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Genetic variation of resistance to Viral Nervous Necrosis and genetic correlations with production traits in wild populations of the European sea bass (*Dicentrarchus labrax*)

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Abstract :

Viral Nervous Necrosis (VNN) disease is considered as one of the most serious threats for European sea bass cultured in Mediterranean Sea, with no simple and effective procedures to treat this disease. In this study, 1472 offspring resulting from artificial full factorial mating of western Mediterranean dams with sires from four different wild populations of European sea bass (Northern Atlantic, NAT; Western Mediterranean, WEM; Northern-East Mediterranean, NEM; and Southern-East Mediterranean, SEM) were challenged by experimental infection to W80 betanodavirus strain in order to evaluate genetic variations for VNN resistance among populations and genetic correlations between VNN resistance and production traits. The results showed a large variation of VNN resistance between the four populations tested as well as between sire families within strain. The survivals between pure wild populations SEM, NEM, WEM and NAT were estimated at 99%, 94%, 62%, and 44%, respectively. A moderate intra-population heritability of VNN resistance, calculated based on liability scale with sire model, was recorded for the first time in European sea bass ($h2u = 0.26 \pm 0.11$). Finally, moderate negative genetic correlations between VNN resistance and daily growth coefficient (DGC) and body weight (BW) were also demonstrated (-0.28 ± 0.20, -0.35 ± 0.14, respectively) while the genetic correlation between resistance to VNN and fillet adiposity (FA) was weakly negative and not significant (-0.13 ± 0.19). These results give good prospects of selective breeding of European sea bass for improved resistance to VNN disease.

Highlights

► VNN disease is a major disease for marine fishes, and especially sea bass. ► For the first time, we show that different natural populations of European sea bass differ widely for their resistance to VNN disease. ► We also provide the first estimates of heritability for VNN resistance in European sea bass.

Keywords : VNN disease, VNN resistance, Heritability, Genetic correlation

European sea bass (*Dicentrarchus labrax*) is a major aquaculture species in the Mediterranean Sea and the Atlantic Ocean with 153,182 tons of annual aquaculture production (mostly from the Mediterranean), compared to 9,000 tons from capture fisheries (FAO, 2014). One of the most important issues of the sea bass aquaculture industry is the outbreak of diseases, and especially viral nervous necrosis (VNN) disease (Haffray et al. 2006; Chavanne et al. 2008). VNN disease, also known as viral encephalopathy and retinopathy (VER), caused by nervous necrosis virus (NNV), a RNA virus belonging to betanodavirus genus, has been considered one of the most serious viral threats for almost all of marine aquaculture fish species, causing serious economic losses in the marine aquaculture industry throughout the world (Doan et al. 2016). Historically, betanodaviruses were responsible for mass mortality in marine fish hatcheries, especially warm water fish such as European sea bass larvae and juveniles, in Martinique and French Mediterranean farms in 1985-1990 (Breuil et al. 1991). This disease, then, has become the main threat for European sea bass aquaculture in the Southern Mediterranean (Haddad-Boubaker et al. 2013; Toffan et al. 2016). With global warming, VNN may become an even more serious issue for European sea bass culture, because the mass mortality caused in sea bass by the RGNNV (Redspotted Grouper Nervous Necrosis Virus) genotype, the most common NNV type in the Mediterranean, is usually reported when water temperature is in the 25 to 30°C range (Le Breton et al. 1997; Toffan et al. 2016).

Despite numerous investigations, no simple and effective procedures are available to treat this disease in fish (Doan et al. 2016). Applying vaccination may be an effective way to prevent disease occurrence (Gomez-Casado et al. 2011). However, no commercialized vaccines against VNN in European sea bass presently exist (Brudeseth et al. 2013; Doan et al. 2016). Selective breeding could be an alternative option, with a general potential to gain at least 12.5% survival per generation for most diseases reported in aquaculture (Gjedrem 2015).

for this trait in the population (Falconer & Mackay 1996). Significant genetic variation has been demonstrated for resistance of farmed fish to most viral diseases studied, with moderate to high heritability estimates (Ødegård et al. 2011). Especially, high to extremely high heritability (up to 0.75) was recorded for resistance of Atlantic cod *Gadus morhua* against another nodavirus genotype, the barfin flounder nervous necrosis virus (BFNNV), hitting cold water species (Ødegård et al. 2010; Bangera et al. 2011; Bangera et al. 2013). Ødegård et al. (2010) also showed very large genetic variations among wild cod populations for VNN resistance (range 10-56% among coastal cod, Northeast Arctic cod and F_1 cross between them). This led us to consider that genetic differences for NNV resistance among natural populations of the European sea bass would be worth investigating. European sea bass is divided into three main populations by population genetics studies, namely Atlantic, Western Mediterranean and Eastern Mediterranean, the latter including two subpopulations: North-Eastern Mediterranean and South-Eastern Mediterranean (Naciri et al. 1999; Bahri-Sfar et al. 2000; Castilho & Ciftci 2005). Selective breeding in this species has been carried out from the mid-1980s in France, Spain, Italy and Israel (Haffray et al. 2006). Although genetic parameters have been estimated to support selective breeding for production traits in many studies, most of them were concentrated on the improvement of growth (Saillant et al. 2006; Dupont-Nivet et al. 2008), sex ratio (Vandeputte et al. 2007; Vandeputte et al. 2012; Saillant et al. 2002), and carcass quality (Saillant et al. 2009). Significant genetic variability for growth traits has been demonstrated within population as well as between wild populations (Vandeputte et al. 2009; Vandeputte et al. 2014; Dupont-Nivet et al. 2008).

The primary aim of this study was to estimate the amount of genetic variation between and within wild populations for resistance against VNN, as well as to estimate genetic correlations with growth-related traits.

2. Materials and methods

Four different wild populations were used to design the experiments, with broodstock captured from the Northern Atlantic (NAT), the Western Mediterranean (WEM), the North-eastern Mediterranean (NEM) and the South-eastern Mediterranean (SEM). The precise origin of the broodstock was detailed by Vandeputte et al. (2014). WEM dams were kept under natural photoperiod and temperature conditions during maturation process at the IFREMER experimental facility of Palavas (at Palavas-les-Flots, France). The NAT and WEM sires were reared at Palavas, their sperm being cryopreserved according to Fauvel et al. (1998), from 2004 until present, while the SEM sires were stocked at IOLR (Eilat, Israel) and their sperm cryopreserved using the methodology in 2005 (Sansone et al. 2002). The sperm of NEM sires was similarly stripped and cryopreserved as described by Sansone et al. (2002) at the Beymelek Lagoon (Turkey) during 2005.

