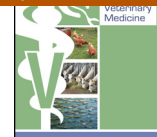




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Associations between piscine reovirus infection and life history traits in wild-caught Atlantic salmon *Salmo salar* L. in Norway

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ABSTRACT

Piscine Reovirus (PRV), the putative causative agent of heart and skeletal muscle inflammation (HSMI), is widely distributed in both farmed and wild Atlantic salmon (*Salmo salar* L.) in Norway. While HSMI is a common and commercially important disease in farmed Atlantic salmon, the presence of PRV has so far not been associated with HSMI related lesions in wild salmon. Factors associated with PRV-infection were investigated in returning Atlantic salmon captured in Norwegian rivers. A multilevel mixed-effect logistic regression model confirmed clustering within rivers and demonstrated that PRV-infection is associated with life-history, sex, catch-year and body length as a proxy for sea-age. Escaped farmed salmon (odds ratio/OR: 7.32, $p < 0.001$) and hatchery-reared salmon (OR: 1.69 $p = 0.073$) have higher odds of being PRV-infected than wild Atlantic salmon. Male salmon have double odds of being PRV infected compared to female salmon (OR: 2.11, $p < 0.001$).

Odds of being PRV-infected increased with body-length measured as decimetres (OR: 1.20, $p = 0.004$). Since body length and sea-age are correlated ($r = 0.85$ $p < 0.001$), body length serves as a proxy for sea-age, meaning that spending more years in sea increases the odds of being PRV-infected.

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1. Introduction

Infectious diseases are major constraints for the Atlantic salmon industry and thus a significant area of research in most salmon producing countries. The possible role of infectious diseases in the decline of many wild Atlantic salmon (*Salmo salar* L.) populations is a less developed field of research (Raynard et al., 2010).

Piscine reovirus (PRV) has been described as the putative causative agent of heart and skeletal muscle inflammation (HSMI) and is prevalent in both farmed and wild Atlantic salmon in Norway (Palacios et al., 2010; Garseth et al., 2013). While HSMI is a common and commercially important disease in farmed salmon (Alarcon et al., 2012), the presence of PRV has so far not been associated with HSMI related lesions in wild salmon (Garseth et al., 2013).

PRV was described in 2010 after ten years of search for the causative infectious agent of HSMI (Palacios et al., 2010). There are still unanswered questions regarding the role of PRV in the development of HSMI, but the identification of a possible causative agent has initiated several studies in both farmed and wild populations that will contribute to filling these knowledge gaps.

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Reoviruses are non-enveloped with icosahedral capsid and a double-stranded RNA genome comprised of 10–12 segments. The host range of *Reoviridae* extends from insects, fungi and plants to fish, molluscs, reptiles, birds and mammals (Fenner et al., 1993). The *Reoviridae* consists of fifteen genera divided into the two subfamilies *Spinareovirinae* and *Sedovirinae* (Carstens and Ball, 2009). PRV is described as equally distant to the genera *Orthoreovirus* and *Aquareovirus* in the *Spinareovirinae* subfamily and may therefore represent a new *Reoviridae* genera (Palacios et al., 2010).

Palacios et al. (2010) identified PRV by high-throughput sequencing and detected the virus in tissue from farmed Atlantic salmon undergoing natural HSMI-outbreaks as well as in tissue from salmon with experimentally-induced HSMI. The link to disease development was further established by in situ hybridisation where viral nucleic acids were detected in histopathological lesions, and by the detection of elevated virus loads in association with outbreaks of HSMI (Palacios et al., 2010). Subsequent studies have strengthened the confidence that there is a causal relationship between PRV and development of HSMI (Finstad et al., 2012; Lovoll et al., 2012; Mikalsen et al., 2012). Hence, PRV seems to be a predisposing necessary but not sufficient factor in the development of HSMI. Piscine reovirus has common features with aquareoviruses. In aquareoviruses, pathogenicity varies among strains and seems to be dependent on age and species of infected fish. Most aquareoviruses have been isolated from apparently healthy fish and shellfish during routine examinations (Lupiani et al., 1995).

Heart and skeletal muscle inflammation (HSMI) was first described in farmed Atlantic salmon in a Norway in 1999 and was initially confined to a limited geographic area (Kongtorp et al., 2004a,b). Since then the disease has been diagnosed in all salmon producing counties in Norway and has also been described from Scotland (Ferguson et al., 2005).

