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GENETIC AND GENOMIC VARIATION OF RESISTANCE TO VIRAL NERVOUS NECROSIS IN WILD POPULATIONS OF EUROPEAN SEABASS (*Dicentrachus labrax*)

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List of publications and manuscripts

Publications

Doan, Q.-K., Vandeputte, M., Chatain, B., Morin, T., Allal, F., 2017. Viral encephalopathy and retinopathy in aquaculture: a review. *J. Fish Dis.,* **40***,* 717-742.

Khanh Doan Q., Marc Vandeputte, Béatrice Chatain, Pierrick Haffray, Alain Vergnet, Gilles Breuil and François Allal, 2017. Genetic variation of resistance to Viral Nervous Necrosis and genetic correlations with production traits in wild populations of the European seabass (Dicentrarchus labrax). *Aquaculture*, **478**, 1-8.

Manuscripts

Doan, Q.-K., Marc Vandeputte, Béatrice Chatain, Pierrick Haffray, Alain Vergnet, François Allal. Construction of a medium-density SNP linkage map and mapping of QTL for resistance to viral nervous necrosis of European seabass.

Doan, Q.-K., Marc Vandeputte, Béatrice Chatain, Pierrick Haffray, Alain Vergnet, François Allal. Genome-wide association study and genomic evaluations for resistance to viral nervous necrosis of European seabass.

Conference papers

Doan Q.K., Vandeputte M., Chatain B., Vergnet A., Allal F., 2015. Combining VITASsIGN and COLONY: An efficient practical procedure for parentage assignment with missing parent genotypes. Poster presentation, International symposium on genetics in aquaculture XII, Spain, June 21st-17th, 2015

Doan Q.K. 2015. Selective Breeding: The perspective procedure adapting to climate change in Aquaculture. *International Conference on "Livelihood Development and Sustainable Environment Management in the Context of Climate Change". November 13-14, 2015* at TUAF, Thai Nguyen City, Vietnam.

Doan, Q-K., Vandeputte, M., Chatain, B., Morin, T., Allal, F., 2015. Selective Breeding for Resistance to Viral Nervous Necrosis Disease: Prospective Procedure for Sustainable Development in Aquaculture. *Sustainable Fishery Development Workshop, November 17 – 28, 2015 in* Taiwan.

Allal F., **Doan Q.K.**, Chatain B., Vergnet A. and Vandeputte M. 2015. Combining vitassign and colony for pedigree reconstruction in a case of factorial mating with missing parental genotypes. Conference: Aquaculture Europe 2015, At Rotterdam, The Netherlands.

Table of contents

Résumé substantiel en français

L'aquaculture durable et ses défis

Le secteur de l'aquaculture et de la pêche joue un rôle important dans la sécurité alimentaire mondiale. En 2014, le montant de la production aquacole (à l'exclusion des plantes aquatiques) était de 73,8 millions de tonnes pour une valeur totale estimée à 160,2 milliards de dollars, contre 93,4 millions de tonnes de pêche de capture (FAO 2016). Aujourd'hui, l'aquaculture fournit plus de 50% des poissons destinés à la consommation humaine (FAO 2016). Alors que la consommation humaine de poisson devrait fortement augmenter à court terme, on s'attend à ce que le volume de la pêche soit plus ou moins stable. Ainsi, en 2025, la production aquacole prévue pour la consommation humaine (112 millions de tonnes) dépassera largement la production des pêches de capture (FAO 2016). Par conséquent, l'aquaculture est et sera une clé majeure pour aborder la sécurité alimentaire mondiale.

Alors que l'aquaculture a continuellement augmenté au cours des deux dernières décennies, que ce soit concernant la production totale ou les zones cultivées (FAO 2016), elle fait face à de nombreux défis. Le réchauffement climatique devrait conduire à une augmentation des épidémies de maladie dans certains domaines spécifiques (Cochrane et al., 2009). La pollution de l'eau et l'eutrophisation causée par la production aquacole (aliments pour animaux, déchets) constituent un défi encore plus large. En outre, dans un contexte de stagnation des pêches, il est essentiel d'assurer son indépendance vis-à-vis des prises de poisson par des pêcheries industrielles, transformées en farine de poisson et d'huile de poisson destinées à nourrir les poissons d'élevage. Enfin, la réduction des épidémies de maladies menant à l'utilisation d'antibiotiques et d'autres médicaments dans l'aquaculture est un défi majeur pour l'acceptabilité sociale, les bénéfices économiques et la protection de l'environnement. Ainsi, les épidémies (maladies infectieuses et parasitaires) constituent l'une des principales menaces pour l'aquaculture durable (Gjedrem 2015, FAO 2016). Parmi les stratégies existantes pour réduire les épidémies et leurs effets négatifs sur l'environnement, soit directement (utilisation excessive d'antibiotiques, transmission de pathogènes à des poissons sauvages), soit indirects (utilisation de ressources océanique pour élever des poissons qui ne seront pas consommés), l'amélioration génétique est l'une des plus prometteuses (Gjedrem 2015). En particulier, la sélection génomique (GS), permettant d'améliorer la précision de la sélection (Yáñez et al., 2014, Vallejo et al., 2017), est maintenant possible même dans les espèces «mineures» du fait de la forte baisse des coûts de génotypage, qui devrait se poursuivre dans le futur.

L'amélioration génétique comme clé pour un développement durable de l'aquaculture

Pour atteindre une aquaculture durable, l'amélioration génétique par sélection est particulièrement intéressante, car elle améliore durablement les performances animales. En effet, la sélection génétique permet une amélioration génétique cumulative et continue des traits vers un objectif souhaité. De plus, cet objectif visé peut être déplacé ou combiné avec d'autres avec le temps, au fur et à mesure que les priorités évoluent, afin d'optimiser la rentabilité et de réduire les impacts environnementaux.

Parmi les trois principales stratégies d'amélioration, la sélection massale reste la plus utilisée pour les espèces qui font des pontes de masse et pour les espèces à valeur économique limitée (Vandeputte et al., 2009a). En sélection massale, les performances individuelles des animaux sont la seule information nécessaire, ce qui en fait une méthode simple et relativement peu coûteuse. Une deuxième méthode est la meilleure prédiction linéaire non biaisée (BLUP) qui utilise des informations sur les parents pour augmenter la précision de sélection, permettant également la sélection pour les phénotypes létaux qui ne peuvent être enregistrés sur le candidat à la reproduction (rendement du filet, résistance aux maladies). Enfin, la sélection génomique (Meuwissen et al., 2001) comprend des informations génomiques avec les mêmes avantages, mais une augmentation accrue de la précision. L'amélioration génétique est pratiquée depuis longtemps en aquaculture (Vandeputte et al. 2009b, Gjedrem 2015). Cependant, si la croissance a tout d'abord été fortement ciblée comme caractère d'intérêt, d'autres caractères sont attendus mieux adaptés au développement durable de l'aquaculture. En particulier, le rendement du filet (partie de la croissance investie dans la production de chaire comestible), l'efficacité alimentaire (proportion de l'apport alimentaire transformé en gain de poids) et la résistance aux maladies, peuvent être classés comme des caractères d'efficacité à volume de production constant. Si au niveau mondial, l'utilisation d'espèces aquatiques améliorées reste faible (8,2% du volume total d'aquaculture) (Gjedrem 2015), en Europe, la situation est différente avec un pourcentage de production de poissons issues de ressource génétique améliorés estimé entre 80 et 83% du volume total de cinq espèces principales (saumon atlantique, truite arc-en-ciel, le bar, la dorade et le turbot) (Janssen et al., 2017). Si l'on considère maintenant le cas spécifique de sélection pour la résistance aux maladies, de nombreuses études génétiques et génomiques pour la résistance aux maladies ont été menées dans le bétail (Bishop & Woolliams 2014), et maintenant chez de plus en plus d'animaux aquatiques (Gjedrem 2015), même avec une application pratique dans les programmes de sélection (Chavanne et al., 2016).

Le caractère de résistance aux maladies est assez spécifique, car il n'est pas souhaité de sélectionner des poissons survivants à un épisode de mortalité en raison du risque de transmission verticale de pathogènes, ce qui empêche une utilisation efficace de la sélection massale. Pour contourner le problème de transmission des agents pathogènes, il est possible d'utiliser la sélection sur apparentés par BLUP, où les candidats sont sélectionnés en fonction de leur relation avec des individus pour lesquels un phénotype a été enregistré. Typiquement, dans la sélection sur apparentés pour la résistance aux maladies, les candidats à sélections sont conservés dans un environnement sans agents pathogènes, tandis que des individus des mêmes familles sont confrontés au pathogène. Les candidats sont ensuite choisis en utilisant des valeurs génétiques estimées en fonction des performances de survies des collatéraux de la famille. Bien que efficace, cette méthode de sélection prend du temps et tous les candidats issus de la même famille sont estimés équivalents en termes de valeur génétique. Cela limite l'intensité de sélection car les individus ne peuvent pas être classés au sein des familles, et il est nécessaire de conserver un nombre suffisant de familles afin de contenir la consanguinité à un niveau raisonnable dans un programme de sélection. Des alternatives plus récentes pour améliorer la résistance aux maladies sont la sélection assistée par marqueur (MAS) ou la sélection génomique (GS). Avec le MAS, les candidats sont sélectionnés en fonction de leur génotype à des loci à effet fort (QTL) liés aux phénotypes résistants aux maladies. La GS, elle est effectuée à partir de marqueurs génotypés sur l'ensemble du génome, qui ne sont pas forcément liés à la résistance à la maladie, mais qui sont assez nombreux pour que toute partie du génome avec un effet mineur sur la résistance soit en déséquilibre de liaison avec les SNP génotypés. Avec ces méthodes, les candidats peuvent être choisis plus précisément, et potentiellement plus tôt dans la vie, en fonction de leur seul génotype.

Le bar: biologie, production et marchés

Le bar vit dans les eaux côtières de l'océan Atlantique du sud de la Norvège (60 ° N) au Sahara occidental (30 ° N) et dans toute la Méditerranée et la mer Noire, dans laquelle il est également appelé « loup ». L'espèce a été divisé en trois populations principales basées sur l'étude de sa diversité génétique, soit la population atlantique, la population de la Méditerranée occidentale et la population de la Méditerranée orientale (Naciri et al., 1999, Bahri-Sfar et al., 2000). Parmi ces groupes principaux, il a été montré que la population de la Méditerranée orientale était subdivisée en deux sous-populations: la population Nord-Est Méditerranée et la population Sud-Est Méditerranée (Castilho & Ciftci 2005). Contrairement à cette observation, aucune subdivision significative n'a été trouvée dans les populations de l'Atlantique et de la

Méditerranée occidentale (Haffray et al., 2006). Le bar est une espèce eurythermale et euryhaline. Par conséquent, ils peuvent fréquenter les eaux côtières, et se produisent également dans les estuaires et les lagunes d'eau saumâtre. Parfois, ils s'aventurent en amont dans l'eau douce. La saison de reproduction se déroule en hiver dans les populations méditerranéennes (décembre à mars) et jusqu'en juin dans la population de l'Atlantique (Perez-Rufaza et Marcos 2014). Les bars sont des prédateurs et leur régime alimentaire comprend les petits poissons, les crevettes, les crabes et les seiches.

En ce qui concerne l'aquaculture, le bar est l'un des poissons cultivés à plus haute valeur ajoutée de la région méditerranéenne. Sa production a atteint 49% du volume total de poissons marins méditerranéens en 2015, avec 317,029 tonnes produites (rapport annuel de FEAP, 2016). Bien qu'il soit cultivé actuellement au Royaume-Uni, en France, au Portugal, en Italie, en Croatie, en Tunisie, en Israël, à Oman et aux Émirats arabes unis, la principale production provient de la Turquie, de la Grèce et de l'Espagne (FEAP, 2016). Le prix de détail moyen du bar a fortement diminué de 1985 à 2005 en raison de l'augmentation de la production aquacole, il est progressivement passé de 8,37 euros en 2005 à 11,13 euros en 2014 (Monfort 2007, marché européen de la pêche, édition 2016). Bien que la France soit un producteur mineur dans l'aquaculture du bar avec seulement 2,244 tonnes en 2014, la consommation française de bar d'élevage est la plus élevée, couvrant 64% du volume total débarqué (7 000 tonnes) et 67% de la valeur totale (79 millions d'euros) en 2014. L'aquaculture du bar doit cependant être comparée au secteur de la pêche dans l'Union européenne, qui tend à diminuer légèrement ou à stagner (selon les pays de compte) avec une capture moyenne de moins de 8 000 tonnes par an au cours de la dernière décennie, la France étant le pays le plus actif avec près de 5 000 tonnes capturées par an.