2.2. Production and rearing of the fish

A full factorial mating scheme was done by artificial fertilization, where 60 sires (15 per origin) were crossed with nine WEM dams at the IFREMER experimental facility of Palavas. Dams were selected following an estimation of their maturation status by biopsy. The 19 dams that had reached the appropriate maturation stage were hormonally injected (LHRHa, 10µg/kg). The stripping of 9 dams was successful at 72h post-injection. Nine hundred ml of high quality eggs (100 ml of eggs per dam) were pooled and gently mixed, and 600 ml of these eggs were divided into 60 tubes (10 ml/tube). Thawed sperm from each sire was used to fertilize each tube separately (1 tube per sire). Ovules and sperm were gently mixed together (ratio: 10 ml eggs: 62.5 µl sperm), then 5ml of seawater was added to provoke sperm activation and fertilization. Finally, all fertilized eggs were pooled, and a subsample of 100 ml of fertilized eggs was incubated in two different incubators (50ml per incubator) for 48h at 13.5–14^{o}C and 37‰ salinity. At 48 h post-fertilization (hpf), sinking (unfertilized or dead)

hatching and larval rearing (36,000 eggs per tank).

Offspring were reared in a common garden until NNV challenge. The larvae were reared at 16.5°C and 25‰ salinity during 58 d post-hatching (dph). Then the temperature was increased gradually from 16 to 20°C in seven days. The fish were reared at the mean of temperature 21.5°C (18.1 – 22.4°C) and 30‰ salinity until 102 dph, where 5000 fish from one of the larval tanks were transferred to 5 juvenile tanks in the same zone. During juvenile stage, the mean of the temperature of water environment was 22.1°C (ranging from 15.5 – 27.9°C). Tagging, collection of fin samples for further genetic analysis as well as biometry of the fish (2100 individuals, 420 fish per pre-growing tank) were performed at 180 dph. At 202 dph, 1472 fish were transferred into three tanks for NNV injection (see below) while 628 fish were kept uninfected in a single tank to collect growth-related traits. Initial and further biometric measurements of the fish were done following the ATOL fish trait ontology database (http://www.atol-ontology.com/index.php/en/lesontologies-en/visualisation-en, Golik et al., 2012): the body weight measured to the nearest 0.1 g (BW, ATOL: 0000351); the fork body length measured to the nearest 0.1 mm (BL, ATOL: 0001658); and fillet adiposity estimated as the mean of four measurements (two on each side) with a fish fat meter (FM692, Distell, UK) (FA, ATOL: 0001663). In total, individual performance could be collected on 545 uninfected fish at 431 dph because 37 fish were no covered pedigree and 46 fish died during growth period. The fish were fed *ad libitum* with a self-feeder and the sea water was filtered, UV-treated and renewed through the bottom of the tank in a recirculated system.

2.3. NNV challenge

A total of 1472 individuals (whose sire's population of origin was unknown at the time of challenge) were randomly transferred into three tanks (493 fish, 486 fish and 486 fish, respectively) after betanodavirus injection at 202 dph. All individuals had been tagged,

and a mean BL of 111.2 mm (77.1 – 149.1 mm, CV = 11%).

The W80 betanodavirus strain (belonging to the RGNNV genotype - Thiéry et al. 2004), was used in the infection trial. Strain W80 was propagated in the SSN-1 cell line, and the titer was determined as previously described by Castric et al. (2001). At the time of challenge, all fish were anesthetized by benzocaine in two steps. Firstly, fish were anesthetized in the rearing tanks by 15ppm benzocaine (0.1 ml/L of stock solution of 150g/L benzocaine in ethanol) for 30 m before fishing then they were fished is small batches and deeply anaesthetized with 37.5ppm benzocaine (0.25 ml/L of stock solution of 150g/L benzocaine in ethanol). The inoculation of the virus suspension was performed using intraperitoneal injection of 0.2 ml of viral suspension (10^9 virus/ml), diluted 100-fold in physiologic solution, thus with a virus dose of $2 .10^7$ virus/fish. During the challenge test, filtered, oxygenated, UV-treated natural salinity seawater (34.3 – 37.1‰) was provided, and the temperature was kept close to 25.5°C. Fish were fed *ad libitum* under continuous light throughout the whole challenge. Fish behavior, clinical signs of disease, and mortality were recorded daily for 24 d post-injection. VNN detection relied on the observation of characteristic clinical signs (erratic swimming pattern such as spiraling or whirling, lying down at the bottom, rapid swimming, swim-bladder hyperinflation) associated with an impairment of the nervous system. The ELISA method described by Breuil et al. (2001), was used to analyze the brains of 24 fish with clinic signs of NNV disease and the dead fish (jumped outside the tanks) to confirm that they were caused by betanodavirus. In addition, the brains of 20 survivors at the end of the experiment were also analyzed by ELISA. The mortality was considered stable after 24 d post-challenge, and the challenge was terminated. All fish were euthanized with an overdose of benzocaine and considered as survivors in the analysis.

2.4. Genotyping and parentage assignment

Josas, France) for DNA extraction and genotyping of 12 microsatellite markers (DLA0003, DLA0006, DLA0016, DLA0104, DLA0105, DLA0106, DLA0112, DLA0119, Labrax17, Labrax29, Labrax3 and Labrax8).

VITASSIGN, an exclusion-based parentage assignment software, was run as described by Vandeputte et al. (2006) with two allelic mismatches tolerated to recover pedigree.

2.5. Daily growth coefficient

Although 628 fish were kept for related growth traits, 37 fish had no correct pedigree and 46 fish died during growth period. Therefore, 545 uninfected fish had data for related growth traits at 431 dph (117 from ♀WEM x ♂NAT, 125 from ♀WEM x ♂WEM, 146 from ♀WEM x ♂NEM and 160 from ♀WEM x ♂SEM). They were derived from a total of 272 full-sib families (1-8 fish per family), 58 sire half-sib families (2-25 fish per family) and 8 dam half-sib families (8-161 fish per family).