Clinical signs of HSMI are abnormal swimming behaviour, anorexia, almost 100% morbidity and up to 20% mortality. Histopathological findings are epi-, endo- and myocarditis, myocardial necrosis, myositis and necrosis of red skeletal muscle (Kongtorp et al., 2004b; Kongtorp, 2008).

HSMI is one of the most frequently diagnosed diseases in Norwegian salmon aquaculture: there were 160 outbreaks in seawater sites and two in fresh water plants with seawater intake in 2011. This represents a 20% increase in the number of outbreaks compared to 2010 (Alarcon et al., 2012). HSMI is a commercially important disease in Norway due to the high number of outbreaks and the biological effects of mortality, reduced growth and increased management costs.

The Scientific Advisory Committee for Atlantic Salmon Management in Norway has listed infectious diseases in farmed Atlantic salmon as a potential threat to the survival and sustainability of wild Atlantic salmon stocks. The committee has also expressed concerns about the lack of knowledge on how infectious diseases in farmed populations affect wild salmon (Anonymous, 2012).

In Garseth et al. (2013) the widespread presence of PRV in Atlantic salmon and sea-trout (*Salmo trutta* L.) captured in Norwegian rivers was described. The prevalence of PRV was 13.4% in wild, 24.0% in hatchery-reared and 55.2% in escaped farmed salmon. Preliminary statistical analysis also indicated associations between PRV-infection and other traits registered at the individual level. Thus the objective of the current study was to examine factors associated with PRV-infection in returning Atlantic salmon caught in Norwegian rivers.

2. Materials and methods

2.1. Study sample

The study sample was derived from a biobank previously described in Garseth et al. (2013) that comprised samples from 1207 Atlantic salmon from 36 Norwegian rivers. Out of the total, 1093 salmon were broodfish captured in 31 rivers for restocking and restoration purposes over the period 2007 to 2009, while 114 salmon from 5 rivers were sampled during recreational and commercial fishing in 2008. The purpose of the latter sampling was disease surveillance in wild populations during viral haemorrhagic septicaemia (Møre & Romsdal) and infectious salmon anaemia (ISA) (Nord-Trøndelag) outbreaks in aquaculture sites in the proximity of the rivers. Broodfish were captured by net, rod or electrofishing from September to November and were kept in tanks onshore or in cages in the rivers until stripping 0–3 months later. Head-kidney samples were collected by authorised fish health personnel during routine post mortem examination. Salmon caught during disease surveillance by rod and bag nets were immediately euthanized and sampled by trained anglers.

2.2. Fish-level data collection

The Norwegian Veterinary Institute (NVI) manages the *Broodfish-database* as part of the Norwegian gene bank programme for wild Atlantic salmon operated by the Norwegian Directorate for Nature Management. A tailor-made PRV-database was developed by merging data from the *Broodfish database* and results from PRV-specific quantitative RT-PCR-analysis (Garseth et al., 2013). Body length (mm) and sex were recorded by stock enhancement hatcheries and anglers who also performed the collection of scales. Biologists at NVI and Norwegian Institute for Nature Research performed the scale reading providing information on life history, smolt-age and sea-age (Antere and Ikonen, 1983; Lund and Hansen, 1991; Fiske et al., 2004). Fish health personnel performed post mortem examination, tissue sampling and confirmed body length and sex. Finally the PCR-laboratory at NVI-Trondheim provided the qRT-PCR results based on analysis of head-kidney tissue (Garseth et al., 2013).

2.3. Sample collection, RNA-extraction and qRT-PCR

Head kidney samples were collected by authorised fish health personnel or trained anglers. To avoid cross-contamination during field sampling, one of two protocols were used; either one set of disposable single use scalpels and forceps was used per salmon, or steel forceps and scalpels were sterilised between each salmon sampling by the use of a portable Bunsen burner (e.g. Flameboy from INTEGRA Bioscience AG). In the latter protocol, steel scalpel blades were in addition changed between every five to six salmon.

Samples were fixed in RNAlater® (Ambion® INC., Austin, TX, USA) and transported frozen on ice to the Norwegian Veterinary Institute (NVI) for further analysis. RNA was extracted from head kidney tissue as described in Garseth et al. (2013). RNA was isolated from approximately 20 mg of tissue with MagMAX™-96 Total RNA Isolation Kit (cat #1830, Ambion). The subsequent RNA extraction was performed with the same kit according to the manufacturers' recommendations. The magnetic-based separation was performed with a KingFisher (Labsystems Oy). RNA concentration and purity was measured after elution by use of NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). All samples had OD260/280 ratios between 1.97 and 2.12 (mean 2.06).