L'encéphalopathie et rétinopathie virale dans l'aquaculture du bar

L'une des principales menaces pour l'aquaculture de bar est les épidémies, et en particulier la nécrose nerveuse virale (VNN) (Doan et al., 2017). Cette pathologie causée par les betanodavirus, également connue sous le nom d'encéphalopathie et rétinopathie virale (VER) (Thiéry et al., 2011), se caractérise par des mortalités importantes dues à des lésions du système nerveux central et de la rétine. C'est l'une des menaces virales les plus graves pour les espèces de poissons marins en général, et en particulier pour les bars dans la région méditerranéenne (Poisa-Beiro et al., 2007; Terlizzi et al., 2012; OIE 2013). En effet, bien que des mortalités graves aient également été signalées chez les poissons adultes, la maladie affecte principalement les stades larvaires et juvéniles et peut induire une mortalité à 100% (Breuil et

al., 1991; Breton et al., 1997; Dalla Valle et al. , 2000; Curtis et al., 2001; Munday et al., 2002; Gomez-Casado et al., 2011; OIE 2013). La nodavirose ne se limite pas au bar, car les betanodavirus ont été isolés dans plus de 70 espèces aquatiques sauvages et élevées à travers le monde, dans des environnements d'eau froide et chaude, principalement marins mais aussi d'eau douce (voir Doan et al., 2017 pour plus de détails).

Malgré de nombreuses études menées pour trouver les meilleures façons de limiter les maladies virales, aucune procédure simple et efficace n'est disponible pour traiter la plupart des pathologies virales chez les poissons (Gomez-Casado et al., 2011; Doan et al., 2017). À ce jour, la nodavirose ne peut être contrôlée qu'en utilisant des méthodes de diagnostic efficaces pour surveiller les géniteurs, et des processus de désinfection (ozone ou autres produits chimiques) pour contrôler l'environnement d'élevage. Cependant, le suivi à long terme de ces mesures est souvent difficile et, en tout cas, ne peut pas éviter les infections sur les sites de grossissement (Mushiake et al., 1994). L'application de la vaccination peut être un moyen efficace de prévenir la maladie (Mushiake et al., 1994; Thiéry et al., 2006; Kai & Chi, 2008; Gomez-Casado et al., 2011). Cependant, cet outil n'est actuellement pas efficace en raison de plusieurs raisons (Nath et al., 2004):

• les inconvénients spécifiques des méthodes de vaccination existantes (oral, immersion, injection), (Bjarnheidur et al., 2007);

• la difficulté de protéger efficacement les stades larvaires précoces (Chi et al., 1999) en raison de la faisabilité pratique et de la maturité insuffisante du système immunitaire (Dos Santos et al., 2000);

• la diversité des virus VNN pour lesquels au moins quatre génotypes différents ont été décrits (Nishizawa et al., 1997; Skliris et al., 2001; Mori et al., 2003) alors que la vaccination traditionnelle visait généralement un type de génotype, potentiellement conduisant à la sélection de populations virales résistantes ou insensibles (Gjedrem 2015; Nath et al., 2004).

Les vaccins à ADN présentent de nombreux avantages par rapport aux vaccins antigéniques traditionnels et semblent être très attrayants pour l'industrie de l'aquaculture (Heppell & David, 2000). Cependant, le transfert horizontal de l'ADN transgénique des vaccins vers l'environnement est possible (Myhr et Dalmo, 2005). Par conséquent, aucune autorisation n'a été délivrée à ce jour pour ces vaccins à ADN dans les fermes piscicoles Européennes (Heppell & Davis, 2000; Gomez-Casado et al., 2011). À ce jour, un seul vaccin RGNNV inactivé existe comme vaccin commercial pour le nodavirus chez un mérou Japonais (Brudeseth et al., 2013), alors qu'un nouveau vaccin a commencé à être testé en Europe en 2016.

Défis et opportunités pour l'amélioration pour la résistance à la nodavirose

Comme suggéré dans la section 1.2. de cette introduction générale, l'amélioration génétique pour la résistance aux maladies des espèces de poissons est une opportunité (Bishop & Woolliams 2014; Gjedrem 2015). L'amélioration génétique du bar a été initiée depuis le milieu des années 1980 en France, en Espagne, en Italie et en Israël (Haffray et al., 2006). Une héritabilité significative a été estimée pour la croissance (Saillant et al., 2006; Dupont-Nivet et al., 2008), pour le sex-ratio (Vandeputte et al., 2007, 2012; Saillant et al., 2002; Palaiokostas et al., 2015) , et la qualité de la carcasse (Saillant et al., 2009), mais à ce jour, aucune étude publiée n'a porté sur la variation génétique de la résistance à la nodavirose. Il est important de noter que la variabilité génétique des traits de croissance a été démontrée dans la population et entre les populations sauvages (Vandeputte et al., 2009, 2014, Dupont-Nivet et al., 2008). Cela nous a amené à considérer que les variations génétiques de la résistance contre la nodavirose chez les populations naturelles bar méritaient d'être étudiées. L'amélioration d'un caractère par sélection artificielle exige une variation génétique suffisante dans la population (Falconer et Mackay, 1996). Des variations génétiques importantes ont été montrées pour la résistance des poissons d'élevage à la plupart des maladies virales étudiées, avec des estimations d'héritabilité modérées à élevées (Ødegård et al. 2011; Gjedrem 2015). En particulier, Ødegård et al. a montré de très grandes variations génétiques parmi les populations de cabillaud sauvage pour la résistance à la nodavirose (gamme 10-56% entre la morue côtière et la morue d'Arctique nord-est) ainsi qu'une héritabilité très élevée (0,75 ± 0,11 sur l'échelle sous-jacente) (Ødegård et al., 2010). De plus, d'autres études ont montré des estimations d'héritabilité modérée à élever pour d'autres maladies virales, telles que la résistance au VHSV chez la truite arc-en-ciel, allant de 0,57 à 0,63 (Dorson et al., 1995; Henryon et al., 2005), la résistance à l'ISAV dans les salmonidés (0,19 à 0,40) (Gjøen et al., 1997; Ødegård et al., 2007b, Kjøglum et al., 2008; Gjerde et al., 2009) et la résistance à l'IPNV dans les salmonidés (0,16 à 0,55) (Guy et al., 2006, 2009; Wetten et al. 2007; Kjøglum et al., 2008). Ces rapports illustrent que la résistance aux maladies virales peut être améliorée de manière significative sélection génétique.

Comme mentionné précédemment, un avantage majeur de la sélection assistée par marqueur (MAS) et de la sélection génomique (GS) par rapport à la sélection traditionnelle (basée sur le génotype et le phénotype seul) est que les animaux peuvent être choisis avec précision au début de leur vie, en fonction de leurs prédictions génomiques et pour des traits difficiles ou coûteux à mesurer tels que la résistance aux maladies (Massault et al., 2008; Zhang et al., 2011; Hayes et al., 2013). Pour la nodavirose, les QTL majeurs expliquant 11% de la variation phénotypique

totale pour a été observée chez le barramundi (Liu et al., 2017). Cependant, une limitation de la MAS est qu'elle nécessite une connaissance préalable des allèles favorables, qui doivent en outre être validés dans les populations sous sélection. En outre, la MAS exploite seulement une partie limitée des différences génétiques entre les individus, car elle n'exploite pas la variation du fond polygénique, qui peut représenter une grande partie de la variance génétique (Meuwissen, Hayes et Goddard 2016). Une approche alternative pour des caractères plus polygéniques est la GS.

La sélection génomique est une méthode qui prédit la valeur génétique totale d'un individu à partir d'enregistrements phénotypiques utilisant un génotypage dense et des estimations des effets SNP (Meuwissen et al., 2001). Ainsi, la sélection génomique utilise également la composante intra-familiale de la variance génétique (Daetwyler et al., 2007), ce qui lui donne une efficacité supplémentaire par rapport à la sélection familiale. La méthodes de GS la plus utilisées est la meilleure prédiction linéaire génomique sans biais (GBLUP), utilisant la matrice de relation génomique réalisée à partir des marqueurs SNP génotypés et des méthodes bayésiennes (Meuwissen et al., 2001; Habier et al., 2009; Bangera et al. 2017).

La précision des valeurs génétiques prédites par la génomique est souvent sensiblement plus élevée que celles basées sur le pedigree. Par conséquent, de grands efforts ont récemment été consacrés à évaluer la qualité de la prédiction génomique pour la résistance à plusieurs maladies dans l'aquaculture. Cependant, la plupart d'entre eux se sont concentrés sur les salmonidés. Ødegård et al. a évalué la prédiction génomique en utilisant les modèles GBLUP et les modèles d' « identité-par-descendance » (IBD-GS) avec une densité variable de SNP (allant de 1K à 220K) pour la résistance aux poux de mer du saumon. Ils ont montré que, dans tous les cas, la prédiction génomique était plus précise que la prédiction basée sur le pedigree (Ødegård et al., 2014). L'effet de l'apparentement entre la population d'entrainement et la population de validation sur les valeurs génétiques estimées par la génomique a été étudié et a montré que la précision de la prédiction génomique était plus élevée lorsqu'elles étaient proches l'une de l'autre (Tsai et al., 2016). Pratiquement parlant, les avantages des modèles génomiques ont été montrés pour la résistance aux poux de mer et la rickettsiose du saumon (Correa et al., 2017, Bangera et al., 2017), pour la résistance à la maladie bactérienne de l'eau froide (BCWD) chez la truite arc-en-ciel (Vallejo et al., 2017) et pour la résistance à la pasteurellose chez la dorade royale (Palaiokostas et al., 2016), même si aucun SNP individuel significatif lié à la résistance n'a été détecté dans ce dernier cas.

Les objectifs de la thèse

Le but de cette thèse est de décrire la variation génétique et d'évaluer la prédiction génomique pour la résistance à la nodavirose dans les populations sauvages de bar. À cette fin, une revue approfondie de la littérature sur l'encéphalopathie et rétinopathie virale en aquaculture a été réalisée au chapitre 2. Ensuite, la variabilité de la résistance au nodavirus chez quatre populations sauvages différentes de bar (Atlantique Nord, Méditerranée occidentale, Nord-Est Méditerranéenne et Sud-Est de la Méditerranée) a été étudiée au chapitre 3. Sur la base de génotypage de basse densité, la construction de cartes de liaisons et la cartographie QTL ont été explorées au chapitre 4. Enfin, une étude d'association pangénomique (Genome Wide Association Study - GWAS) et le potentiel de prédiction génomique pour la résistance à la nodavirose utilisant divers modèles génomiques a été réalisée au chapitre 5.

Variabilité génétique de la résistance à la nodavirose dans des populations sauvages de bar (*Dicentrarchus labrax*)

Dans cette étude, 1472 descendants résultant d'un croisement factoriel complet de femelle de Méditerranée-Ouest avec des mâles provenant de quatre populations sauvages différentes (Atlantique Nord, NAT, Méditerranée occidentale, WEM, Méditerranée du Nord-Est, NEM et Méditerranée du Sud-Est, SEM) ont été challengés par une infection été infectés par injection intrapéritonéale à 15.8g de poids moyen afin d'évaluer les variations génétiques de résistance à la nodavirose parmi les populations et les corrélations génétiques avec les caractères de production. Les résultats ont montré une grande variation de la résistance entre les populations testées ainsi qu'entre les familles de pères au sein de la souche. Les survies en souches sauvages pures SEM, NEM, WEM et NAT ayant été estimées respectivement à 99%, 94%, 62% et 44%. Une héritabilité modérée de la résistance a été calculée ($h^2u = 0,26 \pm 0,11$). Enfin, des corrélations génétiques négatives modérées ont été montrées entre la résistance et le coefficient de croissance journalier (DGC) et le poids au marquage (BW) (-0,28 ± 0,20, -0,35 ± 0,14, respectivement), tandis que la corrélation génétique entre la résistance à la VNN et le gras musculaire (FA) était faiblement négatif et non significatif (-0,13 ± 0,19). Ces résultats donnent de bonnes perspectives d'amélioration la résistance à la nodavirose du bar.