The daily growth coefficient (DGC, ATOL:0002174) of uninfected fish over the growth period 180-431 dph was calculated as described by Cho (1992). The formula was as follows:

$$DGC_{180\text{-}431} = 100*(BW_{431}^{1/3} - BW_{180}^{1/3})/D$$

Where $DGC_{180\text{-}431}$ is daily growth coefficient from 180 dph to 431 dph; BW_{431} is final body weight (g) recorded at 431 dph; BW_{180} is initial body weight (g) collected at 180 dph; and D is total number of days between BW_{431} and BW_{180} (251 days).

2.6. Statistical analysis

The effect of the sires' genetic group (WEM , NAT, NEM, & SEM) was tested with a linear mixed effect model for all traits fitted in lme4 R package implemented in R 3.2.5 (Bates et al. 2015):

$$y_{ijklm} = \mu + O_i + T_j + S_{k(i)} + D_l + \varepsilon_{ijklm}$$

for dead, body weight (BW_{180}), daily growth coefficient ($DGC_{180-431}$), or fillet adiposity (FA_{431})) of individual m of sire k belonging origin i and dam l in tank j, μ is the overall mean, O_i is the fixed genetic effect of sires' origin i, T_j is the fixed effect of pregrowing tank j, $S_{k(i)}$is the random additive genetic effect of sire k within the origin i, Dam_l is the random effect of dam l, and ε_{ijklm} is the random residual effect associated with individual m. The tank for VNN challenge had no effect on VNN resistance, so it was omitted from the model. Multiple comparison of means was performed with the Kenward-Roger test in lmer Test R package (Kuznetsova et al. 2016).

Variance components were estimated based on univariate (to estimate heritability) and multivariate (to estimate phenotypic and genetic correlations between traits) linear mixed sire models fitted by restricted maximum likelihood (REML) in VCE 6.0 (Groeneveld, Kovac & Mielenz 2008):

$$y_{ijklm} = \mu + O_i + T_j + S_k + D_l + \varepsilon_{ijklm}$$

With the same notation as above, the only difference being that the sire effect is not explicitly nested within origin in this case. Furthermore, inter-population heritability was also estimated based on the same model without the origin effect.

Heritability was calculated from the estimated variance components as follows:

$$h^2_o = 4\sigma^2_{sire} / (\sigma^2_{sire} + \sigma^2_e)$$

Values of heritability (h^2_o) estimated on the observed scale for the binary trait VNN resistance were transformed to the values on the liability scale according to Dempster & Lerner (1950) using the following equation,

$h^2_u = h^2_o * p(1-p)/z^2$ (Dempster & Lerner 1950; Lynch & Walsh 1998)

estimate on the observed scale, p is the proportion of affected individuals and z is the value of the normal distribution density at the threshold point.

2.7. Estimating the potential resistance to VNN in pure strains.

Based on the VNN resistance of pure WEM population and the other hybrid populations, the survival in all pure strains was computed under the hypothesis of an equivalent additive effect of sire and dam origin. However, percentage scale variances vary with the mean. Therefore, firstly, incidences of VNN resistance were converted to mean (normally distributed) liabilities as follows: $m_i=probit(s_i)$ with m_i the mean liability, *probit* the inverse of the cumulative distribution function of the standard normal distribution and s_i the survival in origin *i*. Origin refers to the pure WEM population in the case of WEM, and to WEM x *i* hybrids for the other origins. Then the difference in liability between the hybrids and the WEM population were calculated as $y_i = m_i - m_{WEM}$. Under the hypothesis of an additive genetic effect, the liability of the pure populations was calculated as $m2_i = m_{WEM} + 2y_i$. This liability was back transformed to a proportion of survivors in the pure populations $p2_i = \Phi(m2_i)$, where Φ is the cumulative distribution function of the standard normal distribution.

3. Results

3.1. Pedigree recovery

96% (2011 of 2100 individuals) were assigned to a unique parental pair and 4% (89 individuals) were not assigned. The number of fish per population cross combination at tagging was rather balanced (447 individuals in the ♀WEM x ♂NAT, 457 individuals in ♀WEM x ♂WEM, 519 individuals in the ♀WEM x ♂NEM, and 588 individuals in the ♀WEM x ♂SEM).

The number of fish of each population, which were challenged for NNV, was also determined *a posteriori*. It varied from 326 to 407 (average of 355).

3.2. ELISA results

optical density (OD) values of 2.00 in the brains from inoculated fish sampled at day 6during the peak of mortality. At that period, the level of betanodavirus protein reached 867 ng/ml, showing a high multiplication of the virus in the brain associated with fish mortality and clinical signs of the disease. The betanodavirus load decreased rapidly at day 10 post-challenge (OD= 0.15) just before the end of the peak of mortality. A low but detectable amount of virus was sill detected at day 20 (OD=0.07) on fish showing clinical signs of the disease, but no virus was detected by ELISA in brains from surviving fish at day 30 (OD= 0.04, lower than the cut-off limit of the ELISA).

3.3. Performance of populations

The mortality of all populations started at fourth day post-challenge (dpc) and reached a peak at 6 dpc. Then they decreased sharply from 7 dpc to 10 dpc. After that, they fluctuated until 24 dpc, where no more mortality happened. General survival was 73% at the end of the test. There were highly significant differences (P<0.001) in offspring survival between sire origins (ranging from 53 to 90% - Figure 1). The survival of offspring from SEM sires was the highest (90%), whereas in contrast, the lowest survival (53%) was in the offspring of NAT sires. The survival of the offspring belonging to NEM sires and WEM sires were 83% and 62%, respectively. The survival proportion between the progenies of SEM sires and NEM sires and between progenies of NAT sires and WEM sires were not statistically significant (table 1). There was a large variation of survival between sire families within population (P<0.001). The ranges of sire family survival within origins NAT, NEM, SEM and WEM were 23.8-73.3%, 42.1-88%, 65-100% and 81-100%, respectively (Figure 2).

Because of lacking of other dam populations, the results of this experiment only showed the large variations in VNN resistance between three hybrid progeny populations with WEM dams source (WEM x NAT, WEM x NEM, WEM x SEM) and pure WEM population. However, based on the results of pure WEM population and the other hybrid populations, the

equivalent genetic of VNN resistance between sire and dam. There were still more large variations between pure populations: 44% of NAT, 62% of WEM, 94% of NEM, and 99% of SEM.