Primers and MGB probe (for genome segment L1) were the same as used in Palacios et al. (2010). The following cycling conditions were performed for all the samples: 50 °C for 10 min (reverse transcription), 95 °C for 5 min, followed by 45 cycles of 95 °C for 15 s, 58 °C for 30 s and 72 °C for 15 s (Garseth et al., 2013).

2.4. Description of variables

The outcome of our study, PRV-infection was dichotomised by setting a cut-point at cycle threshold (Ct) 40. Hence, samples with Ct-values below 40 and an exponential curve were regarded as positive (PRV-present = 1). Samples with no amplification or with Ct values equal to or higher than 40 were regarded as negative (PRV not present = 0) (Lovoll et al., 2012; Garseth et al., 2013). PRV was recently described and efforts to culture the virus has so far failed. Sensitivity and specificity of the used qRT-PCR assay (Palacios et al., 2010) has not been established.

The variable *river* represents where the salmon were captured, but in wild and hatchery-reared salmon it also has genetic and ecologic significance. Since most Atlantic salmon spawners return to the river they left as smolts, each river has its own genetically distinct salmon stock with unique features or even several distinct populations in one river (Verspoor et al., 2005). Three to six percent of returning wild salmon and 15% of hatchery-reared salmon may stray to other rivers (Stabell, 1984; Jonsson et al., 2003). Depending on the contribution of strayed salmon in neighbouring rivers several rivers may constitute one population (Verspoor et al., 2005).

The variable *life-history* describes whether the salmon is of wild, hatchery-reared, escaped farmed or uncertain origin. As described in Garseth et al. (2013) scale-circuli

patterns and additional information such as smolt size, smolt age, general growth pattern and knowledge of local cultivation and release practices were used to distinguish between the different groups (Antere and Ikonen, 1983; Lund and Hansen, 1991; Fiske et al., 2004). Hence, the term *wild* describes individuals that are the result of natural spawning and recruitment in the river, the term *escaped farmed* describes individuals displaying scale-circuli patterns of salmon escaped from commercial aquaculture, while the term *hatchery-reared* describes individuals that are offspring of wild parents, and that are hatchery reared and released for stock enhancement or restoration purposes.

The original sample collection contained salmon that were classified as “unreadable” or “uncertain” with regards to scale category. These were discarded from the study sample presented in Garseth et al. (2013). Scales from all salmon in the original sample collection were reread in 2012 using new high quality equipment (Leica binocular M60, Leica camera DFC 450 and Leica software LAS v4.0) enabling reclassification and amending registrations of sea-age and smolt-age. Fourteen individuals remained uncertain with regards to scale category, but were kept in the study sample.

Smolt-age and *sea-age* were recorded from scale-circuli patterns. Smolt-age is the number of winters spent in freshwater before migrating to the sea, and sea-age is the number of winters spent in sea-water before returning to freshwater to spawn (Aas et al., 2010). Both age-categories may vary between rivers.

Body length was measured from tip of the snout to the tip of the caudal fin and registered as total length (in mm), and converted to decimetres in the final dataset.

A variable called *broodfish tank* was constructed to test if cohabitation of broodfish in tanks constituted a risk factor for PRV-infection. Broodfish were cohabitated in tanks onshore or in cages in the river for 0–3 months prior to sampling of head kidney. In contrast salmon caught for disease surveillance were killed and sampled immediately after capture. As displayed in Table 1 the proportion of PRV-positive broodfish of wild origin is higher than that of wild salmon caught during disease surveillance, 14.4% (12.2–16.9) versus 5.6% (1.8–12.5). During sequencing of PRV segments S1, S2 and S4 as part of phylogenetic analysis, pairwise identical sequences for all three segments were detected from broodfish kept in three stock enhancement hatcheries. Two hatcheries confirmed cohabitation of salmon with identical sequences, while the third had recorded that salmon had been moved between several tanks.

2.5. Data management and statistical analysis

The final dataset was analysed for associations between PRV-infection status (the outcome variable) and available predictors using Stata release 12 (Stata Corp., College Station, TX, USA). Descriptive statistics (mean, range, proportions) were calculated for predictors. To investigate collinearity between predictor variables the variance inflation factor (VIF) (command *collin*) and pairwise correlation coefficients (*r*) (command *pwcorr*) were computed.