Construction d'une carte de liaison moyenne densité et cartographie de QTL de résistance contre la nodavirose chez le bar

Chez le bar, des études antérieures ont identifié des QTL liées à des traits de production (morphologie, croissance et réponse au stress) et à la détermination du sexe. Cependant, aucun QTL lié à la résistance à la nodavirose n'a été publiée. Nous avons d'abord construit une carte

de liaison moyenne densité à partir de 1274 SNP utilisant 1650 individus issus de 397 familles de plein-frères. Au total, 1174 marqueurs SNP ont été mappés avec succès dans 24 groupes de liaison. Le nombre moyen de marqueurs par LG individuel était de 49 marqueurs (allant de 27 pour LG24 à 65 pour LG4). La longueur de la carte génétique mâle, de la carte femelle et de la carte moyenne était respectivement de 1287,8, 1609,7 et 1409,3 cM. La carte femelle était au total 1,25 fois plus longue que la carte mâle. En plus de cela, la distance moyenne entre deux marqueurs (IM) de la carte féminine (1,40 cM) était également plus longue que celle de la carte masculine (1,13 cM). Ceci illustre que les événements de recombinaison sont plus fréquents pendant la méiose chez les femelles que chez les mâles. Des cartes de liaison origine-spécifiques ont également été construites. La taille totale de la carte de liaison Atlantique était la plus longue (1091,4 cM) suggérant plus de processus de recombinaison en moyenne dans cette population. Malheureusement, aucun QTL significatif lié à la résistance VNN n'a pu être identifié.

Étude d'association pangénomique et évaluation génomique de la résistance à la nodavirose du bar

Chez le bar, la sélection génomique (GS) n'est toujours pas utilisée dans la pratique, et sa performance doit être évaluée. Dans ce chapitre, utilisant les même marqueurs que ceux décris pour la cartographie de QTL, une étude pangénomique d'association (GWAS) en deux étapes a été réalisée. Un première GWAS non pondérée a été effectuée dans le logiciel BLUPF90, puis utilisant un poids dérivé de l'effet des SNPs dans l'estimation des valeurs génétique, une GWAS pondérée (wGWAS) a été effectuée. Suite à cette wGWAS, un SNP significatif expliquant 3.11% de la résistance et appartenant au LG9 a été identifié. Le potentiel de prédiction génomique de la résistance, utilisant les différents modèles génomiques a été réalisé. L'évaluation génomique et la précision des modèles GBLUP et SNP-BLUP implémentés dans BLUPF90 et GS3 ont comparés à la prédiction par pedigree (PBLUP). La précision des valeurs génétique prédites en fonction des modèles génomiques était similaire à celle du modèle traditionnel (PBLUP). Cela suggère que notre schéma expérimental n'était pas optimal pour tenir compte de la variation de la résistance en utilisant des informations génomiques et que la résistance à la nodavirose pourrait être améliorée plus efficacement avec des évaluations génétiques incorporant des informations génotypiques plus dense et dans une population avec des familles plus grande.

List of Tables

List of figures

List of symbols and acronyms

Units and measurements

°C	Degree Celsius
µg	Microgram
Kb	Kilo base-pair
cm	Centimeter
cM	centiMorgan
KDa	Kilo dalton
kg	Kilogram
ml	Mililiters
mm	Milimeters
nm	Nanometers

Disease and virus name

BCWD	Bacterial Cold Water Disease
BFNNV	Barfin Flounder Nervous Necrosis
IPNV	Infectious Pancreatic Necrosis Virus
ISAV	Infectious Salmon Anaemia Virus
KHV	Koi herpesvirus
MrNV	Machrobrachium rosenbergii nodavirus
RGNNV	Red-spotted grouper nervous necrosis virus
SJNNV	Striped jack nervous necrosis virus
SPDV	Salmon pancreases disease virus
TPNNV	Tiger puffer nervous necrosis
VER	Viral encephalopathy and retinopathy
VHSV	Viral haemorrphic septicaemia virus
VNN	Viral nervous necrosis
WTD	White tail disease

Others

AFLP	Amplified-Fragment Length Polymorphism
BPI-France	Banque Publique d'Investissement
BV	Breeding value
BW	Body weight
CIM	Composite interval mapping
CV	Cross-validation
DGC	Daily growth coefficient
DNA	Deoxyribonucleic acid
dph	day post-hatching
EBV	Estimated breeding value
ELISA	Enzyme-linked immunosorbent assay
EU	Europe
FA	Fillet adiposity
FUI	Fonds Unique Interministériel

GBLUP	Genomic Best Linear Unbias Prediction
GEBV	Genomic Estimated Breeding Value
GS	Genomic selection
GWAS	Genome Wide Association Study
wGWAS	Weighted Genome Wide Association Study
hpf	Hours post-fertilization
IBD	Identity by Descent
IBS	Identity by State
IFAT	Indirect fluorescent antibody test
IFREMER	Institut français de recherche pour l'exploitation de la mer
iQTLm	Interactive QTL mapping
iQTLm-GW	iQTLm genome-wide
LASSO	Least absolute shrinkage and selection operator
LD	Linkage disequilibrium
LG	Linkage group
LHRHa	Luteinizing Hormone–Releasing Hormone Analog
MAF	Minor allele frequency
MAS	Marker Assisted Selection
MI	marker interval
NAT	Northern Atlantic
NEM	North-Eastern Mediterranean
NGS	Next Generation Sequence
OD	Optical density
OIE	World Organization for Animal Health
ORF	Open reading frame
PBLUP	Pedigree-based Best Linear Unbiased Prediction
PCR	Polymerase chain reaction
dpc	post-day challenge
QTLs	Quantitative Trait Loci
RAPD	Random amplified polymorphic DNA
RdRp	RNA-dependent RNA polymerase
REML	Restricted Maximum Likelihood
RE-SIST	Amélioration par selection de la résistance des poissons d'élevage aux agents pathogènes
RNA	Ribonucleic acid
rr-BLUP	Ridge regression Best linear unbiased prediction
RT-PCR	Real time polymerase chain reaction
SEM	South-Eastern Mediterranean
SNP	Single nucleotide polymorphism
GBLUP	genomic best linear unbiased prediction
WEM	Western Mediterranean

Chapter 1:
General introduction

Selective Breeding for Resistance to Viral Nervous Necrosis Disease of European seabass: Prospective method for Sustainable Development in seabass cultured industry

1.1. Sustainable aquaculture and its challenges

The aquaculture and fisheries sector plays an important role in world food security. In 2014, the amount of aquaculture production (excluding aquatic plants) was 73.8 million tons for a total estimated value of $160.2 billion, compared to 93.4 million tons of capture fisheries (FAO 2016). Today, aquaculture supplies more than 50 percent of all fish for human consumption (FAO 2016). While human consumption of fish is predicted to sharply rise in the short-term, the volume of fisheries is predicted to be more or less stable. Thus, in 2025, the predicted aquaculture production for human consumption (112 million tons) will largely exceed the production of capture fisheries (FAO 2016). Therefore, aquaculture is and will be a major key to address for the world food security.

While, the aquaculture industry continuously increased during the last two decades, regarding either the total production or the cultured areas (FAO 2016), it faces many challenges. Global warming is expected to lead to an increase of disease outbreaks in some several specific areas (Cochrane et al. 2009). Containing water pollution and eutrophication caused by aquaculture production (feed, waste) is an even wider challenge. Besides that, in a context of fisheries stagnation, it is crucial to ensure further its independence from fish catches by industrial fisheries, which are processed as fish meal and fish oil used to feed farmed fish. Last but not least, reducing disease outbreaks leading to antibiotics and other drugs use in aquaculture, is a key challenge for social acceptability, economic profits and care for the environment. This makes disease outbreaks (infectious and parasitic diseases) one of the main threats to sustainable aquaculture (Gjedrem 2015; FAO 2016). Among the existing strategies to reduce disease outbreaks and their negative effect on the environment, either direct (antibiotics overuse, transmission of pathogens to wild fish) or indirect (waste of natural resouces consumption for farming fish that will not be consumed), selective breeding is one of the most promising (Gjedrem 2015). In particular, genomic selection (GS) is of special interest due to its expected higher efficiency (Yáñez et al. 2014; Vallejo et al. 2017). It is now possible to consider it even in “minor” species by a high drop in genotyping costs, which is expected to continue in the future.

1.2. Selective breeding as a key for sustainable aquaculture development

To reach a sustainable aquaculture, selective breeding is particularly interesting, as it durably improves the animal performances. Indeed, selective breeding allows cumulative and continuous genetic improvement of traits toward a desired objective. Moreover, this breeding

goal may be shifted or combined with others along time, as priorities evolve, to optimize profitability and reduce environnemental impacts.

Among the major three selective breeding strategies, mass selection remains the most widely used for mass spawning species, and for species with limited economic value (Vandeputte et al. 2009a). In mass selection, the animals' individual performances are the only information needed, making it an easy and relatively cheap method to apply. A second method is the best unbiased linear prediction (BLUP) that uses information on relatives to increase the accuracy of selection, also allowing the selection of animals for lethal phenotypes that cannot be recorded on the live breeding candidate (fillet yield, disease resistance). Finally, genomic selection (Meuwissen et al. 2001) includes genomic information with the same benefits but a further increase in accuracy. Selective breeding has been performed for a long time in aquaculture (Vandeputte et al. 2009b; Gjedrem 2015). Initiallly, the major goals of genetic selection was on production traits, with a special focus on the improvement of growth. But selective breeding of other traits tends to fit better with sustainable development of aquaculture. Traits such as fillet yield (part of growth invested into edible meat production), feed efficiency (proportion of feed intake transformed into weight gain) and disease resistance, which can be categorized as "efficiency" traits rather than quantitative production traits. Efforts need to be done at a global scale in aquatic species to increase the uptake of the benefits of selective breeding, as only 8.2% of total aquaculture volume throughout the world are estimated to come from genetically improved stocks (Gjedrem 2015). However, in Europe, the situation is different, as the percentage of production cultured based on genetically improved fish in Europe was estimated to range from 80 to 83% of the total volume of five major species (Atlantic salmon, rainbow trout, European seabass, gilthead seabream and turbot) of European aquaculture (Janssen et al. 2017). If we consider now the specific case of selection for disease resistance, many genetic and genomic studies for resistance to diseases have been conducted in livestock (Bishop & Woolliams 2014), and now increasingly in aquatic animals (Gjedrem 2015), with even practical application in selective breeding programs (Chavanne et al., 2016).

The disease resistance trait is rather specific, as it is undesirable to select surviviors of challenged fish as breeding due to the risk of vertical transmission of pathogens, preventing an efficient use of mass selection. In order to thwart the pathogen transmission, selective breeding may be based on sib selection using BLUP where the candidates are selected based on their relationship to individuals on which the phenotypes have been recorded. Typically, in sib selection for disease resistance, breeding candidates are kept in a pathogen-free environment,

while individuals from the same families are challenged with the pathogen. Then, breeding candidates are selected using family-wise estimated breeding values obtained from the percentage of survivors of their challenged sibs. Although efficient, this selection method is time consuming and all candidates originated from the same family are estimated as equivalent in terms of breeding value. This limits the selection intensity as individuals within families cannot be ranked, and it is necessary to keep a sufficient number of families in order to keep inbreeding to a reasonable level in a breeding program. The more recent alternatives to select for disease resistance are Marker-Assisted Selection (MAS) or genomic selection (GS). With MAS, the candidates are selected based on their genotype at specific Quantitative Trait Loci (QTLs) linked to the disease resistant phenotypes. Genomic selection is performed based on denseSNP markers genotypes covering the whole genome, which may not all be linked to the disease resistance, but which are dense enough so that any portion of the genome with even a minor effect on the resistance is expected to be in linkage disequilibrium with at least one of the genotyped SNPs. With these methods, the candidates can be chosen more accurately, and potentially earlier in life, based on their sole genotype. In the present study, we want to evaluate the possibilities to select European sea bass, our target aquaculture species, for disease resistance, using genetic and/or genomic selection.