There were significant differences in other performance traits between the sire origins. Offspring from NAT sires were larger and fatter than the other populations (P<0.01), and offspring from SEM sires were larger and fatter than and offspring from WEM sires (P<0.05). There were no differences between SEM and NEM for these traits (Table 1). Meanwhile, significant differences in DGC was only experienced between populations belonging to NAT sires and WEM sires (P<0.05).

3.4. Genetic parameters

3.4.1. Heritability of resistance to VNN and performance traits

The heritability of all traits was significant in both univariate and multivariate linear mixed sire models. The intra-population heritability of VNN resistance was moderate (0.26±0.11) while inter-population heritability (i.e. omitting the origin effect in the model) was higher than 1 (Table 2). The intra-population heritability of DGC and FA was moderate, whereas in contrast, inter-population heritability was high (but lower than 1). The heritability of BW was high both intra and inter-population (Table 2). In addition, the heritability of VNN resistance was also estimated separately in each population with a univariate linear mixed sire model. The heritability estimates for VNN resistance in the NAT, WEM and SEM populations were 0.20±0.21, 0.23 ±0.25 and 0.19±0.22, respectively, while that of the NEM population was high (0.42±0.31). However, standard errors were high, so that they were not significant.

3.4.2. Genetic and phenotypic correlations among traits

The genetic correlations between VNN resistance and the growth-related traits were negative, whereas in contrast, the genetic correlations between growth-related traits were positive (Table 3). To be more specific, the genetic correlation between VNN resistance and BW (-

between VNN resistance and FA was not significant (-0.13±0.19). The genetic correlations between DGC and FA (0.28±0.21) and between DGC and BW (0.42±0.13) were moderate. Meanwhile, there was a strong genetic correlation between FA and BW (0.71±0.11) (Table 3).

4. Discussion

In this experiment, the first major result is the demonstration of substantial differences in resistance to VNN between four wild European sea bass populations. These results are coherent with their genetic differentiation, as reported in population genetics studies (Naciri et al. 1999; Bahri-Sfar et al. 2000). There were significant and large differences in VNN resistance between all populations except between NEM and SEM and between NAT and WEM (Table 1). The absence of difference between NEM and SEM could be explained by their limited genetic differences, as they are both subpopulations of the Eastern Mediterranean population (Castilho & Ciftci 2005). The strong level of differentiation between East and West Mediterranean is seen in several species and would be linked to limited circulation through the Siculo-Tunisian Strait (Bahri-Sfar et al. 2000), while the differentiation between Atlantic and Mediterranean population would be linked to the existence of two different glacial refuges during Quaternary glaciations, as also proposed in meagre *Argyrosomus regius* (Haffray et al., 2010). Further to this divergence period, a secondary contact would be the cause of the greater proximity between the WEM and ATL populations, when compared to Easter Mediterranean populations (Tine et al. 2014). In addition, it can be noted that VNN resistance here is also proportional to the summer temperature, which is higher in the eastern Mediterranean than in the Atlantic or in the West Mediterranean. It was previously shown that the SEM population was better adapted than the others to grow at high temperature (Vandeputte et al. 2014), and it could be that part of the resistance observed here is linked to a better tolerance to high water temperatures (25°C) at which the NNV challenge was performed.

variability for resistance to VNN, and more specifically to the RGNNV genotype in a warm water fish species, as demonstrated by a moderate heritability estimate (0.26±0.11). The inter-population heritability was also estimated ignoring the origin effect, thus considering the sea bass as a vast meta-population. However, this appeared irrelevant, as because of the difference of mean survival in the different population, the value of inter-population heritability was higher (1.09±0.22) than the theoretical maximum of 1. In addition, the heritability of VNN resistance in each separate population was estimated, and although estimates were moderate to high (0.19-0.42), they were not significant. It may be because the number of fish of separate populations were not enough to reliably estimate genetic parameters (range 326-407 individuals per population). Heritability for resistance to RGNNV in European sea bass is much lower than the heritability reported for resistance to the BFNNV genotype of NNV in Atlantic cod, which ranges from 0.68 to 0.75 (Ødegård et al. 2010; Bangera et al. 2011). However, similar to Atlantic cod, we evidenced large differences in the resistance of different natural populations. The heritability of resistance to viral diseases have been shown to be moderate to high in few farmed fish, mainly in salmonids. For instance, the moderate to high heritability for ISAV and IPNV (0.24-0.55) have been also experienced (Doan et al. 2016). To our knowledge, this was the first attempt to estimate heritability of resistance to any infectious disease in the European sea bass. Taken altogether, selective breeding for resistance to VNN in European sea bass is thus a good perspective as done for other viral diseases in salmonids (Yáñez et al. 2014), and genetic gain in NNV resistance can be expected both by selection between populations or between families within populations.

The phenotypic and genetic correlations between VNN resistance and growth-related traits (including DGC, FA and BW) were not significant except the correlations between VNN resistance and BW. Different correlations between viral disease resistance and growth traits have been reported in previous studies. Even though positive genetic correlations have been recorded between growth traits and most diseases (Gjedrem 2015), several negative

and BL (Henryon et al. 2002) in rainbow trout, between WSSV and TSV and growth in whiteleg shrimp (Gitterle et al. 2005; Argue et al. 2002). Meanwhile, the correlation between NNV and BW was not significant and close to zero in Atlantic cod (Bangera et al. 2011). In our study, there were moderate and negative genetic correlations between VNN resistance and growth traits, so that selection for fast growth may lead to an increased susceptibility to VNN. This should be taken with appropriate caution as the standard errors of the correlations are rather large. However, this negative correlation was also true at the population level, as we showed that the mean BW of Northern Atlantic population was the highest, while their resistance to VNN was the lowest. However, their resistance could be improved using final cross-breeding with wild Eastern Mediterranean. Resistance could also be improved by genetic introgression of SEM and NEM populations in the WEM or NAT stocks.