Table 1

The proportion of PRV-positives with 95% CI in final study sample comprised of wild, hatchery-reared and escaped farmed Atlantic salmon (*Salmo salar* L.) broodfish and adult subjects caught during disease surveillance.

	Broodfish (2007–2009)		Disease surveillance (2008)		Overall	
	<i>n</i>	Proportion PRV-positive % (95% CI)	<i>n</i>	Proportion PRV-positive % (95% CI)	<i>n</i>	Proportion PRV-positive % (95% CI)
Wild	897	14.4 (12.2–16.9)	90	5.6 (1.8–12.5)	987	13.6 (11.5–15.9)
Hatchery-reared	123	24.4 (17.1–33.0)	0	–	123	24.4 (17.1–33.0)
Escaped farmed salmon	39	46.2 (30.1–62.8)	15	66.7 (38.4–88.2)	54	51.9 (37.8–65.7)
Uncertain origin	13	23.1 (5.0–53.8)	1	0 (0–97.5)	14	21.4 (4.7–50.8)

Predictors with correlation coefficient >0.7 were not entered into the model together. Uni-variable association between the outcome variable and predictor variables was assessed by mixed-effect models with river as random effect. Predictors with *p*-values <0.20 in were kept for further analysis.

2.6. Building the model

The dataset has a binary outcome variable and a two-level hierarchical structure with salmon nested within rivers. Clustering was thus accounted for by modelling river as a random effect in multilevel logistic regression. All predictor variables were recorded at the salmon level and were modelled as fixed effects. The xtmelogit command in Stata was used, and the final model was developed by a manual, forward, stepwise procedure inserting predictor variables one by one. Between each step a likelihood ratio test (LR-test) and Akaike’s information criterion (Akaike, 1974) was used to evaluate the model performance, where LR-test is described to be superior to AIC in the evaluation of nested models (Dohoo et al., 2009). Variables were included in the model if AIC was reduced by five units or more and LR-test had *p*-values <0.05. Finally, model selection was verified by stepwise backward selection where all included variables had *p*-values <0.005. Table 3 displays relative AIC and likelihood ratio-test *p*-values from comparing full model with reduced models where individual variables were omitted. Random effect models produce subject specific estimates (β^{SS}) and should be interpreted as how changes in fixed effects affect a single river (subject). Approximate population averaged estimates were calculated to ease the interpretation of how changes in fixed effects affect the whole population (Dohoo et al., 2009). The random effect resulted in a modest reduction in estimated odds ratio when subject specific estimates were converted to population averaged estimates. We therefore continue to refer to odds ratios from the initial subject specific estimates.

A latent response approach was used to interpret the random effect in terms of variance components and inter-class correlation coefficient (ICC) (Dohoo et al., 2009).

Procedures for goodness of fit-tests for multilevel logistic regression models are not readily available; hence the Hosmer–Lemeshow test of the fit of the corresponding single-level multivariable logistic regression model was used. The assumption of linearity for the continuous variable body-length (*ldm*) was graphically assessed and confirmed to be linear using the function lincheck in Stata.

3. Results

3.1. Descriptive statistics and unconditional associations

Mean VIF was 1.85 indicating a collinearity problem. Body-length and sea-age had VIF of 3.80 and 3.66, respectively, while the other predictors had VIF between 1.02 and 1.13. Body-length and sea-age were correlated ($r = 0.85$, $p < 0.01$), while all other significant correlations ($p < 0.05$) had correlation coefficient of 0.3 or lower. Body-length and sea-age was assumed to be collinear as $r > 0.7$ and was consequently not entered into the model together. Smolt-age and sea-age could not be reliably recorded in salmon escaped from aquaculture (Håvard Lo and Bjørn Florø-Larsen pers. communication) and were missing in most escaped farmed salmon in the dataset. Hence, body-length was included in the final model. Since variables sea-age and body-length were positively correlated with a near linear relationship (Fig. 1) body length could serve as a proxy for sea-age in the final model.

The original sample set contained six precocious males (parr) from river Lærdal. These were omitted from the study since the life of precocious maturing male parr diverge substantially from other subjects (Fleming and Einum, 2011). All precocious males were PRV-negative.