1.3. European seabass: biology, production, markets

The European seabass lives in coastal waters of the Atlantic Ocean from South of Norway (60°N) to Western Sahara (30°N) and throughout the Mediterranean Sea and the Black Sea. It has been divided into three main populations based on genetic diversity, which are the Atlantic population, the Western Mediterranean population and the Eastern Mediterranean population (Naciri et al. 1999; Bahri-Sfar et al. 2000). Among these main groups, the Eastern Mediterranean population was shown to be subdivided in two different subpopulations, the North-Eastern Mediterranean and South-Eastern Mediterranean populations (Castilho & Ciftci 2005). Contrary to this observation, no significant subdivision has been found within the Atlantic and the Western Mediterranean populations (Haffray et al. 2006). European seabass is a eurythermal and euryhaline species. Therefore, they are able to frequent coastal inshore waters, and also occur in estuaries and brackish water lagoons. Sometimes they venture upstream into freshwater. The breeding season takes place in the winter in the Mediterranean populations (December to March), and up to June in the Atlantic population (Perez-Rufaza & Marcos 2014). Seabass are predators and their feeding range includes small fish, prawns, crabs and cuttlefish.

Regarding aquaculture, European seabass is one of the most valuable cultured fish in the Mediterranean area. Its production reached 49% of the total volume of marine Mediterranean finfish in 2015, with 317.029 tons produced (Annual report of FEAP, 2016). While it has been also currently cultured in UK, France, Portugal, Italy, Croatia, Tunisia, Israel, Oman and the United Arab Emirates, the major production originates from Turkey, Greece and Spain (FEAP, 2016). Though the average retail price of seabass had strongly decreased from 1985 to 2005 due to the increase of aquaculture production, it has gradually increased from 8.37 Euros in 2005 to 11.13 Euros in 2014 (Monfort 2007; The EU fish market, 2016 Edition). Although the total volume of seabass only ranked 7th of the aquaculture products in the EU, its total value was the 5th in 2013 (European Commission 2016). Although France is a minor producer in seabass aquaculture with only 2.244 tons in 2014, the French consumption of farmed seabass was the highest, covering 64% of the total landed volume (7.000 tons) and 67% of the total value (€ 79 million) in 2014, most of total volumes imported, leading to the major effect on the price increase trend of European seabass market (The EU fish market, 2016). The seabass aquaculture has also to be compared to wild catch sector in European Union that tends to slightly decrease or stagnates (depending on countries repoorts) with a mean catch of less than 8.000 tonnes per year over the last decade, France being the most active country with nearly 5.000 tonnes caught per year.

1.4. Viral encephalopathy and retinopathy in European seabass aquaculture

One of the main threats to the European seabass industry is disease outbreaks, especially the viral nervous necrosis (VNN) disease (Doan et al. 2017). This pathology caused by betanodaviruses, otherwise known as viral encephalopathy and retinopathy (VER) (Thiéry et al., 2011), is characterized by significant losses associated to vacuolating lesions of the central nervous system and the retina. It is one of the most serious viral threats to marine fish species in general, and particularly to European seabass in the Mediterranean region (Poisa-Beiro et al., 2007; Terlizzi et al., 2012; OIE 2013). In the mid-1980s, in Martinique and French Mediterranean hatcheries, betanodaviruses were already reported as responsible of mass mortality in European seabass larvae and juveniles (Breuil et al., 1991; Bellance and Gallet de Saint-Aurin, 1988). Indeed, although serious mortalities were also reported in market-size and adult fish, the disease mainly affects larval and juvenile stages and can induce 100% mortality (Breuil et al., 1991; Le Breton et al., 1997; Dalla Valle et al., 2000; Curtis et al., 2001; Munday et al., 2002; Gomez-Casado et al., 2011). VNN is not limited to European seabass as betanodaviruses have been isolated in more than 70 wild and cultured aquatic species

throughout the world, in cold and warm water environments, mostly marine but also freshwater, (see Doan et al. 2017 for details).

While numerous studies investigated the best ways to control viral diseases, no simple and effective procedures are available to treat most viral pathologies in fish (Gomez-Casado et al. 2011; Doan et al. 2017). To date, VNN disease can only be controled by using efficient diagnostic methods to monitor the breeders, together with disinfection processes (ozone or other chemicals) to control the environment during hatchery rearing. However, the long-term monitoring of such measures is often difficult, and they in any case cannot avoid VNN infections on grow-out sites (Mushiake et al. 1994). Applying vaccination may be an effective way to prevent the disease (Mushiake et al. 1994; Thiéry et al., 2006; Kai & Chi, 2008; Gomez-Casado et al., 2011). However, this tool is presently not effective due to several reasons (Nath et al., 2004):

- the specific drawbacks of existing vaccination methods (oral, immersion, injection), (Bjarnheidur et al., 2007);
- the difficulty to efficiently protect early larval stages (Chi et al., 1999) due to practical feasibility and insufficient maturity of the immune system (Dos Santos et al., 2000);
- the diversity of the VNN viruses for which at least four different genotypes were described (Nishizawa et al., 1997; Skliris et al., 2001; Mori et al., 2003) while traditional vaccination generally targeted one type of genotype, also potentially leading to the selection of resistant or insensitive viral populations (Gjedrem 2015; Nath et al., 2004).

DNA vaccines have numerous advantages compared to traditional antigen vaccines, and seem to be very attractive for the aquaculture industry (Heppell & David, 2000). However, horizontal gene transfer may occur from transgenic DNA from the vaccines to the environment (Myhr and Dalmo, 2005). Therefore, no license has been delivered to date for potential applications of DNA vaccines in commercial fish farms in some areas such as Europe (Heppell & Davis, 2000; Gomez-Casado et al., 2011). To date, only one inactivated RGNNV vaccine exists as commercial vaccine for NNV in seven-band grouper in Japan (Brudeseth et al. 2013), while a new vaccine started to be tested in Europe in 2016.

1.5. Challenges and Opportunities for selective breeding for resistance to VNN

As suggested in section 1.2. of this general introduction, selective breeding for disease resistant in fish species is an opportunity (Bishop & Woolliams 2014; Gjedrem 2015). European seabass selective breeding has been performed since the mid-1980s in France, Spain, Italy and Israel

(Haffray et al., 2006). Significant heritability has been estimated for growth (Saillant et al., 2006; Dupont-Nivet et al., 2008), sex ratio (Vandeputte et al., 2007, 2012; Saillant et al., 2002; Palaiokostas et al. 2015), and carcass quality (Saillant et al., 2009), but to date no published studies focused on the genetic variation of resistance to VNN. Importantly, genetic variability for growth traits has been demonstrated within population as well as between wild populations (Vandeputte et al., 2009, 2014; Dupont-Nivet et al., 2008). This led us to consider that genetic variations for resistance against NNV among natural populations of the European seabass would be worth investigating. Improving a trait by artificial selection basically requires the existence of sufficient genetic variation for this trait in the population (Falconer and 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; Gjedrem 2015). In particular, Ødegård et al. showed very large genetic variations among wild cod populations for VNN resistance (range 10–56% among coastal cod, Northeast Arctic cod and F1 cross between them) as well as a very high heritability (0.75±0.11 on the underlying liability scale) (Ødegård et al. 2010). Moreover, other noticeable studies showed moderate to high heritability estimates for other viral diseases, such as VHSV resistance in rainbow trout, ranging from 0.57 to 0.63 (Dorson et al. 1995; Henryon et al. 2005), ISAV resistance in salmonids (ranging from 0.19 to 0.40) (Gjøen et al. 1997; Ødegård et al. 2007b; Kjøglum et al. 2008; Gjerde et al. 2009) and IPNV resistance in salmonids (ranging from 0.16 to 0.55) (Guy et al. 2006, 2009; Wetten et al. 2007; Kjøglum et al. 2008). These reports illustrate that resistance to viral diseases can be improved significantly based on selective breeding in farmed fish.

As mentioned previously, a major advantage of Marker-assisted Selection (MAS) and Genomic Selection (GS) over traditional selection (based on pedigree and phenotype alone) is that animals can be selected accurately early in life, based on their genomic predictions, and for traits that are difficult or expensive to measure such as disease resistance (Massault et al., 2008; Zhang et al., 2011; Hayes et al., 2013). In order to apply MAS for selective breeding programs, QTL mapping has been conducted for most serious viral diseases of several major commercial aquatic species as reported in **Chapter 1**. For the VNN disease, major QTLs, which explained 11% of the total phenotypic variation for resistance to VNN were found in Asian sea bass (Liu et al. 2017). However, the limitation of MAS is that it requires prior knowledge of alleles that are associated with the traits of interest, which moreover have to be validated in the specific populations or even families under selection. Furthermore, MAS exploits only a limited part of

the genetic differences between individuals, as it does not exploit the polygenic background variation, which may account for a large part of the genetic variance (Meuwissen, Hayes & Goddard 2016). An alternative approach for more polygenic traits is GS.

Genomic selection is a method that predicts the total genetic value of an individual from phenotypic records using dense single nucleotide polymorphism (SNP) marker genotyping and estimates of SNP effects (Meuwissen et al., 2001). Thus, genomic selection also utilizes the within-family component of the genetic variance (Daetwyler et al., 2007), giving it extra efficiency when compared to family selection. Many GS methodologies varying with respect to assumptions about marker effects have been proposed for the genome-enabled prediction of estimated breeding values (GEBVs). The most widely used GS methods are the genomic best linear unbiased prediction (GBLUP) approach using the realized genomic relationship matrix calculated from the genome-wide SNP markers, and Bayesian methods (Meuwissen et al. 2001; Habier et al. 2009; Bangera et al. 2017). The performance of each of these GS methods varies according to the true underlying genetic architecture of the traits and to model assumptions.

The accuracy of genomic-based predicted breeding values is often substantially higher than pedigree-based breeding values. Therefore, large efforts have recently been devoted to evaluate the quality of genomic prediction for resistance to several diseases in aquaculture. However, most of them were carried out in salmonids, for which adequate genotyping tools have been developed earlier than in other species. Ødegård et al. has illustrated genomic prediction using GBLUP and identity-by-descent GS (IBD-GS) models with different density of SNPs (ranging from 1K to 220K) for salmon lice resistance. They showed that in all cases genomic prediction was more accurate than pedigree-based prediction (Ødegård et al. 2014). Furthermore, the effect of relationship between training and validation population on the genomic estimated breeding values was studied, and showed that the accuracy of genomic prediction was highest when the training and validation sets were close to each other (Tsai et al. 2016). Practically speaking, the advantages of genomic models have been reported for resistance against sea lice as well as salmon rickettsia syndrome in Atlantic salmon (Correa et al. 2017; Bangera et al. 2017), for bacterial cold-water disease (BCWD) resistance in rainbow trout (Vallejo et al. 2017) and for pasteurellosis resistance in gilthead sea bream (Palaiokostas et al. 2016) even though no significant individual SNPs linked to resistance were detected in this latter case.

Concerning European sea bass, in 2014 a panel of expert deplored “no or poor availability of high quality genomics resources (e.g. reference genomes, and high-density SNP chips)” in

European sea bass, "although they are valuable for increasing knowledge on fish biology, gene mapping and selection accuracies" (Aquaculture Europe 14, 2014). But since then, next generation sequencing technologies is offering every day the opportunity to discover larger numbers of markers, mostly single nucleotide polymorphism (SNPs), at a reasonable (and decreasing) cost. With the publication of the genome of the European sea bass (Tine *et* al. 2014), the potential use of genomics in selective breeding of European sea bass has clearly raised. The availability of such genomic resources offers new insights to go further in the description and in the exploitation of genetic variability in fish. Based on these tools, genomic selection for disease resistance will offer new prospects, especially in a context of business competition, cost containment and rationalization of inputs such as chemitry (Hayes et al. 2007, 2013).