Direct selection of survivors may be more efficient than family-based selection among unchallenged sibs. However, because of the risk of vertical transmission of pathogens, it is not desirable (Gheyas et al. 2010). Recent advances in genomic tools and procedure, promise to overcome this limitation, enabling the evaluation of sibs within families, either using the genotyping at specific QTLs (quantitative trait loci) linked to the resistance in Marker-assisted selection (MAS), or using random massive genotype data to perform Genomic Selection (GS) (Ødegård et al. 2011, Vandeputte & Haffray 2014). QTLs for viral disease resistance have been identified in most viral diseases in salmonids. Several QTLs responsible for VNN resistance were also reported in Atlantic cod (Baranski et al. 2010; Yu et al. 2014) and in Asia sea bass (Liu et al. 2016). Identification of such QTLs VNN resistance in European sea bass may help to perform Marker-Assisted introgression, and is therefore of particular relevance for the future development of selection for VNN resistance in European sea bass.

5. Conclusion

were 99%, 94%, 62%, and 44%, respectively, as well as between sire families within strain of NNV resistance. Furthermore, the within-population heritability of resistance to NNV was recorded for the first time in European sea bass. The large and significant variations in survival, the moderate heritability estimated on liability scale give good prospects of selective breeding of European sea bass for improved resistance to VNN disease.

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Table 1: Differences in survival and production traits in the offspring of European sea bass from four sire origins (NAT: WEM, NEM and SEM) mated to the same WEM dams. Origins with different superscripts are statistically different ($P<0.001$). Estimated survival as pure strains is based on an additive liability model.

Table 2: Heritability (intra- and inter-populations) of resistance to VNN and production traits. h^2_o is the heritability estimated on the observed scale, h^2_u is the heritability on the liability scale. The heritability estimation of DGC based on the data calculated from 180 dph to 431 dph while that of BW lied on the data at 180 dph. Meanwhile that of FA based on data recorded at 431 dph.

Table 3: Genetic (above the diagonal) and phenotypic (below the diagonal) correlations among traits. $DGC_{180\text{-}431}$ was calculated from 180 dph to 431 dph while BW was collected at 180 dph. FA data was recorded at 431 dph.

Figure 1: Evolution of cumulated survival in the offspring derived from 4 populations of European sea bass sires (NAT: North Atlantic, NEM: North-East Mediterranean; SEM: South-East Mediterranean; WEM: West Mediterranean), mated with WEM dams, following experimental infection by NNV.

Figure 2: The variations of survival of sire families within and between populations during NNV test. North Atlantic in red, North-East Mediterranean in green, South-East Mediterranean in blue and West Mediterranean in yellow.

Table 1: Differences in survival and production traits in the offspring of European sea bass from four sire origins (NAT, WEM, NEM and SEM) mated to the same WEM dams. Origins with different superscripts are statistically different (P<0.05). Estimated survival as pure strains is based on an additive liability model.

Sire origin	Total number of fish at tagging	Mean BW_{180} at tagging (SD)	Mean BL_{180} at tagging (SD)	Total number of fish at NNV challenge	Mean BW_{180} of NNV challenge (SD)	Mean BL_{180} of NNV challenge (SD)	Survival ratio (%)	Estimated survival as pure strain (%)	Total number of unchallenged fish with parentage	Mean of $DGC_{180\text{-}431}$ of unchallenged fish (SD)	Mean of FA_{431} of unchallenged fish (SD)
ATOL		ATOL:0000351	ATOL:0001658		ATOL:0000351	ATOL:0001658				ATOL:0002174	ATOL:0001663
NAT	447	17.7(7.0)^{a}	113.3(13.9)^{a}	326	18.2(7.3)	114.2(14.0)	53^{a}	44	117	0.96(0.15)^{a}	3.98(1.63)^{a}
WEM	457	14.2(4.6)^{c}	107.8(10.6)^{b}	327	14.6(4.8)	109.0(10.8)	62^{a}	62	124	0.92(0.11)^{b}	2.73(1.21)^{b}
NEM	519	15.6(5.3)^{bc}	109.9(11.6)^{b}	360	15.9(5.5)	110.7(11.7)	83^{b}	94	144	0.96(0.13)^{ab}	2.89(1.45)^{bc}
SEM	588	15.7(5.7)^{b}	109.8(12.3)^{b}	407	16.3(5.8)	110.7(12.4)	90^{b}	99	160	0.92(0.14)^{ab}	3.30(1.55)^{c}
Total	**2011**	**15.8(5.8)**	**110.1(12.3)**	**1420**	**16.3(6.0)**	**111.2(12.4)**	**73**		**545**	**0.94(0.13)**	**3.21(1.54)**

related traits. h^2_o is the heritability estimated on the observed scale, h^2_u is the heritability on the liability scale. The heritability estimation of DGC was based on the data calculated from 180 dph to 431 dph while that of BW on the data at 180 dph. Meanwhile that of FA was estimated based on data recorded at 431 dph.

Multivariate model

	Intra-population		Inter-populations	
Traits	**h^2_o**	**h^2_u ± SE**	**h^2_o**	**h^2_u ± SE**
VNN resistance	0.14	0.26 ± 0.10	0.61	1.09±0.20
$DGC_{180-431}$		0.47±0.12		0.55±0.10
FA_{431}		0.44±0.13		0.78±0.15
BW_{180}		0.54±0.10		0.76±0.12

Univariate model

	Intra-population		Inter-populations	
Trait	**h^2_o**	**h^2_u ± SE**	**h^2_o**	**h^2_u ± SE**
VNN resistance	0.14	0.26 ± 0.11	0.61	1.09±0.22
$DGC_{180-431}$		0.48±0.16		0.52±0.16
FA_{431}		0.37±0.14		0.67±0.18
BW_{180}		0.55±0.11		0.76±0.13

among traits. $DGC_{180-431}$ was calculated from 180 dph to 431 dph while BW was collected at 180 dph. FA data was recorded at 431 dph.

Traits	VNN resistance	$DGC_{180-431}$	FA_{431}	BW_{180}
VNN resistance		-0.28±0.20	-0.13±0.19	-0.35±0.14
$DGC_{180-431}$	-0.02		0.28±0.21	0.42±0.13
FA_{431}	-0.01	0.16		0.71±0.11
BW_{180}	-0.14	0.34	0.28	

Argue, B.J., Arce, S.M., Lotz, J.M., Moss, S.M., 2002. Selective breeding of Pacific white shrimp (*Litopenaeus vannamei*) for growth and resistance to Taura Syndrome Virus. Aquaculture 204, 447–460.