All salmon lacking information on sex and body-length were omitted from the final analysis. Most of these were assessed to be missing information at random, as the failure to register sex and body-length was deemed to be independent both of observable variables and of unobservable

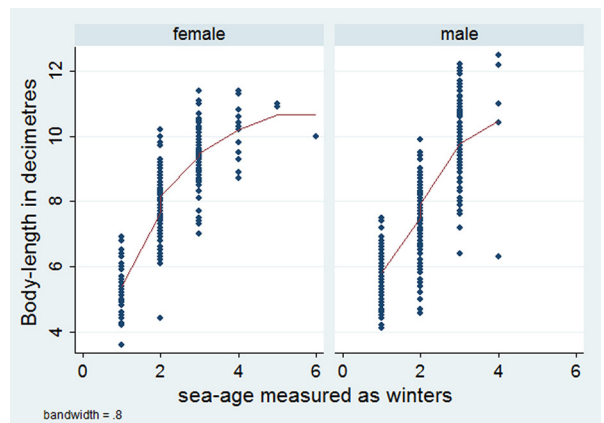


Fig. 1. Body length (decimetres) and sea-age are correlated ($r = 0.85$) and has a near linear relationship in both sexes. Body length can serve as a proxy for sea-age in the final model.

parameters of interest and was thus considered not to influence the final results. An exception from this was the missing data on body length in six escaped farmed salmon captured in river Nidelva in 2009. These data were assessed to be missing because these salmon were identified as escaped. Since four of these salmon were PRV-positive one cannot exclude the possibility that omitting these subjects has influenced the estimates.

The final study sample including proportion of PRV-positives in each catch-category and life-history group is described in Table 1, while Table 2 describes the variables including unconditional associations with the outcome.

3.2. Multilevel model

The final two-level mixed-effect logistic regression model was specified as:

$$\log_{it}P(Y_i = 1|X) = \beta_0 + \beta_1 \text{ life history} + \beta_2 \text{ sex} + \beta_3 \text{ year} + \beta_4 \text{ body length} + u_{\text{river}(i)}$$

where $u_{\text{river}(i)}$ is the random effect of the river (r) containing salmon i , assumed to be $\sim N(0, \sigma^2_r)$ (Dohoo et al., 2009).

Robustness against model-misclassification was assessed by evaluating the presence of extreme values at the salmon level and by assessing the residuals at the river level. The assumption of normality was verified by a normality plot of river-level residuals.

Table 3 displays the final model including random effect and fish-level fixed effects associated with piscine reovirus infection. In the same table results from final model evaluation are presented. AIC in the full model was 981.6, while relative AIC- estimates (difference between full and reduced model for the different variables) were between 5.5 and 31.6. p -Values from LR-tests (reduced model nested in full model) were all <0.01 , justifying the inclusion of life-history, sex, body-length and catch year as fixed effects in the final model.

3.3. Evaluation of clustering

The dataset comprised 36 rivers (clusters) with 1–121 salmon in each river (Table 2). The random effect variance (σ_r^2) was 0.361 with S.E. 0.186 and 95% CI 0.132–0.990, confirming that there was clustering within rivers. A value of zero would indicate no variation between rivers and therefore no clustering (Dohoo et al., 2009). The intra-class correlation coefficient (ICC) calculated post estimation in Stata was 0.099, with S.E. 0.046 and 95% CI 0.039–0.231 (Table 3). This indicates that the proportion of variance at the river level was approximately 10%. The clustering effect may be considered as moderate, but the Stata likelihood ratio statistic for testing the null hypothesis that $\sigma_r^2 = 0$ (comparing xtlogit and logistic regression) supports using a two-level model at the sacrifice of a single-level model (χ^2 (1df): 15.09 $p=0.0001$). Failing to account for clustering can lead to incorrect β estimates, too narrow confidence intervals and too small standard errors and erroneous p -values.

Table 2 Descriptive statistics of the variables considered in multilevel modelling. Unconditional uni-variable associations with outcome in model with river as random effect are demonstrated with p -values.

