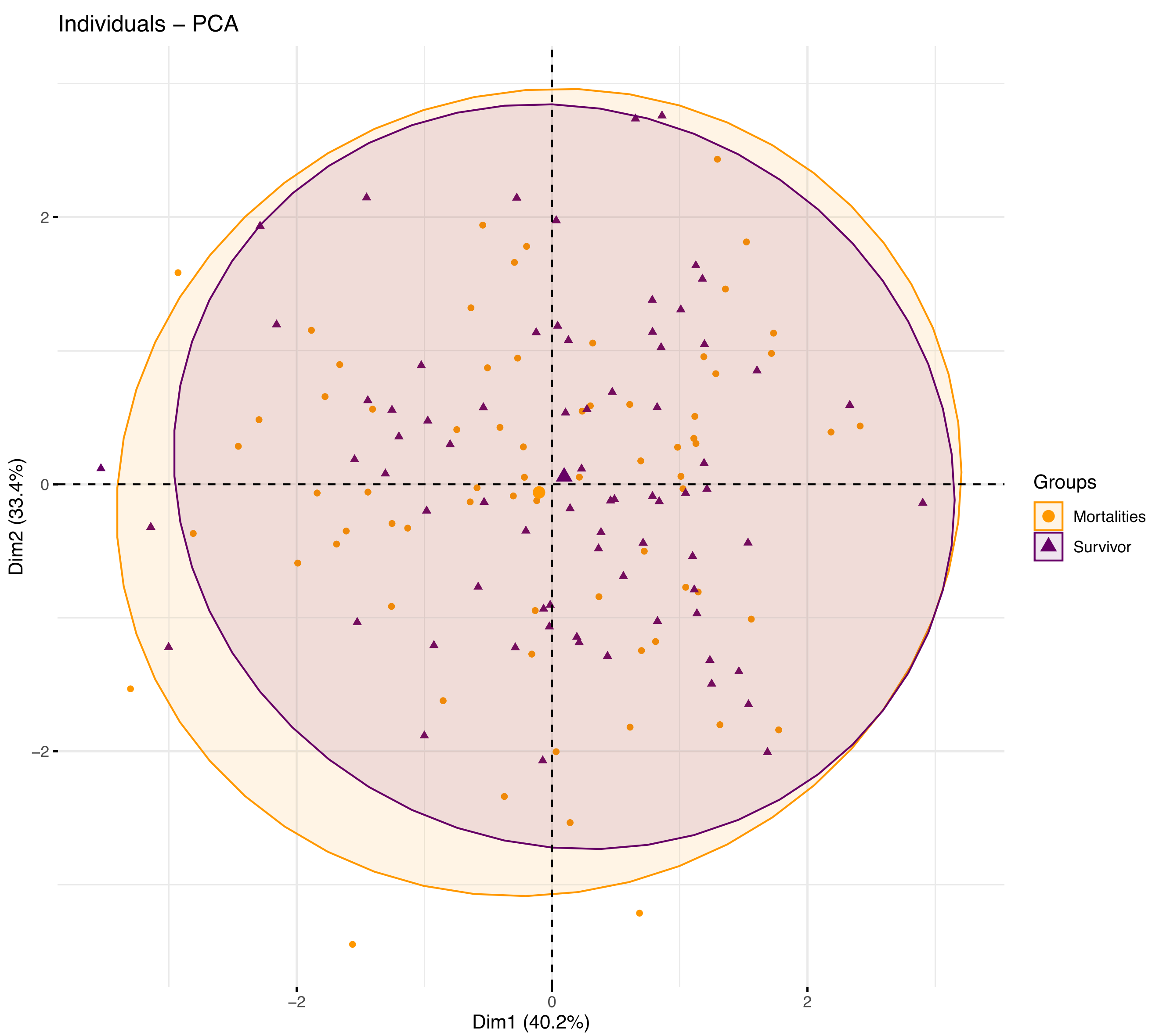

Fig. 6. A cluster plot of the principal component analysis (PCA) on the pooled material for the purpose of illustrating clustering of fish that died (Mortalities, red circle) or survived (Survivor, blue triangle) following four mechanical delousing events at three different commercial sea locations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