Bahri-Sfar, L., Lemaire, C., Hassine, O.K.B., Bonhomme, F., 2000. Fragmentation of sea bass populations in the western and eastern Mediterranean as revealed by microsatellite polymorphism. Proceedings of the Royal Society of London Series B 267, 929–935.

Bangera, R., Ødegård, J., Præbel, A.K., Mortensen, A., Nielsen, H.M., 2011 Genetic correlations between growth rate and resistance to vibriosis and viral nervous necrosis in Atlantic cod (*Gadus morhua* L.). Aquaculture 317, 67–73.

Bangera, R., Ødegård, J., Nielsen, H.M., Gjøen, H.M., Mortensen, A., 2013. Genetic analysis of vibriosis and viral nervous necrosis resistance in Atlantic cod (*Gadus morhua* L.) using a cure model. Journal of Animal Science 91, 3574–3582.

Baranski, M., Præbel, A.K., Sommer, A., Kirste, K.H., Wesmajervi, M., 2010. Major quantitative trait loci for viral nervous necrosis resistance in Atlantic cod (*Gadus morhua* L.). In: *Proceedings*, 9th World Congress of Genetics Applied to Livestock Production, August 1st–6th, 2010, Leipzig, Germany.

Bates, D.M., Machler, M., Bolker, B.M., Walker, S.C., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67, 1–48. Available at: http://lme4.r-forge.r-project.org/lMMwR/lrgprt.pdf.

Le Breton, A., Grisez, L., Sweetman, J., Ollevier, F., 1997. Viral nervous necrosis (VNN) associated with mass mortalities in cage-reared sea bass, *Dicentrarchus labrax* (L.). Journal of Fish Diseases 20, 145–151.

Breuil G., Mouchel, O., Fauvel, C., Pepin, J.F., 2001. Sea bass Dicentrarchus labrax nervous necrosis virus isolates with distinct pathogenicity to sea bass larvae. Diseases of Aquatic Organisms 45, 25–31.

Breuil, G., Bonamib, J.R., Pepin, J.F., Pichot, Y., 1991. Viral infection (picorna-like virus) associated with mass mortalities in hatchery-reared sea bass (*Dicentrarchus labrax* L.) larvae and juveniles. Aquaculture 97, 109–116.

Brudeseth, B.E., Wiulsrød, R., Fredriksen, B.N., Lindmo, K., Løkling, K.E., Bordevik, M., Steine, N., Klevan, A., Gravningen, K., 2013. Status and future perspectives of vaccines for industrialised fin-fish farming. Fish and Shellfish Immunology 35, 1759–1768.

Castilho, R., Ciftci, Y., 2005. Genetic differentiation between close eastern Mediterranean *Dicentrarchus labrax* (L.) populations. Journal of Fish Biology 67, 1746–1752.

Castric, J., Thiéry, R., Jeffroy, J., Kinkelin, P.D., Raymond, J.C., 2001. Sea bream *Sparus aurata*, an asymptomatic contagious fish host for nodavirus. Diseases of Aquatic Organisms 47, 33–38.

Chavanne, H., Chatain, B., Haffray, P., 2008. Review on breeding and reproduction of European sea bass aquaculture species. http://shkola.of.by/docs/index-28803.html

Cho, C.Y., 1992. Feeding systems for rainbow trout and other salmonids with reference to current estimates of energy and protein requirements. Aquaculture 100, 107–123.

Dempster, E.R., Lerner, I.M., 1950. Heritability of threshold characters. Genetics 35, 212–236.

Doan, Q-K., Vandeputte, M., Chatain, B., Morin, T., Allal, F., 2016. Viral Encephalopathy and Retinopathy in Aquaculture: A Review. *Journal of Fish Diseases, doi:10.1111/jfd.12541*

Chatain, B., 2008. Heritabilities and GxE interactions for growth in the European sea bass (*Dicentrarchus labrax* L.) using a marker-based pedigree. Aquaculture 275, 81–87.

Falconer, D.S., Mackay, T.F.C., 1996. Introduction to quantitative genetics, 4th edition. Longman Group.

FAO (2014) http://www.fao.org/3/a-i3720e.pdf

Fauvel, C., Suquet, M., Dreanno, C., Menu, B., 1998. Cryopreservation of sea bass (*Dicentrarchus labrax*) spermatozoa in experimental and production simulating conditions. Aquatic Living Resources 11, 387–394.

Gheyas, A.A., Houston, R.D., Mota-Velasco, J.C., Guy, D.R., Tinch, A.E., Haley, C.S., Woolliams, J.A., 2010. Segregation of infectious pancreatic necrosis resistance QTL in the early life cycle of Atlantic salmon (*Salmo salar*). Animal Genetics 41, 531–536.

Gjedrem, T., Robinson, N., 2014. Advances by Selective Breeding for Aquatic Species: A Review. Agricultural Sciences 5, 1152-1158.

Gjedrem, T., 2015. Disease resistant fish and shellfish are within reach: a review. Journal of Marine Science and Engineering 3, 146–153.

Golik, W., Dameron, O., Bugeon, J., Fatet, A., Hue, I., Hurtaud, C., Reichstadt, M., Meunier-Salaün, M.C., Vernet, J., Joret, L., Papazian, F., Nédellec, C., Le Bail, P.Y., 2012. ATOL: the multi-species livestock trait ontology. 6th International Conference on Metadata and Semantic Research, 28–30 Nov.2012, Cadiz, Spain.

Gomez-Casado, E., Estepa, A., Coll, J.M., 2011. A comparative review on European-farmed finfish RNA viruses and their vaccines. Vaccine 29, 2657–2671.

Groeneveld, E., Kovac, M., Mielenz, N., 2008. VCE User's Guide and Reference Manual Version 6.0.

Haddad-Boubaker, S., Bigarré, L., Bouzgarou, N., Megdich, A., Baud, M., Cabon, J., Chéhida N.B., 2013. Molecular epidemiology of betanodaviruses isolated from sea bass and sea bream cultured along the Tunisian coasts. Virus genes 46, 412–422.

Haffray, P., Tsigenopoulos, C.S., Bonhomme, F., Chatain, B., Magoulas, A., Rye, M., Triantafyllidis, A., Triantaphyllidis, C., 2006. European sea bass - *Dicentrarchus labrax*. Report geneimpact 3, 40–46.