Variable	Observations	# Categories	Mean	Min	Max	p -Value	Description
PRV-infected ^a	1178	2		0	1		PRV-positive = Ct <40, PRV-negative \geq 40 or NA in qRT-PCR
River ^b	1178	36		1	121		
Life-history	1178	4		1	4	$<0.001^c$	Wild ($n=987$), hatchery-reared ($n=123$), escaped farmed* ($n=54$), uncertain ($n=14$)
Year	1178	3		2007	2009	0.028 ^c	2007 ($n=222$), 2008* ($n=560$), 2009 ($n=396$)
Sex	1178	2		0	1	0.001	Female ($n=613$), male ($n=565$)
Broodfish tank	1178	2		0	1	0.538	No (from disease surveillance, $n=106$), yes (broodfish, $n=1072$)
Sea-age	1042	6		1	6	0.445	Winters at sea before returning to spawn
Smolt-age	960	6		1	6	0.420	Winters in freshwater prior to migration as smolt
Body-length	1178	-		3.6	13.92	0.013	Body-length in decimetres

^a Outcome-variable.

^b Cluster variable.

^c p -Value is shown for category with lowest value (see * in description) versus the reference.

Table 3 Final model of fish-level variables associated with piscine reovirus infection. Also displaying results from AIC and LR-test used to evaluate model and include variables. AIC in the full model was 981.6.

Variable	Subject specific estimates				Model evaluation			
	Coefficient (SE)	Odds ratio (SE)	95%CI	p-Value	AIC	Relative AIC if deleted from model	LR-test	p-Value
Life history	0	1			31.6			<0.00001
Wild	0.527 (0.294)	1.694 (0.498)	0.952–3.014	0.073				
Hatchery reared	1.990 (0.326)	7.315 (2.383)	3.863–13.852	<0.001				
Escaped farmed	0.757 (0.682)	2.131 (1.453)	0.560–8.110	0.267				
Uncertain					16.4			<0.00001
Sex	0	1						
Female	0.746 (0.177)	2.109 (0.372)	1.492–2.980	<0.001				
Male	1.195 (0.074)	1.058 – 1.349	0.004		6.5			0.0036
Body length (dm) proxy for sea-age	0	1			5.5			0.0087
Catch year								
2007	–0.477 (0.246)	0.620 (0.153)	0.383–1.005	0.053				
2008	0.110 (0.242)	1.116 (0.270)	0.695–1.793	0.649				
2009	–3.557 (0.594)	0.029 (0.017)	0.009–0.091	<0.001				
Cons	σ^2	S.E.	95% CI					
Random effects								
River	0.361	0.186	0.132–0.990					

4. Discussion

4.1. PRV-infection and the effect of sea-age and catch-year

Increasing body length (measured in decimetres) was associated with increasing odds for PRV-infection (OR 1.20, $p=0.004$), meaning that a 10 cm increase in body length increases the odds of PRV-infection by 1.2. Body length can serve as a proxy for sea-age but is influenced by many factors. For example body length differs between wild stocks (rivers) and between different life histories as farmed salmon are larger than wild fish of the same age. Hence, we cannot relate length directly to sea-age. A coarse interpretation would be that increasing body-length is an indicator of increasing sea-age and that spending more years in the sea increases the odds of being PRV-infected. This is again an indication of pathogen transmission and infection occurring in the sea-phase. However there might be factors not included in the model that could modify this picture. A proportion of Atlantic salmon is iteroparous breeders; hence they will return to the river more than once to spawn and may be infected in the near-coastal areas or in the river. Iteroparous breeding was not reliably recorded in the dataset and could not be included in the model.

Salmon captured in 2008 had a 38% reduction in odds of being PRV-infected compared to salmon captured in 2007 (OR: 0.62, $p=0.053$). As the study period only covers three years it is too early to conclude that an annual trend exists. A true annual trend could be elucidated if rivers were sampled over several years.

4.2. PRV-infection and the effect of sex and sexual maturation

Controlled for river-effects, life-history, sea-age (body-length) and catch year, male salmon had double the odds of being PRV-infected compared to female salmon (OR: 2.11, $p<0.001$). Other studies have reported differences in pathogen resistance and disease occurrence between sexes. Lawlor et al. (2009) reported that during experimental *Listonelle anguillarum* challenge tests, the survival rates were higher among male than female parr and that survival was not affected by maturation in male parr. Significant differences between sexes were found for epidermal hyperplasia/papilloma during a survey of dab *Limanda limanda* in the German Bight (male/female OR: 0.57 with 95% CI: 0.39–0.83) (Vethaak et al., 1992). Weakening of male salmonid spawners caused by high energy costs and demanding interaction with other salmonids were the key explanations behind differences between sexes during outbreaks of furunculosis in Norwegian river Eidselva in 1990. Altogether 110 deceased wild Atlantic salmon were detected; of these 106 were male spawners. Likewise, all 8 dead sea-trout were male (Sættem, 1991; Johnsen and Jensen, 1994). Both male and female spawners invest a large proportion (45–60%) of their total energy during reproduction (Jonsson and Jonsson, 2003; Fleming and Einum, 2011) but the allocation of energy is different between sexes. Female spawners allocate 30–31% of their total energy to egg production; this