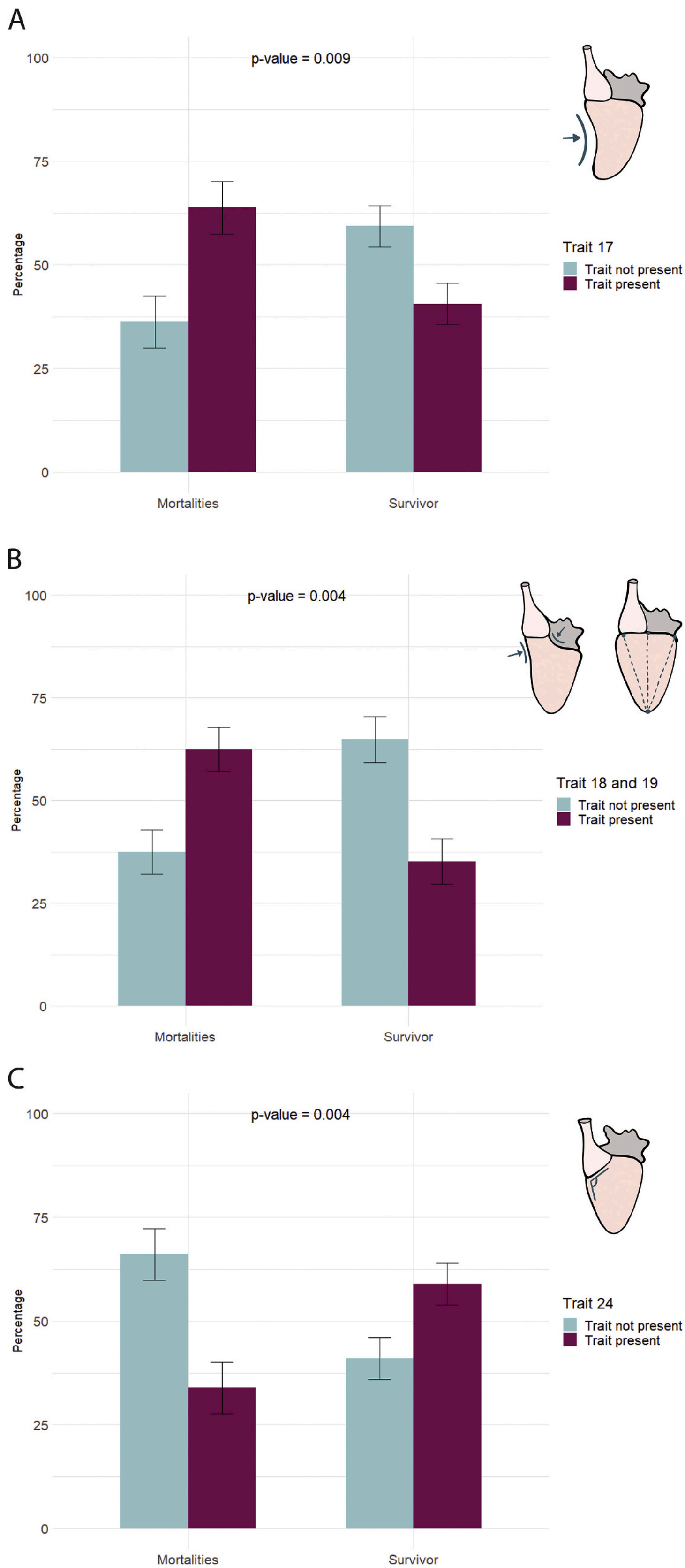

Fig. 7. Presence or absence (in percent) of qualitative morphological traits number A) 17 (Ventroventricular ridge curved inwards), B) 18 (Narrowing of the ventricle at ventriculobulbar junction) and 19 (Bulbus positioned flat on left oblique ventricular ridge), and C) 24 (Skewed bulbus) in mortalities and survivors possessing. Statistical differences were tested by chi-square and Fisher's exact tests.

angle. This is in accordance with quantitative measurements showing that survivors in event no. 2 exhibited larger VS angles compared to mortalities (Fig. S8).