Haffray, P., Malha, R., Ould Taleb Sidi, M., Prista, N., Hassan, M., Castelnaud, G., Karahan-Nomm, B., Gamsiz, K., Sadek, S., Bruant, J-S., Balma, P., Bonhomme, F., 2012. Very high genetic fragmentation in a large marine fish, the meagre *Argyrosomus regius* (Sciaenidae, Perciformes): Impact of reproductive migration, oceanographic barriers and ecological factors. Aquatic Living Resources 25, 173-183.

Henryon, M., Jokumsen, A., Berg, P., Lund, I., Pedersen, P.B., Olesen, N.J., Slierendrecht, W.J., 2002. Genetic variation for growth rate, feed conversion efficiency, and disease resistance exists within a farmed population of rainbow trout. Aquaculture 209, 59–76.

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2016. lmerTest: Tests in Linear Mixed Effects Models. R package version 2.0-33. https://CRAN.R-project.org/package=lmerTest

Liu, P., Wang, L., Wan, Z.Y., Ye, B.Q., Huang, S., Wong, S.M., Yue, G.H., 2016. Mapping QTL for resistance against viral nervous necrosis disease in Asian seabass. Marine Biotechnology 18, 107–116.

Lynch, M., Walsh, B., 1998. Genetics and Analysis of Quantitative Traits. Sinauer Associates, Sunderland, MA.

Atlantic/Mediterranean transition in sea bass (*Dicentrarchus labrax*). Journal of Heredity 90, 591–596.

Ødegård, J., Sonesson, A.K., Yazdi, M.H., Meuwissen, T.H.E., 2009. Introgression of a major QTL from an inferior into a superior population using genomic selection. Genetics, selection, evolution : GSE 41, 38.

Ødegård, J., Baranski, M., Gjerde, B., Gjedrem, T., 2011. Methodology for genetic evaluation of disease resistance in aquaculture species: challenges and future prospects. Aquaculture Research 42, 103–114.

Ødegård, J., Sommer, A.-I.,Præbel, A.K., 2010. Heritability of resistance to viral nervous necrosis in Atlantic cod (*Gadus morhua* L.). Aquaculture 300, 59–64.

Saillant, E., Dupont-Nivet, M., Haffray, P., Chatain, B., 2006. Estimates of heritability and genotype–environment interactions for body weight in sea bass (*Dicentrarchus labrax* L.) raised under communal rearing conditions. Aquaculture 254, 139–147.

Saillant, E., Dupont-Nivet, M., Saboureult, M., Haffray, P., Laureau, S., Vidal, M-O., Chatain, B., 2009. Genetic variation for carcass quality traits in cultured sea bass (*Dicentrarchus labrax*). Aquatic Living Resources 22, 105–112.

Saillant, E., Fostier, A., Haffray, P., Menu, B., Thimonier, J., Chatain, B., 2002. Temperature effects and genotype-temperature interactions on sex determination in the European sea bass (*Dicentrarchus labrax* L.). Journal of Experimental Zoology 292, 494–505.

Sansone, G., Fabbrocini, A., Ieropoli, S., Langellotti, A.L., Occidente, M., Matassino, D., 2002. Effects of extender composition, cooling rate, and freezing on the motility of sea bass (*Dicentrarchus labrax* L.) spermatozoa after thawing. Cryobiology 44, 229–239.

Thiery, R., Cozien, J., de Boisseson, C., Kerbart-Boscher, S., Nevarez, L., 2004. Genomic classification of new betanodavirus isolates by phylogenetic analysis of the coat protein gene suggests a low host-fish species specificity. The Journal of General Virology 85, 3079–3087.

Tine, M., Kuhl, H., Gagnaire, P-A., Louro, B., Desmarais, E., Martins, R.S.T., Hecht, J., Knaust, F., Belkhir, K., Klages, S., Dieterich, R., Stueber, K., Piferrer, F., Guinand, B., Bierne, N., Volckaert, F.A.M., Bargelloni, L., Power, D.M., Bonhomme, F., Canario, A.V.M., Reinhardt, R., 2014. European sea bass genome and its variation provide insights into adaptation to euryhalinity and speciation. *Nature Communications* **5,** 5770.

Toffan, A., Panzarin, V., Toson, M., Cecchettin, K., Pascoli, F., 2016. Water temperature affects pathogenicity of different betanodavirus genotypes in experimentally challenged *Dicentrarchus labrax*. Diseases of Aquatic Organisms 119, 231–238.

Vandeputte, M., Dupont-Nivet, M., Chavanne, H., Chatain, B., 2007. A polygenic hypothesis for sex determination in the European sea bass *Dicentrarchus labrax*. Genetics 176, 1049–1057.

Vandeputte, M., Garouste, R., Dupont-Nivet, M., Haffray, P., Vergnet, A., Chavanne, H., Laureau, S., Ron, T.B., Pagelson, G., Mazorra, C., Ricoux, R., Marques, P., Gameiro, M., Chatain, B., 2014. Multi-site evaluation of the rearing performances of 5 wild populations of European sea bass (*Dicentrarchus labrax*). Aquaculture 424-425, 239–248.

Vandeputte, M., Dupont-Nivet, M., Haffray, P., Chavanne, H., Cenadelli, S., Parati, K., Vidal, M-O., Vergnet, A., Chatain, B., 2009. Response to domestication and selection for growth in the European sea bass (*Dicentrarchus labrax*) in separate and mixed tanks. Aquaculture 286, 20–27.

Vandeputte, M., Haffray, P., 2014. Parentage assignment with genomic markers: a major

farmed aquatic animals. Frontiers in genetics 5:432.

Vandeputte, M., Mauger, S., Dupont-Nivet, M., 2006. An evaluation of allowing for mismatches as a way to manage genotyping errors in parentage assignment by exclusion. Molecular Ecology Notes 6, 265–267.

Vandeputte, M., Quillet, E., Chatain, B., 2012. Are sex ratios in wild European sea bass (*Dicentrarchus labrax*) populations biased? Aquatic Living Resources 25, 77–81.

Yáñez, J. M., Houston, R.D., Newman, S., 2014. Genetics and genomics of disease resistance in salmonid species. Frontiers in genetics 5:415.

Yu, X.Z., Meuwissen, T.H.E., Baranski, M., Sonesson, A.K., 2014. Selective breeding against infectious diseases in Atlantic cod with whole genome sequence data. In: Proceedings, 10th World Congress of Genetics Applied to Livestock Production, August 17th–22th, Vancouver, BC, Canada.