constitutes 60% of the reproductive energy investment (Jonsson and Jonsson, 2003) and female reproductive behaviour is focused towards increasing offspring survival e.g. nest digging (Økland et al., 2000). Intra-sexual aggression is rare in female spawners. Male spawners lose 47–49% of their total energy while in the river for spawning, and gamet production constitutes less than 10% of that energy loss. The remainder is devoted to development of secondary sexual traits and to energy demanding breeding-activities such as chasing, aggressive display and courting (Økland et al., 2000; Jonsson and Jonsson, 2003). These differences may partly explain the differences in odds of PRV-infection between sexes since male behaviour to a larger extent than female behaviour may include interaction and confrontation with other, potentially infected salmon. It is not likely that cohabitation of male spawners in tanks in hatcheries will mitigate this effect.

4.3. PRV-infection was not statistically associated with broodfish being held in tanks

Fish caught during disease surveillance were killed and sampled immediately after they were caught while broodfish are kept together in tanks before stripping (removal of eggs and milt) and autopsy. Transmission of PRV in broodfish tanks has been rendered possible by identical PRV-S1, S2 and S4 sequences in broodfish kept in the same tanks. Attempts to include *broodfish tank* as a fixed effect was not significant in the multilevel mixed-effect logistic regression model. This may be due to the effect actually not being significant, or because the model interprets the difference as a river effects since none of the rivers had both broodfish and fish caught during disease surveillance.

The fact that the proportion of PRV-positive escaped farmed salmon was higher in the disease surveillance group than in broodfish probably also influences the interpretation. A proportion of broodfish was not identified as escaped farmed salmon until scale reading results were provided, an indication that time had elapsed between the actual escape and sampling. It is plausible to suggest that these individuals may have lower PRV-proportion than in the disease surveillance group either because they are no longer infected with the PRV virus or because they represent the survivors of PRV-infection.

The difference between broodfish and the disease surveillance group may indicate that the proportion of PRV-positives is overestimated in wild broodfish.

4.4. PRV-infection is associated with life history

Atlantic salmon escaped from aquaculture sites had higher odds of being PRV-infected than wild salmon (OR: 7.32, $p < 0.001$). The model also indicated that salmon released from stock enhancement hatcheries may have higher odds for being PRV-infected than wild salmon (OR: 1.69, $p = 0.073$).

Farming creates a favourable environment for transmission of pathogens, within and between sites through high stocking densities, movement of fish, open cages during the sea-water phase, sharing of equipment and personnel and by locating sites with a high number of susceptible hosts

close to each other. Higher odd of PRV-infection in escaped farmed salmon is therefore plausible and also expected based on the number of outbreaks in farmed fish. Investigation of horizontal transmission of HSMI, conducted prior to description of PRV, emphasise distance to nearest HSMI-affected farm, sharing a contact network with an affected farm and previous HSMI diagnosis on same farm as risk factors, but also that HSMI seemed to be introduced by an external factor such as smolt (Aldrin et al., 2010). Receiving smolt from several smolt-producers has been identified as a risk-factor for development of infectious pancreatic necrosis (Jarp et al., 1995) and ISA (Jarp and Karlsen, 1997). A study quantifying PRV at different stages of the Atlantic salmon production cycle followed 14 cohorts from fresh-water smolt production to slaughter. PRV-positive pre-smolts were detected in 5 of the 14 cohorts before transfer to sea-water (approximately 36%). PRV was detected in 29 of 50 (58%) samples from PRV-positive freshwater farms (max 10, min 1), and 7 of 29 (24%) of the positive samples had Ct-values less than 25 (Lovoll et al., 2012). PRV-infection in freshwater plants could be caused by vertical transmission from broodfish, or by horizontal transmission e.g. by use of sea-water in the smolt production (Kongtorp et al., 2006; Alarcon et al., 2012). In the same study none of the 14 cohorts remained PRV-negative through the sea-water phase, and a general increase in viral load was observed after transfer to sea. The highest viral loads were detected closest to HSMI outbreaks (Lovoll et al., 2012).