1.6. The objectives of thesis

The purpose of this thesis is to describe the genetic variation and to investigate genomic prediction for resistance to viral nervous in wild populations of European seabass. To this end, a deep literature review of viral encephalopathy and retinopathy in aquaculture has been done in **Chapter 2**. Then, the variability of resistance to Betanodavirus among four different wild populations of European sea bass (North Atlantic, Western Mediterranean, North-Eastern Mediterranean and South-Eastern Mediterranean) was investigated in **Chapter 3**. Based on low-density genome scans, multiple family linkage map construction and QTL mapping were explored in **Chapter 4**. Finally, Genome Wide Association Study (GWAS) and potential for genomic prediction for resistance against VNN using various genomic models were performed in **Chapter 5**.

Chapter 2: Viral encephalopathy and retinopathy in aquaculture: a review

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Abstract

Viral encephalopathy and retinopathy (VER), otherwise known as viral nervous necrosis (VNN), is a major devastating threat for aquatic animals. Betanodaviruses have been isolated in at least 70 aquatic animal species in marine and in freshwater environments throughout the world, with the notable exception of South America. In this review, the betanodavirus main features, including its diversity, its distribution and its transmission modes in fish are firstly presented. Then, the existing diagnosis and detection methods, as well as the different control procedures of this disease are reviewed. Finally, the potential of selective breeding, including both conventional and genomic selection, as an opportunity to obtain resistant commercial populations, is examined.

Keywords: Betanodavirus, NNV, disease resistance, selective breeding, genetics.

2.1. Introduction

Although there is presently no strong evidence highlighting a possible raise of fish disease outbreaks due to climate change, increasing temperatures are expected to induce the spread of pathogens towards higher latitudes and to provoke negative impacts on fish physiology (Cochrane et al. 2009). Among others, the viral encephalopathy and retinopathy (VER), otherwise known as viral nervous necrosis (VNN), is considered one of the most serious viral threats for almost all marine aquaculture fish species, and requires a special focus due to the fact that outbreaks mostly happen in warm conditions. This disease, detected in at least 70 cultured or wild marine and fresh water species, already caused serious economic losses in the aquaculture industry in the past decades, and we can anticipate larger impacts of this disease because of global warming.

No simple and effective procedures are available to treat this disease in fish. It is, therefore, important to develop tools and set up new approaches to limit the occurrence and impacts of VNN episodes in aquaculture farms.

To stress that need, we present here an extensive review about VNN disease in aquaculture, including the features of the virus, the available procedures to control this disease, and the potential of selective breeding and genomic selection for resistance to viral diseases, as a prospective way to prevent VNN disease in fish.

2.2. Nervous Necrosis Virus

The causative agent of VNN, the Nervous Necrosis Virus, was classified as a member of the Nodaviridae family (Mori et al. 1992) which contains two genera: alphanodavirus and betanodavirus (Van Regenmortel et al., 2000). The species of the first genus were originally isolated from insects (figure 2.1), but appear to infect both vertebrates and invertebrates, and to cause the death of insect and mammalian hosts (Adachi et al. 2008). Betanodaviruses usually affect the nervous system of marine fish, leading to behavioral abnormalities and extreme high mortalities (Munday et al. 2002). In mammals, the pathogenicity of betanodaviruses is poorly reported, but mice have been demonstrated as non-susceptible, and human cells as not permeable to that genus (Adachi et al. 2008). Recently, a new emerging disease, the white tail disease (WTD) which affects the giant freshwater prawn and the whiteleg shrimp *Penaeus vannamei* has been demonstrated to be caused by the *Macrobrachium rosenbergii* nodavirus (MrNV). Sequence analysis of this virus suggests the existence of a new genus, gammanodavirus, infecting crustaceans (Qian et al. 2003; Senapin et al. 2012 - figure 2.1).

2.2.1. General morphology:

Betanodavirus virions were first described as non-enveloped, spherical in shape, and have icosahedral symmetry, with a diameter around 25nm and a capsid formed by 180 copies of a single protein of 42 Kda (Mori et al. 1992). A similar virus of 20-34 nm in diameter was detected in infected Asian seabass *Lates calcarifer* larvae, striped jack *Pseudocaranx dentex*, turbot *Scophthalmus maximus*, European seabass *Dicentrarchus labrax* (Yoshikoshi & Inoue 1990; Glazebrook et al. 1990; Bloch et al. 1991; Munday et al. 1992) and many various fish species through the world were subsequently recorded to be infected by betanodaviruses (Munday et al. 2002; Shetty et al. 2012).

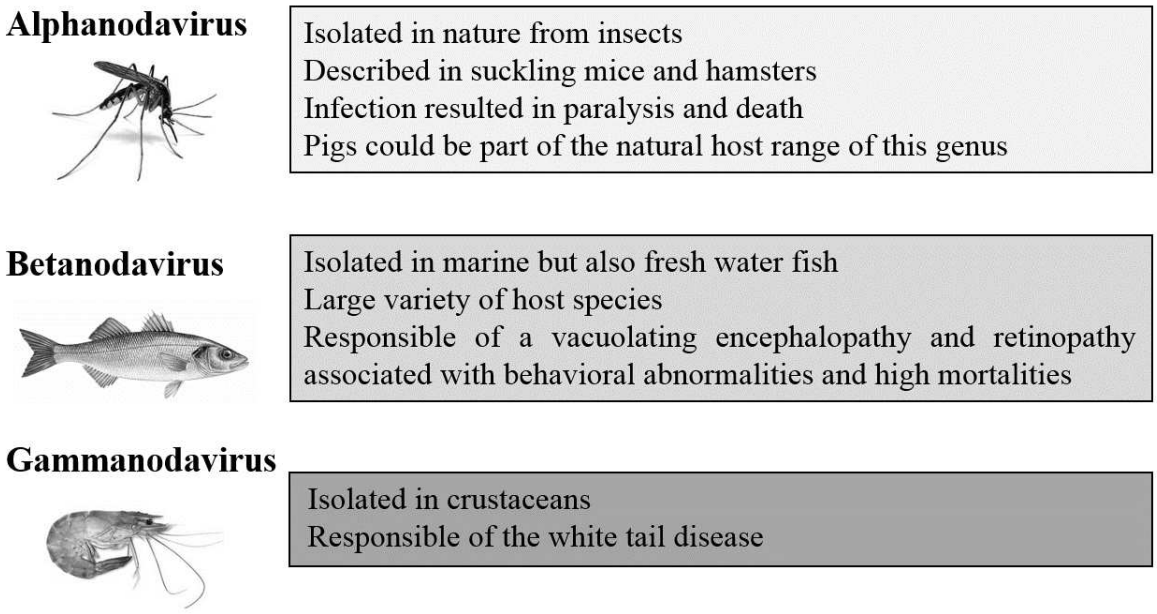

Figure 2.1: Three genera of Nodaviridae

2.2.2. Molecular structure:

Betanodavirus contains a bi-segmented genome composed of two single-stranded, positive-sense RNA molecules (Mori et al. 1992). The sequence of RNA1 is about 3.1 kb, and includes an open reading frame (ORF) encoding a RNA-dependent RNA polymerase (RdRp) of 110 kDa catalyzing the replication of the virus, also named protein A (Nagai & Nishizawa 1999). The sequence of RNA2 (1.4 kb) encodes the capsid protein (37kDa) which may have a function in the induction of cell death (Guo et al. 2003). In addition, during the virus replication, a sub-genomic RNA (RNA3) is synthesized from the 3'-terminus of RNA1 (Ball & Johnson 1999). This RNA3 encodes two other nonstructural proteins, B1 (111 amino acids) and B2 (75 amino acids). Protein B1 displays anti-necrotic property enhancing the viability of viral host cell (Sommerset & Nerland 2004).

Protein B2 is an inhibitor of host RNA silencing in either alphanodavirus or betanodavirus, but could also promote mitochondrial fragmentation and cell death induced by hydrogen peroxide production (Su et al. 2014).

2.2.3. Classification:

Betanodavirus was described for the first time from infected larval stripped jack. The name striped jack nervous necrosis virus (SJNNV) was consequently adopted (Mori et al. 1992). Subsequently other agents of VNN were isolated from diseased fish species (Munday et al. 2002). The first comparative studies between viral strains isolated from different marine fish species were done in the middle of the 1990s, where Nishizawa *et al.* reported the sequence of SJNNV and four different fish Nodaviruses as well as four different insect Nodaviruses (Nishizawa et al. 1995). From a phylogenetic analysis of the RNA2 T4 variable region, betanodaviruses were classified into four different species designed as the SJNNV-type, the barfin flounder nervous necrosis virus (BFNNV)-type, the red-spotted grouper nervous necrosis virus (RGNNV)-type, and the tiger puffer nervous necrosis virus (TPNNV)-type (Nishizawa et al. 1997). These species partially correlate with three different serotypes determined from virus neutralization using polyclonal antibodies (serotype A for SJNNV species, B for TPNNV species and C for BFNNV and RGNNV species) (Morit et al. 2003). Each species corresponds to different host fish and different *in vitro* optimal growth temperatures (table 2.1). RGNNV is the most popular species because a variety of fish species, distributed in warm-water, are affected (optimal growth temperature of 25−30ºC) (Asian seabass, European seabass, groupers…), whereas BFNNV is restricted to cold-water (15−20ºC) marine fish species (Atlantic halibut *Hippoglossus hippoglossus*, Atlantic cod *Gadus morhua*, flounders…) and TPNNV infects a single species (Tiger puffer *Takifugu rubripes*) at an intermediate temperature (20ºC). The SJNNV type was initially known to affect a few species cultured in Japan at 20-25ºC (Iwamoto et al. 2000; Munday et al. 2002; Nishizawa et al. 1995; Toffan et al. 2016). However, it was also recently described in some fish species cultured in Southern Europe such as Senegalese sole *Solea senegalensis* in Spain, gilthead sea bream *Sparus aurata* and European seabass in the Iberian Peninsula (Thiéry et al. 2004; Cutrín et al. 2007). This capacity to infect such warm water fish species is probably associated to reassortant RGNNV and SJNNV strains (Iwamoto et al. 2004; Toffolo et al. 2007; Panzarin et al. 2012; Toffan et al. 2016, see also Phylogenetic relationshipc paragraph). Phylogenetic analysis of betanodaviruses was also made based on the T2 region, which covers a larger RNA2 sequence than T4 (Chi et al. 2003; Johansen et al. 2004). This taxonomy has been used to genetically characterized new isolates in various fish species as well as in different areas (Aspehaug et al. 1999; Starkey et al. 2000; Dalla Valle et al. 2001; Tan et al. 2001; Skliris et al. 2001; Johnson et al. 2002; Chi et al. 2003; Gagné

et al. 2004; Sommerset & Nerland 2004; Thiéry et al. 2004; Johansen et al. 2004; Ransangan & Manin 2012; Vendramin et al. 2013). Because NNV is detected in many new species as well as new regions, description of new isolates and sequences are regularly published and could lead to evolution in the classification (table 2.1). For example, an additional genotype including a turbot betanodavirus strain (TNNV) was described in 2004. This species is currently awaiting classification (Johansen et al. 2004).

An alternative classification has been proposed (Thiéry et al. 2004). However, this numerical nomenclature (cluster I, II, III and IV), independent from the host species origin, is not extensively used because viruses from different clusters could infect a same host species, for example European seabass (Thiéry et al. 1999) and the classification was not consistent with geographical areas (Dalla Valle et al. 2001; Thiéry et al. 2004; Cutrín et al. 2007).

1.5.2.4. Phylogenetic relationships:

Among the different species of betanodaviruses, amino acid sequences of RdRp protein and capsid protein share 87 to 99% and 77 to 100% of identity respectively (82 to 98% for the complete RNA1 nucleic sequence and 76 to 99% for the RNA2 segment (Okinaka & Nakai 2008). The topology of phylogenetic trees based on RNA1 and RNA2 distinguishes several clades, suggesting a high diversity despite relatively strong purifying selection on most codons (Panzarin et al. 2012). This important variability can be explain by a significant substitution rate but also by a re-assorting process specific to segmented viruses (Panzarin etal. 2012).