It is noteworthy that no morphological traits were consistently overrepresented in either group at any events. Morphology typically varies from location to location, as illustrated in our material (Fig. S10) and as such the same morphological deviations will not necessarily develop at all locations, and separate locations may suffer from distinct deviations. Adding to this complexity, one morphological deviation may be more benign at one point in the production cycle, but if it inflicts increased strain on the heart, it may become a clinically important deviation later in the production cycle.

To confidently establish morphological traits as either risk factors or compensatory, qualitative scoring of a considerably larger material is imperative. Future endeavours to establish morphological risk factors should also strive to identify cardiac failure as the cause of death. Coming studies should also strive to better understand the physiological importance of the varying morphological deviations. Indeed, deviations that appear anatomically severe, may have limited effects on function and vice versa. In addition, certain traits could be correlated with each other. As illustrated in the correlation heatmap (Fig. S11) different traits show varying degree of correlation. Some of the stronger positive associations are likely related to the same traits being detected in different projections (e.g., traits related to short ventricles; 4 + 5 and 20 or 21), or because some traits are related. For example, a heart with a skewed bulbus (trait 24) will typically also have an extended left dorsal ventricular apex (trait 15). Apart from such positively associated traits, few strong positive correlations were observed. Still, even weak correlations could have anatomical and/or physiological significance and future studies should aim to elucidate how one anatomical trait contribute to development of another.

Nonetheless, this study supports that morphological deviations of the salmonid heart are indeed associated with mortality, and efforts to identify causes of morphological deviations in farmed salmonids should be made.

The current study demonstrates the potential for how this method can be used in risk assessment prior to planned operations commonly associated with mortality. It also illustrates how morphological deviations overlooked by quantitative methods can be detected when combining the two methods presented here. Hopefully, this qualitative method can also serve as a tool to understand both causes and consequences of heart deformities in salmon. It is relatively easy and inexpensive to use and offers a standardized protocol for recording and monitoring heart morphology.

The collection of qualitative traits included in the scoring system is expected to largely cover the variation in cardiac deformities present in Norwegian farmed salmon. Of note, although our library of photographs was extensive and consisted of heart images from farmed salmon collected from multiple production areas in Norway, we recognise that our material is not large enough to guarantee the inclusion of all existing deviations.

Since the method is based on subjective assessment of heart morphology, some user training is required to achieve reliable results. To test reproducibility among different observers, we correlated the test results of five (three relatively experienced and two inexperienced) observers.

We found an overall correlation between all the observers when pooling all traits (Figure 8). When assessing isolated traits (Table S4), good agreement among observers was achieved for most traits (correlation coefficients >0.7 or between 0.7 and 0.5). For some traits,