In 2010, thirty-six of thirty-eight escaped farmed salmon captured in river Etne were PRV-positive by qRT-PCR (Garseth et al., 2013). These salmon were not included in this study since they were thought to have escaped from the same farm and would represent a selection bias. Nevertheless, the result supports the findings of Lovoll et al. (2012) and the conclusion that escaped farmed salmon not only supply local rivers with unwanted genes (Hindar et al., 1991a,b; Hindar et al., 2006). They probably also supply unwanted pathogens with potential harmful effects.

The odds ratio for PRV-infection in hatchery-reared salmon was borderline non-significant (OR: 1.69, $p = 0.073$) but is reported in this paper because it might be biologically significant. The difference between wild and hatchery-reared salmon can be attributed to differences in exposure during life, differences in genetic resistance or a combination. Hatchery reared salmon are released into the river as eggs, fry, parr or smolt from hatcheries where the production is less intensive than in commercial aquaculture. Eggs are collected from broodfish of local origin, stocking density is lower, water consumption is higher and there is no recirculation of water or use of seawater. Nevertheless, the freshwater phase in a hatchery will differ significantly from the conditions in the river. Hence, hatchery-reared salmon represent semi-domesticated populations and will be expected to harbour some of the features found in salmon from commercial aquaculture. The practice of cohabitating broodfish in tanks before stripping may increase the risk for transmission of pathogens within the broodfish group and will potentially increase the likelihood of introducing PRV to hatcheries by vertical transmission. It is not yet clear if PRV is transmitted

vertically. A small industry-based study does not exclude vertical transmission, but concludes that this probably is not a major route of transmission for PRV (Wiik-Nielsen et al., 2012).

After the release into rivers, hatchery-reared salmon will disperse over a larger area and the relative host density will decrease. This will result in reduced contact between individuals and thus a reduced infection pressure. This is unlike the situation previously described for farmed salmon, where host density remains high throughout the production cycle. Another factor potentially influencing the proportion of PRV-positive is differences in behaviour between wild and hatchery-reared salmon. Salmon released from hatcheries into the Baltic ocean had a shorter feeding migration distance than wild salmon (Jutilla et al., 2003). This could imply that hatchery-reared salmon spend more time near the coast and potentially could more easily be infected by virus from farmed salmon.

Differences in PRV-prevalence between life-history categories could also be caused by differences in pathogen resistance. Reduced pathogen resistance in endangered populations of Atlantic salmon compared to wild and cultured populations has been recorded (Lawlor et al., 2009).

So far there are no indications that PRV-infected wild salmon will develop HSMI. However, PRV-infection is useful as a model of disease interaction since it is widely distributed in both wild and farmed salmon populations. The present study concludes that escaped farmed salmon have higher odds of being PRV-infected than wild salmon. This adds to the negative effects of escapees entering rivers. Possible higher odds of PRV-infection in returning spawners of hatchery-reared origin is an unwanted situation in restocking and restoration of wild salmon stocks and should be investigated further.

The differences observed in proportion of PRV-positives between wild salmon in the broodfish and disease surveillance group might be caused by virus transmission during cohabitation of broodfish in tanks. This may imply that the prevalence is overestimated in wild broodfish caught for stock enhancement purposes. Non-lethal sampling immediately after the fish is caught may be more valuable or may remedy some of the shortcomings of current practise in health monitoring of wild fish.

5. Conclusion

In the present study we conclude that PRV-infection in Atlantic salmon captured in Norwegian rivers is associated with life-history, sex, catch-year and sea-age.

Escaped farmed salmon had higher odds of being PRV-infected than wild salmon. This adds to the negative effects of escapees entering rivers. Higher odds of PRV-infection in returning spawners of hatchery-reared origin is an unwanted situation in restocking and restoration of wild salmon stocks and should be investigated further. Odds of being PRV-infected increased with body-length that served as a proxy for sea-age, meaning that spending more years at sea increases the odds of being PRV-infected.

So far there are no indications that PRV-infected wild salmon will develop HSMI. However, higher odds of PRV-infection in escaped farmed salmon and with increasing

sea-age may be important indicators of transmission routes that could be open also to other, more virulent, pathogens.

Conflict of interest statement

There are no conflicts of interest.

Role of the funding source

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