2.3. Distribution and Transmission

2.3.1. Distribution:

Viral encephalopathy and retinopathy is one of the most widespread viral diseases of marine fish species cultured worldwide. A large number of species have been reported to be affected, especially larval and juvenile stages in which high mortalities were recorded (Munday et al. 2002; Shetty et al. 2012). Based on clinical signs, VNN disease has been documented since 1985 in Japanese parrotfish *Oplegnathus fasciatus* larvae and juveniles in Japan, while the pathogen was first observed in the brain of reared Japanese parrotfish (Yoshikoshi & Inoue 1990). Three years later, it was recorded in European seabass produced in Martinique (West Indies, France) and French Mediterranean (Breuil et al. 1991). Since then, similar clinical signs with encephalitis associated with picorna-like viral particles were observed in the Asian seabass *Lates calcarifer* cultured in Australia (Glazebrook et al. 1990; Munday et al. 2002), as well as in turbot *Scopthalmus maximus* (Bloch et al. 1991), red-spotted grouper *Epinephalus akaara* (Nishizawa et al. 1995),

Table 2.1: Species of the genus Betanodavirus

Species	GenBank accession no.	Optimal temperature For replication	Serotype	Main hosts effected	Key Ref.
Species in the genus Betanodavivus					
Barfin flounder nervous necrosis virus – BFNNV-BF93Hok	RNA1 (EU826137 = NC_011063) RNA2 (EU826138 = NC011064)	15–20°C	C	Atlantic cod (*Gadus morhua*) Barfin flounder (*Verasper moseri*) Atlantic halibut (*Hippoglossus hippoglossus*)	Munday et al. 2002, Iwamoto et al. 2000, Morit et al. 2003, Vendramin et al. 2013
Redspotted grouper nervous necrosis virus – RGNNV -SGWak97	RNA1 (AY324869 = NC_008040) RNA2 (AY324870 = NC_008041)	25–30°C	C	Sevenband grouper (*Epinephelus septemfasciatus*) Redspotted grouper (*Epinephelus akaara*) Kelp grouper (*Epinephelus moara*) Orange spotted grouper (*Epinephelus coioides*) Dragon grouper (*Epinephelus lanceolatus*) Greasy grouper (*Epinephelus tauvina*) Humpback grouper (*Cromileptes altivelis*) Barramundi (*Lates calcarifer*) Japanese seabass (*Lateolabrax japonicus*) European seabass (*Dicentrarchus labrax*)	Munday et al. 2002, Iwamoto et al. 2000, Mori et al. 2003, Vendramin et al. 2013, Vendramin et al. 2014
Striped jack nervous necrosis virus – SJNNV-SJ93Nag	RNA1 (AB056571 = NC_003448) RNA2 (AB056572 = NC_003449)	20–25°C	A	Japanese striped jack (*Pseudocaranx dentex*) Gilthead sea bream (*Sparus aurata*) Senegalese sole (*Solea senegalensis*)	Nishizawa et al. 1997, Iwamoto et al. 2000, Mori et al. 2003, Thiéry et al. 2004, Vendramin et al. 2013 & 2014
Tiger puffer nervous necrosis virus – TPNNV-TPKag93	RNA1 (EU236148 = NC_013640) RNA2 (EU236149 = NC_013641)	20°C	B	Tiger puffer (*Takifugu rublipes*)	Iwamoto et al. 2000, Mori et al. 2003, Vendramin et al. 2013
Other genotypes which have not been approved as species					
Atlantic cod nervous necrosis virus - ACNNV	RNA1 (EF433472) RNA2 (EF433468)	15–20°C	C	Atlantic cod (*Gadus morhua*)	Nylund et al. 2008, Johnson et al. 2002
Atlantic halibut nodavirus – AHNV	RNA1 (AJ401165) RNA2 (AJ245641)	15–20°C	C	Atlantic halibut *(Hippoglossus hippoglossus)*	Grotmoll & Totlandl 2000, Johnson et al. 2002, Sommerset & Nerland 2004
Dicentrarchus labrax encephalitis virus - DIEV	RNA2 (U39876)	25–30°C	C	Seabass (*Dicentrarchus labrax*)	Dalla Valle et al. 2001 Johnson et al. 2002
Dragon grouper nervous necrosis virus - DGNNV	RNA1 (AY721616) RNA2 (AY721615)	25–30°C	C	Dragon grouper (*Epinephelus laceolatus*)	Panzarin et al. 2012 Johnson et al. 2002
Greasy grouper nervous necrosis virus - GGNNV	RNA1 (AF319555) RNA2 (AF318942)	25–30°C	C	Greasy grouper (*Epinephelustauvina*)	Tan et al. 2001, Johnson et al. 2002, Sommerset & Nerland 2004
Japanese flounder nervous necrosis virus - JFNNV	RNA1 (FJ748760) RNA2 (D38527)	25–30°C	C	Japanese flounder (*Paralichthys olivaceus*)	Panzarin et al. 2012, Johnson et al. (2002)
Lates calcarifer encephalitis virus - LcEF	RNA2 (AF175516)	25–30°C	C	Barramundi (*Lates calcarifer*)	Skliris et al. 2001
Malabaricus grouper nervous necrosis virus - MGNNV	RNA2 (AF245003)	25–30°C	C	Malabaricus grouper (*Epinephelus malabaricus*)	Johnson et al. 2002
Seabass nervous necrosis virus - SBNNV	RNA2 (Y08700)	20–25°C	A	Seabass (*Dicentrarchus labrax*)	Thiéry et al. 2004
Solea senegalensis nervous necrosis virus - SSNNV	RNA1 (FJ803911) RNA2 (AJ698113)	20–25°C	A	Senegalese sole (*Solea senegalensis*)	Panzarin et al. 2012, Thiéry et al. 2004
Turbot nodavirus - TNV	RNA2 (AJ608266)	undefined	undefined	Turbot (*Scophthalmus maximus*)	Johansen et al. 2004
Marcrobrachium rosenbergii nodavirus - MrNV	RNA1 (AY231436) RNA2 (AY231437)	25–30°C	undefined	Giant freshwater prawn (*Macrobrachium rosenbergii*)	Senapin et al. 2012, Bonami & Sri Widada 2011
Peneaus vannamei nodavirus - PvNV	RNA1 (FJ751226) RNA2 (FJ751225)	25–30°C	undefined	Whiteleg shrimp (*Litopeneaus vannamei*)	Senapin et al. 2012, Tang et al. 2007

striped jack *Pseudocaranx dentex* (Mori et al. 1992), Japanese flounder *Paralichthys olivaceus* (Nishizawa et al. 1995), tiger puffer *Takifugu rublipes,* kelp grouper *Epinephelus moara* (Munday et al. 2002) and barfin flounder *Verasper moseri* in Japan (Nishizawa et al. 1995), and recently in golden grey mullet *Liza aurata* and leaping mullet *Liza saliens* in the Caspian Sea (Zorriehzahra et al. 2016).

Infections caused by NNV have been detected all around the world, with the notable exception of South America (Crane & Hyatt 2011; Shetty et al. 2012). It was the cause of mass mortality in Atlantic halibut in Norway and Scotland (Grotmol et al. 1997; Starkey et al. 2000) and in juvenile greasy grouper *Epinephelus tauvina* in Singapore (Hegde et al. 2002) and in groupers in Taiwan (Chi et al. 1997). Betanodaviruses have been the cause of high economical losses in aquaculture industry throughout the Mediterranean area. Mass mortalities have been repeatedly recorded since 1991 on larvae and juvenile stages in European seabass in France (Breuil et al. 1991) as well as on grow-out size seabass in Greece, Italia and Tunisia (Le Breton et al. 1997; Bovo et al. 1999; Thiery et al. 2004; Haddad-Boubaker et al. 2013). Grey mullet *Mugil cephalus*, red drum *Sciaenops ocellatus*, and barramundi cultured in Israel were also reported to be affected by NNV (Ucko et al. 2004). Farmed Senagalese sole *Solea senegalensis* were reported as infected by RGNNV and SJNNV in Spain (Thiery et al. 2004, Hodneland et al. 2011). More recently, RGNNV, SJNNV genotypes and reassortant RGNNV/ SJNNV and SJNNV/RGNNV viruses have been reported to infect several fish species (European seabass, sea bream, Senegalese sole) in Mediterranean Sea (Toffolo et al. 2007; Olveira et al. 2009; Hadda-Boubaker et al. 2013; Panzarin et al. 2012; Toffan et al. 2016). A strain belonging to the RGNNV species caused mass mortality in white seabass *Atractoscion nobilis* reared in South California in 1999 (Curtis et al. 2001). NNV was also found in Atlantic cod and haddock *Melanogrammus aeglefinus* juvenile stages on the Atlantic coast of North America (Johnson et al. 2002). Furthermore, betanodaviruses do not only affect reared fish species, but have also been found in a variety of wild fish species, as reported in table 2.2.

Regarding environment, although NNV is mostly known for infecting aquatic animals in marine and brackish water, the reports of freshwater species infected by NNV have been increasing (table 2.2). NNV infection was observed in freshwater eel and catfish aquaculture systems in Taiwan (Chi et al. 2003) as well as in other freshwater species including sturgeon *Acipenser gueldenstaedtii* (Athanassopoulou et al. 2004), tilapia *Oreochromis niloticus* (Bigarré et al. 2009), largemouth bass *Micropterus salmoides*, pike-perch *Sander lucioperca*, striped bass x white bass, *Morone saxatilis* x *Morone chrysops* (Bovo et al. 2011), guppy *Poecilia reticulata* (Hegde et al. 2003), Australian catfish *Tandanus tandanus*, and sleepy cod *Oxyeleotris lineolatus* (Munday et al. 2002). Zebrafish *Danio rerio* and goldfish *Carassius auratus* were also found to be infected

(Binesh 2013). Furthermore, the freshwater blenny *Salaria fluviatili*, which is an endangered species endemic to watersheds of the Mediterranean Basin, was also reported as affected by NNV (Vendramin et al. 2012). To date, the susceptibility of Mandarin fish *Siniperca chuatsi* to RGNNV, an important economical species in freshwater aquaculture in China, has been demonstrated (Tu et al. 2016). At present, at least 70 host species belonging to 32 families of 16 orders have been described as carriers of betanodavirus (table 2.2) and this disease is widely reported all over the world, with the exception of South America.

2.3.2. Transmission:

NNV is characterized by both vertical and horizontal transmission (Munday et al. 2002, see also figure 2.2). Vertical transmission was early described in a number of different fish species where betanodaviruses were detected in broodstock gonads or in early larval stages with typical symptomatic signs. It can occur from broodstock to larvae through germplasm, including the eggs or genital fluids as reported in striped jack, in barfin flounder or in European seabass (Mushiake et al. 1994; Nishizawa et al. 1996; Mori et al. 1998; Watanabe et al. 2000; Dalla Valle et al. 2000; Breuil et al. 2002).

Horizontal transmission is a very difficult route to control because betanodavirus can easily spread during an outbreak via water but also rearing equipment (Mori et al. 1998; Watanabe et al. 1998). Horizontal transmission has been experimentally demonstrated by several routes: contact between healthy fish and diseased larvae (Arimoto et al. 1993), bathing fish in water containing betanodavirus-infected tissue homogenates (Arimoto et al. 1993; Tanaka et al. 1998; Grotmol et al. 1999), contamination using strains isolated from symptomatic fish (Koch postulate) (Thiéry et al. 1997; Peducasse et al. 1999) or contact of healthy fish with asymptomatic carriers (Skliris & Richards 1999; Breuil et al. 2002).