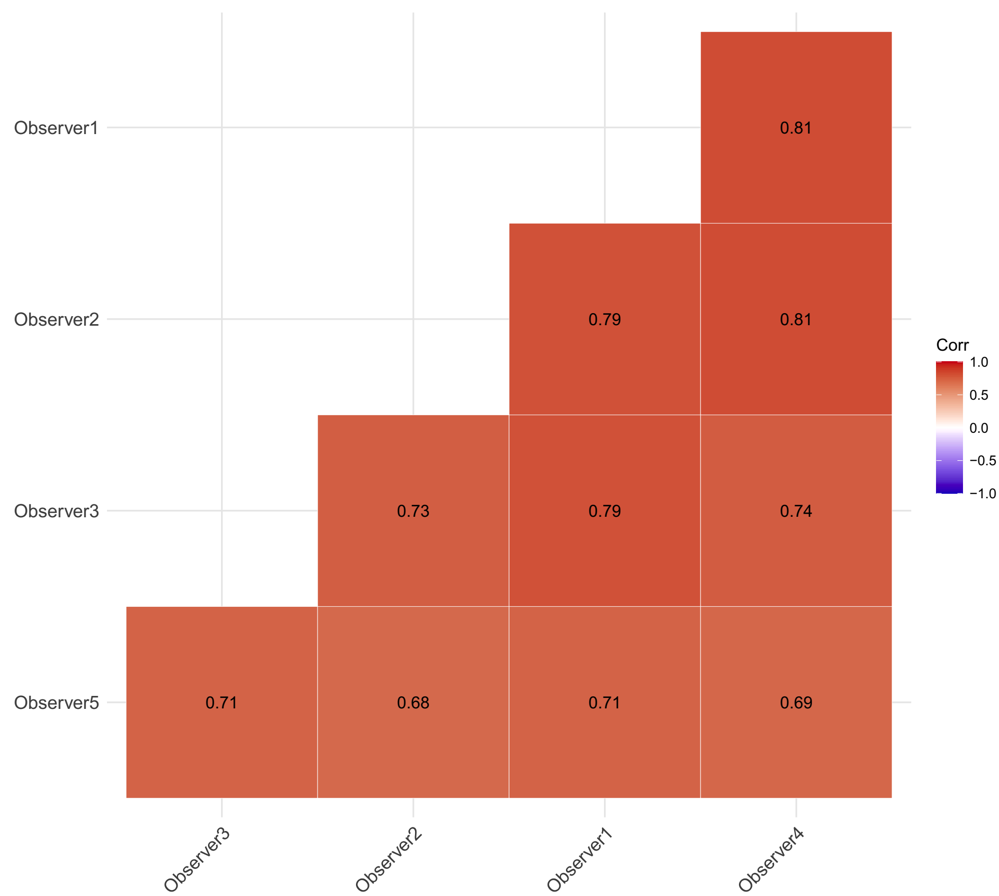

Fig. 8. Correlation coefficients between five observers using the qualitative method to score cardiac phenotypic traits on a material consisting of images of hearts collected from farmed Atlantic salmon at three different sea locations in Norway.

however, correlations between observers were poorer (correlation coefficients <0.5), suggesting that not all traits are equally recognizable. Interestingly, there was a higher correlation between the three observers (observer 1,2 and 4, Figure 8) who were experienced users of the method compared to the inexperienced observers (observer 3 and 5, Figure 8). Combined with the poor correlations observed for certain morphological traits, this suggests that training is necessary before using the method.

4. Conclusion

This study provides updated nomenclature and methodology for collecting morphological data on pyramidally shaped teleost hearts that exceed common standardized measurements (e.g. cardiosomatic index). We have assembled and unified previously published quantitative methods and used the updated nomenclature to standardise method descriptions. Further, we created a qualitative method that enables detection of morphological traits overlooked by the quantitative method. In combination, the two methods can explain a large fraction of the cardiac morphological variation observed in populations of wild and farmed salmonids. Whether deviating heart morphology is caused by heritable factors, environmental factors or others, these tools offer a way of systematising heart health in a new way that we believe can be valuable for the salmonid aquaculture.

Using the quantitative method, we confirmed well-known morphological differences between farmed and wild Atlantic salmon and revealed that these differences are present already in early life stages. Thus, future efforts to understand the causes of morphological deviations should focus on heart development during early production. Further, we identified certain qualitative morphological traits associated with mortality, illustrating its potential as a risk assessment tool.

In summary we found that combining the qualitative and quantitative approaches is recommended to gather sufficient information on morphological deviations, strengthening the method for elucidating true variation in morphological traits. The quantitative method is more objective, suitable for describing morphological development over time and differentiating distinctly contrasting groups (e.g. wild vs. farmed). On the other hand, the qualitative method is superior in detecting extreme deviations and reveals traits overlooked by the quantitative method. Together with the detailed anatomical description of the heart, these tools may represent an important step to understand and eventually mitigate heart deformities in farmed salmonids, which is fundamental for improving animal welfare and ensuring sustainable growth in aquaculture.

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Author contributions

I.B.J., H.R. and A.S.D.: Conceptualization, I.B.J. and K.H.: Project administration, I.B.J. and K.H.: Funding acquisition, V.A.E., A.S.D., H. K., M.F., H.R. and I.B.J.: Investigation, V.A.E., A.S.D., H.T., M.F., K.H., H.R. and I.B.J.: Resources, V.A.E. and I.B.J.: Data curation, V.A.E., A.S. D., H.K., H.R. and I.B.J.: Methodology, V.A.E., A.S.D., M.F., H.R. and I.B. J.: Formal analysis, V.A.E.: Visualization, V.A.E. and I.B.J.: Writing, original draft, V.A.E., A.S.D., H.K., M.F., H.T., K.H., H.R. and I.B.J.: Writing, review and editing.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used OpenAIs ChatGPT, an AI language model to improve readability of parts of the manuscript. After using this tool, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2023.740046.

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