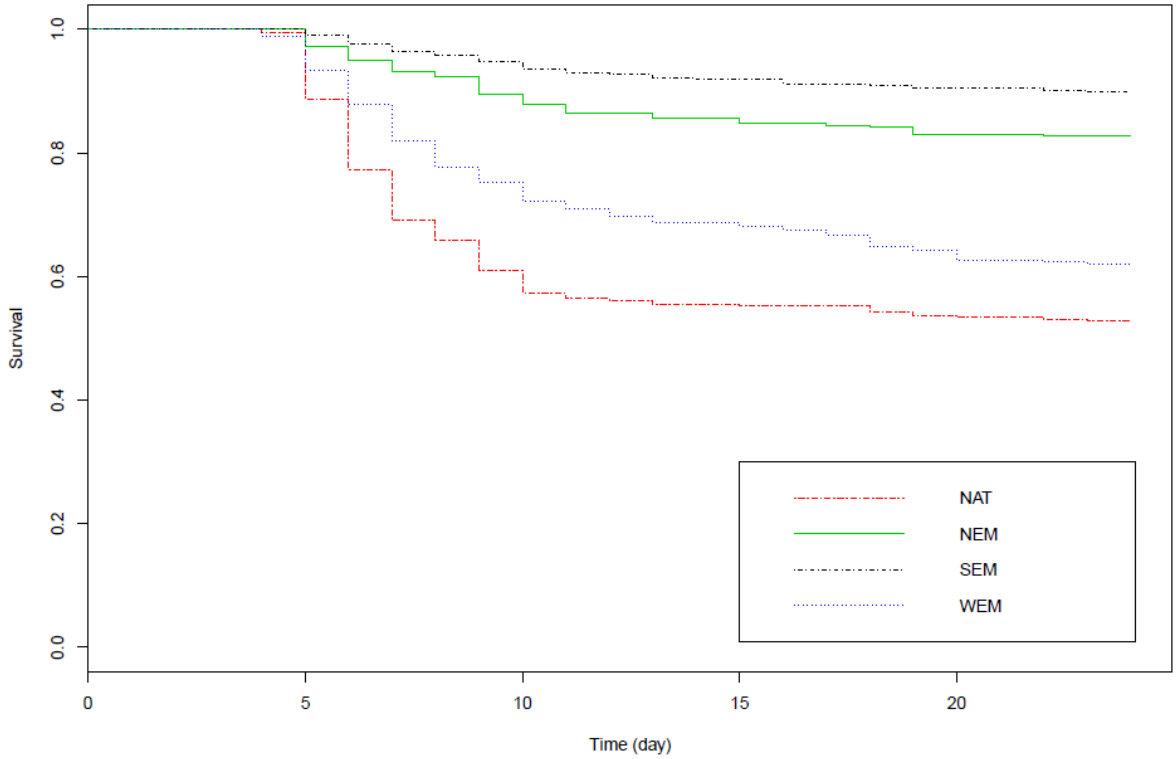

Fig. 1

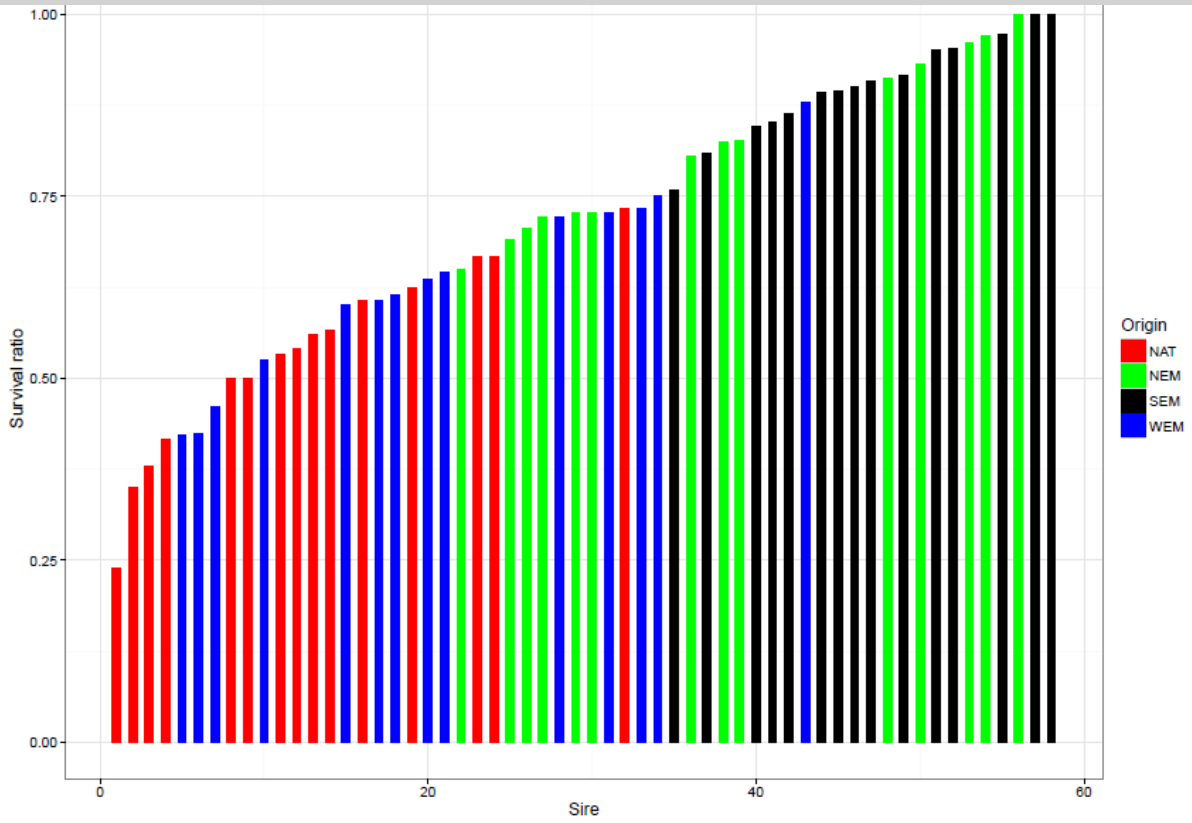

Fig. 2