Once in the aquatic environment, betanodavirus can persist without host for a long time and can be spread widely by tide, aquatic transport means or migration of the wild hosts (Gomez et al. 2004; Gomez et al. 2008; Giacopello et al. 2013). As NNV was reported in sand worms belonging to the family *Nereidae* (Liu et al. 2006a) but also in crabs and mussels (Gomez et al. 2008), several studies are carried out to clarify the existence of non-fish carriers or vectors of NNV such as raw fish (trash fish), brine shrimp *Artemia salina* and mollusks used as feed for marine culture (Gomez et al. 2010; Costa & Thompson 2016). Commercial trade of aquatic animals should also be regarded as an important potential source of virus diffusion (Gomez et al. 2006).

Table 2.2: Fish species influenced by VER/VNN

Host species					Species	Key ref.
Oder		Family	Common name	Latin name		
Marine species						
Farmed species	Decapoda	Penaeidae	Whiteleg shrimp	*Lipopenaeus vannamei*	PvNV	Tang et al. 2007
	Scorpaeniformes	Sebastidae	Black rockfish	*Sebastes inermis*	RGNNV	Gomez et al. 2004
			Oblong rockfish	*S. oblongus*		
			Spotbelly rockfish	*S. pachycephalus*		
	Pempheriformes	Lateolabracidae	Chinese seabass	*Lateolabrax sp.*		
	Perciformes	Sparidae	Red seabream	*Pagrus major*		
			Gilthead sea bream	*Sparus aurata*	SJNNV	Cutrín et al. 2007
		Oplegnathidae	Japanese parrotfish (Barred knifejaw)	*Oplegnathus fasciatus*	SJNNV	Yoshikoshi & Inoue 1990 Nishizawa et al. 1997
		Centropomatidae	Japanese seabass	*Lateolabrax japonicus*	RGNNV	Mori et al. 2003
		Sciaenidae	White seabass	*Atractoscion nobilis*	RGNNV	Curtis et al. 2001
		Percichthydae	European seabass	*Dicentrarchus labrax*	RGNNV/ SJNNV	Breuil et al. 1991 Thiéry et al. 2004
		Scombridae	Pacific bluefin tuna	*Thunnus orientalis*	RGNNV	Sugaya et al. 2009
		Rachicentridae	Cobia	*Rachycentron canadum*	RGNNV	Chi et al. 2003
		Carangidae	yellow-wax pompano	*Trachinotus falcatus*		
			Striped jack	*Pseudocaranx dentex*	SJNNV/ TPNNV	Mori et al. 1992 Nishizawa et al. 1997
			Golden pompano	*Trachinotus blochii*	RGNNV	Ransangan et al. 2011
		Serranidae	Humpback grouper	*Cromileptes altivelis*	RGNNV	Yuasa et al. 2007
			Dragon grouper	*Epinephelus lanceolatus*	RGNNV	Lin et al. 2001
			Red-spotted grouper	*Epinephalus akaara*	RGNNV	Nishizawa et al. 1997
			Black spotted grouper	*Epinephelus fuscogutatus*	RGNNV	Chi et al. 1997
			Sevenband grouper	*Epinephelus septemfasciatus*	SJNNV	Fukuda et al. 1996
			Greasy grouper	*Epinephelus tauvina*	GGNNV	Hegde et al. 2002 Tan et al. 2001
			Orange-spotted grouper	*Epinephelus coioides*	RGNNV	Chi et al. 1999
			Brown-spotted grouper	*Epinephelus malabaricus*	RGNNV	Nishizawa et al. 1997
			Yellow grouper	*Epinephelus awoara*	RGNNV	Lai et al. 2001
			Kelp grouper	*Epinephelus moara*	undefined	Munday et al. 2002
	Tetraodontiformes	Tetraodontidae	Tiger puffer	*Takifugu rubripes*	TPNNV	Nishizawa et al. 1997
	Pleuronectiformes	Soleidae	Senegalese sole	*Solea senegalensis*	SJNNV	Thiéry et al. 2004
		Pleuronectidae	Barfin flounder	*Verasper moseri*	BFNNV	Nishizawa et al. 1995
			Atlantic halibut	*Hippoglossus hippoglossus*	BFNNV	Grotmol et al. 1997
		Paralichthyidae	Japanese flounder	*Paralichthys olivaceus*	SJNNV	Nishizawa et al. 1995
		Scophthalmidae	Turbot	*Scophthalmus maximus*	TNV	Johansen et al. 2004
	Perciformes	Centropomatidae	Barramundi/Asian seabass	*Lates calcarifer*	RGNNV	Bloch et al. 1991
	Gadiformes	Gadidae	Pacific cod	*Gadus macrocephalus*	BFNNV	Mori et al. 2003
			Atlantic cod	*Gadus morhua*	BFNNV	Johnson et al. 2002
			Haddock	*Melanogrammus aeglefinus*	BFNNV	Gagné et al. 2004
Wild species	Perciformes	Epigonidae	Cardinal fish	*Epigonus telescopus*	undefined	Giacopello et al. 2013
		Serranidae	Wild dusky grouper	*Epinephelus marginatus*	RGNNV	Vendramin et al. 2013
			Wild golden grouper	*Epinephelus costae*		
		Sparidae	Bogue	*Boops boops* (L.)	RGNNV	Ciulli et al. 2007
		Mugilidae	Flathead grey mullet	*Mugil cephalus* (L.)		
			Golden grey mullet	*Liza aurata*	RGNNV	Zorriehzahra et al. 2016
			Leaping mullet	*Liza saliens*		
			Red mullet	*Mullus barbatus barbatus* (L.)	RGNNV	Ciulli et al. 2007
		Gobiidae	Black goby	*Gobius niger* (L.)		
		Carangidae	Horse mackerel	*Trachurus trachurus*		
			Japanese scad	*Decapterus maruadsi* (Temminck & Schlegel)	RGNNV	Gomez et al. 2004
	Lepisosteiformes	Lepisosteidae	Garpike (Longnose Gar)	*Lepisosteus osseus*	RGNNV	Ciulli et al. 2007
	Pleuronectiformes	Pleuronectidae	wild winter flounder	*Pleuronectes americanus*	BFNNV	Gagné et al. 2004

Table 2.2: (continued)

Wild species ***(continued)***	Notacanthiformes	Notacanthidae	Shortfin spiny eel	*Notacanthus Bonaparte*	undefined	Giacopello et al. 2013
	Beryciformes	Trachichthyidae	Mediterranean slimehead	*Hoplostethus mediterraneus mediterraneus*		
	Gadiformes	Macrouridae	Glasshead grenadier	*Hymenocephalus italicus* (Giglioli)		
		Gadidae	Whiting	*Merlangi merlangus* (L.)	RGNNV	Ciulli et al. 2007
		Merlucciidae	European hake	*Merluccius merluc- cius* (L.)		
	Clupeiformes	Clupeidae	European pilchard	*Sardina pilchardus (Walbaum)*		
	Scorpaeniformes	Triglidae	Gurnard	*Chelidonichthys lucerna* (L.)		
		Sebastidae	Marbled rockfish	*Sebastiscus marmoratus* (Cuvier)	RGNNV	Gomez et al. 2004
	Tetraodontiformes	Monacantidae	Threadsail filefish	*Stephanolepis cirrhifer* (Temminck & Schlegel)		
			Black scraper	*Thamnaconus modestus* (Gunther)		
	Decapoda	Portunidae	Charybdid crab	*Charybdis bimaculata*	RGNNV	Gomez et al. 2008
		Pandalidae	Southern humpback shrimp	*Pandalus hypsinotus*		
	Mytiloida	Mytilidae	Mediterranean mussel	*Mytilus galloprovincialis*		
Freshwater species						
Farmed species	Acipenseriformes	Acipenseridae	Sturgeon	*Acipenser gueldenstaedi*	SJNNV	Athanassopoulou et al. 2004
	Anguilliformes	Anguillidae	European eels	*Anguilla anguilla*	RGNNV	Chi et al. (2003)
	Siluriformes	Siluridae	Chinese catfish	*Parasilurus asotus*		
			Australian catfish	*Tandanus tandanus*	undefined	Shetty et al. 2012
	Perciformes	Eleotridae	Sleepy cod	*Oxyeleotris lineolatus*	undefined	
		Centrarchidae	Largemouth black bass	*Micropterus salmoides* (Lacepede)	RGNNV	Bovo et al. 2011
		Percidae	Pike-perch	*Sander lucioperca*		
		Cichlidae	Tilapia	*Oreochromis niloticus*	RGNNV	Bigarré et al. 2009
	Decapoda	Palaemonidae	Giant freshwater prawn	*Macrobrachium rosenbergii*	MrNV	Bonami & Widada 2011
Ornamental/model fish species	Cyprinodontiformes	Poeciliidae	Guppy	*Poecilia reticulata*	RGNNV	Hegde et al. 2003
	Cypriniformes	Cyprinidae	Zebrafish	*Danio rerio*	RGNNV	Lu et al. 2008
			Goldfish	*Carassius auratus*	RGNNV	Binesh 2013
	Perciformes	Blenniidae	Freshwater blenny	*Salaria fluviatili*	RGNNV	Vendramin et al. 2012
	Beloniformes	Adrianichthyidae	Medaka	*Oryzias latipes*	RGNNV	Furusawa et al. 2006

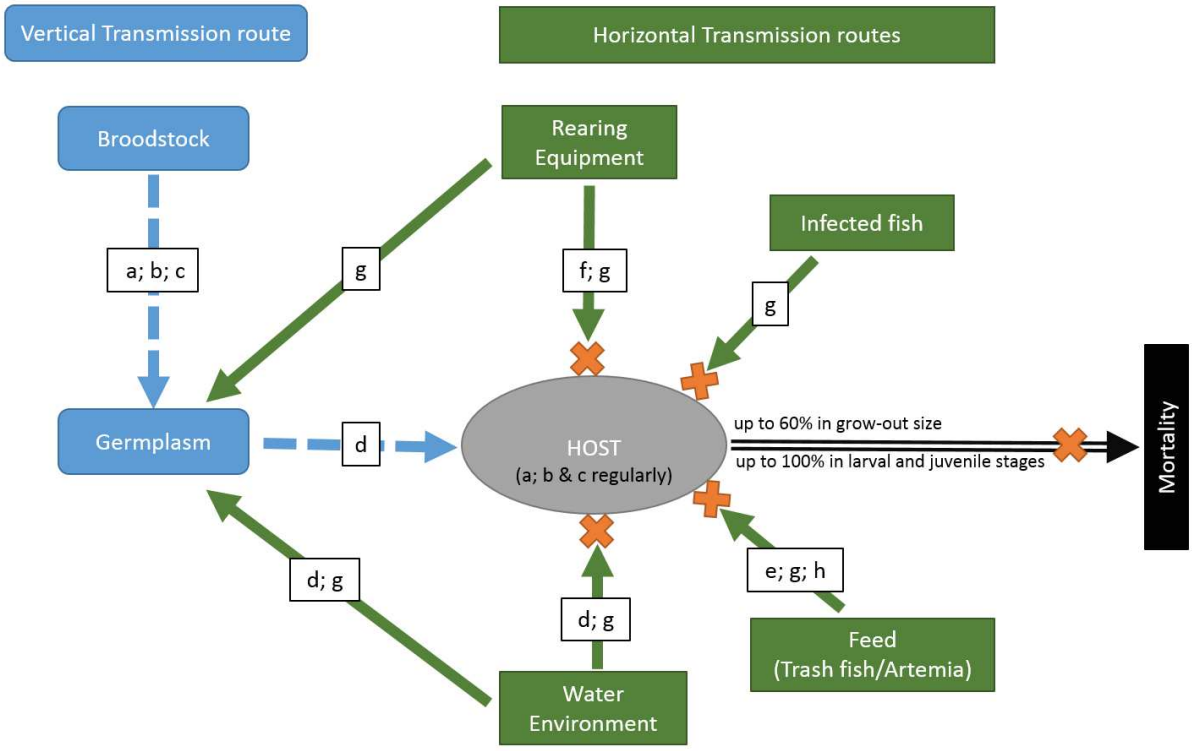

Figure 2.2: The different transmission routes of betanodaviruses and possible prevention modes. Blue discontinuous arrows represent vertical transmission routes; green arrows represent horizontal transmission routes; orange crosses display possible actions of genet- ics (by improving for fish natural barriers to infections or resistance/tolerance – see section 'Selective breeding to nervous necrosis virus (NNV) resistance: prospective procedure'); host represents either larvae/juvenile/grow-out size or broodstock; the possible prevention modes are as follows: a: vaccination; b: serological diagnostic (ELISA) to screen and eliminate seropositive individuals; c: direct diagnostic (RT-qPCR) to screen and eliminate positive individuals or germplasm; d: ozone/UV/bleach water treatments; e: strict control of feed input to avoid NNV infected trash fish; f: unique equipment kit for each tank/pond/cage and adapted decontamination of equipment after use; g: biosecurity measures during all production cycles; h: ozone treatment of artemia before feeding.

2.4. Diagnosis/Detection

2.4.1. First diagnostic approaches:

In the early 1990s, the structure of NNV was already clearly known but virus isolation using cell lines was not successful. Therefore, the method of VNN diagnostic relied on the observation of characteristic clinical signs. VNN is characterized by typical behavioral abnormalities (erratic swimming patterns such as spiraling or whirling, lying down at the tank bottom, rapid swimming, darker coloration…) associated to an impairment of the nervous system (figure 2.3) (Yoshikoshi & Inoue 1990; Breuil et al. 1991; Chi et al. 1997). Gross pathology examination frequently reveals a hyperinflation of the swimbladder and hemorrhages on the brain tissue. The most common microscopical findings consist of vacuolation and necrosis of nervous cells of the spinal cord, brain and/or retina, particularly in larvae and juveniles stages. The infection is rarely accompanied by inflammatory processes. In presence

of these typical signs, diagnosis must be confirmed by a laboratory test. Electronic microscopy allowed observation of virus particles free or membrane bound by endoplasmic reticulum in cells collected from infected organs (brain, retina) and revealed icosahedral, non-enveloped viruses with a commonly reported diameter of 20-34 nm (Yoshikoshi & Inoue 1990; Glazebrook et al. 1990; Breuil et al. 1991; Bloch et al. 1991; Mori et al. 1992; Grotmol et al. 1997). Over two decades, the reference method to detect betanodavirus was isolation in permissive cell culture (striped snakehead cells SSN-1 or E11) followed by immunological (indirect fluorescent antibody test – IFAT, immunohistochemistry, enzyme-linked immunosorbent assay – ELISA; Nuñez-Ortiz et al. 2016) or molecular identification (RT-PCR, Nested RT-PCR, real time RT-PCR). However, cell culture is time consuming, requires a great experience, and some NNV strains are not always easy to detect because of a poor cultivability and/or the absence of induction of clear cytopathic effects. This is why molecular methods, particularly real-time RT-PCR, have been increasingly used (Munday et al. 2002; Shetty et al. 2012).

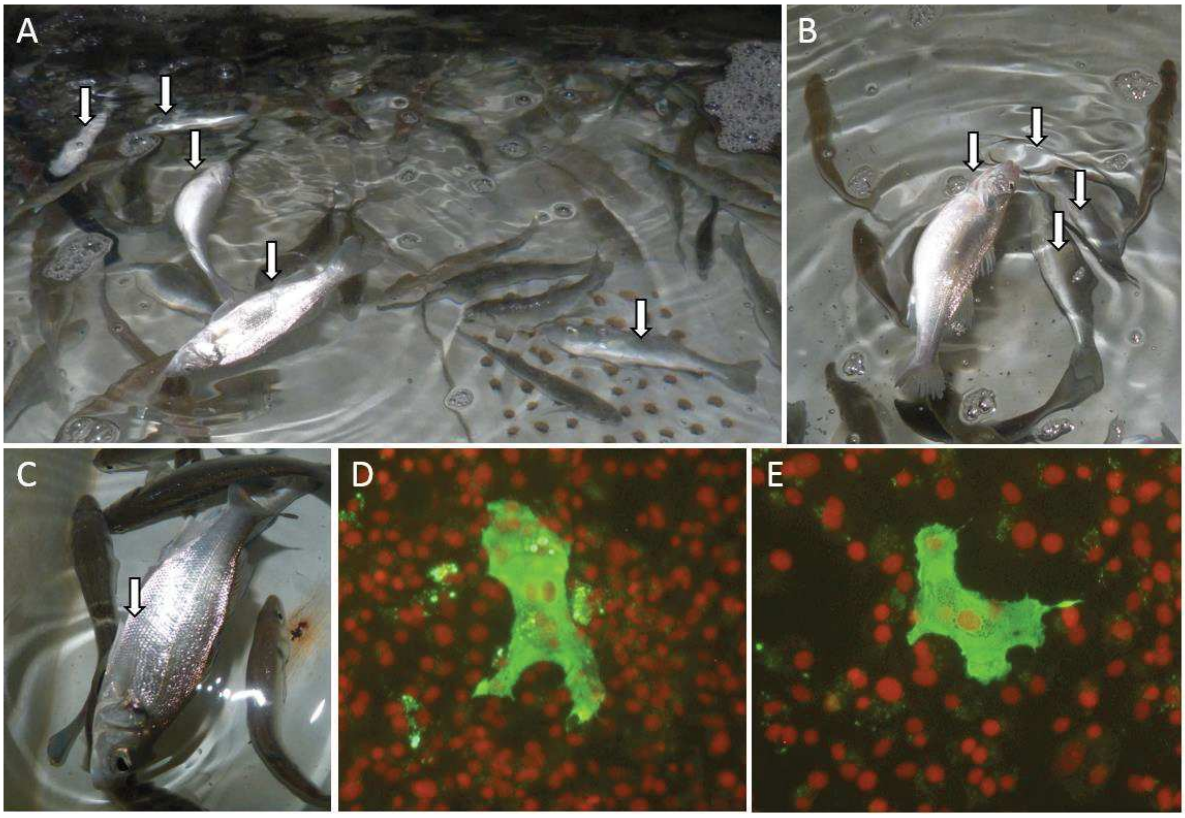

Figure 2.3: (a–c) Typical clinical signs observed during experimental nervous necrosis virus (NNV) infection in European seabass (arrows show impacted fish). (d, e) Positive immunofluorescence antibody test signal (in green) obtained for betanodaviruses on SSN1 cell line. Source: Anses, Ploufragan-Plouzané Laboratory, Viral diseases of fish Unit.

2.4.2. Direct molecular methods:

Numerous RT-PCR protocols have been described for the detection of VNN (table 2.3). The first RT-PCR published designed a set of primers (F2/R3) directed against 430 bp from the

T4 variable region of the RNA2 segment of a SJNNV strain isolated from striped jack (Nishizawa et al. 1994). Later on, the same region was amplified from other isolates, such as red-spotted grouper (Nishizawa et al. 1995). This test, recommended by the World Organization for Animal Health (OIE) until 2006, was extensively used for routine diagnostic and genotyping of betanodavirus and led to the current classification (Nishizawa et al. 1995; Nishizawa et al. 1997). However, the sensitivity of this method is not only limited by a low viral load but also by the genetic diversity of the T4 region that leads to mismatches between the F2/R3 primers and their targets (Nishizawa et al. 1996; Thiéry et al. 1997; Dalla Valle et al. 2001). In some cases, it has been illustrated that betanodavirus in brain could be detected by immunohistochemistry whereas the same samples were negative by RT–PCR (Thiéry et al. 1997). In addition, low or false positive as well as false negative results were reported in different fish species like striped jack, barfin flounder, European seabass, shi drum *Umbrina cirrosa* and gilthead sea bream (Nishizawa et al. 1996; Mori et al. 1998; Thiéry et al. 1999; Watanabe et al. 2000; Dalla Valle et al. 2000). To improve the performance of this test and take into account genetic diversity reported in newly available sequences, further generations of tests were developed. Primers specific to more conserved region of the RNA2 or allowing to discriminate Mediterranean and Atlantic viral strains were published as well as Nested-PCR approaches allowing to improve the sensitivity by at least 100 times (Thiéry et al. 1999; Dalla Valle et al. 2000). More recently, Bigarré and colleagues designed a new set of primers in a highly conserved region (680 bp) named T6 in RNA2 which perfectly matches with a wide range of published sequences and detects at least three of the five described species namely RGNNV, SJNNV and BFNNV (Bigarré et al. 2010).

Since 2005, numerous real-time RT-PCR assays were developed to regularly adapt the primer sets and probes to newly published sequences (Dalla Valle et al. 2005; Fenner et al. 2006a; Panzarin et al. 2010; Hick & Whittington 2010; Hodneland et al. 2011; Baud et al. 2015). These Real-time RT-PCR assays, targeting RNA1 or RNA2, are now currently used for the diagnosis of betanodavirus because they are less time consuming than classical approaches and significantly decrease cross-contamination occurring during post amplification procedures (Hick & Whittington 2010; Hodneland et al. 2011). Recently, a one-step generic TaqMan® method targeting sequences found in a vast majority of known viral genotypes was validated and efficiently used to detect NNV in different geographic regions and host species (Panzarin et al. 2010; Baud et al. 2015), and an optimized loop-mediated isothermal amplification has

been developed to detect NNV in *Epinephelus septemfasciatus* (Hwang et al. 2016). This last method showed improved sensitivity compared to PCR.

Table 2.3: Primers/probes sets used for betanodavirus detection by RT-PCR

Primer/Probe	Target	GenBank accession number [§]	Sequence 5' - 3'	Position	Key Ref.
VNNV1 VNNV2 VNNV3 VNNV4	RNA2	AB056572	ACACTGGAGTTTGAAATTCA GTCTTGTTGAAGTTGTCCCA ATTGTGCCCCGCAAACAC GACACGTTGACCACATCAGT	343-362 953-934 366-383 620-601	Dalla Valle et al. 2000
AH95-F1 AH95-R1	RNA2	AJ245641	AGTGCTGTGTCGCTGGAGTG CGCCCTGTGTGAATGTTTTG	577-596 917-898	Grotmoll & Totlandl 2000
F2 R3	RNA2	AB056572	CGTGTCAGTCATGTGTCGCT CGAGTCAACACGGGTGAAGA	592-611 1017-998	Nishizawa et al. 1994
F'2 R'3	RNA2	Y08700	GTTCCCTGTACAACGATTCC GGATTTGACGGGGCTGCTCA	677-693 970-951	Thiéry et al. 1999
Q-CP-1 Q-CP-2	RNA2	D38636	CAACTGACAACGATCACACCTTC CAATCGAACACTCCAGCGACA	234-256 463-443	Dalla Valle et al. 2005
P1 P2 Probe	RNA2	AJ245641	GGTATGTCGAGAATCGCCC TAACCACCGCCCGTGTT TTATCCCAGCTGGCACCGGC*	141-159 351-335 183-202	Grove et al. 2006
qR2TF qR2TR R2probe2	RNA2	*LcNNV09_07* [ǂ]	CTTCCTGCCTGATCCAACTG GTTCTGCTTTCCCACCATTTG CAACGACTGCACCACGAGTTG*	378-397 470-451 448-428	Hick & Whittington 2010
RNA2 FOR RNA2 REV probe	RNA2	DQ864760	CAACTGACARCGAHCACAC CCCACCAYTTGGCVAC TYCARGCRACTCGTGGTGCVG*	392-410 460-445 422-442	Panzarin et al. 2010
Nod1f Nod1r	RNA2	EF617335; AY744705; AF 175511; AB056572; AJ608266; D38637; D38635	TTCCAGCGATACGCTGTTGA CACCGCCCGTGTTTGC AAATTCAGCCAATGTGC*	322–341[d] 376–391[d] 356-372[d]	Hodneland et al. 2011
Nod2f Nod2r	RNA2	EF617335; AY744705; AF 175511; AB056572; AJ608266; D38637; D38635	CTGGGACACGCTGCTAGAATC TGGTCGTTGTCAGTTGGATCA AAATTCAGCCAATGTGC*	301–321[d] 414–434[d] 356-372[d]	Hodneland et al. 2011
RG-RNA2-F2: RG- RNA2-R2:	RNA2	D38636	CGTCCGCTGTCCATTGACTA CTGCAGGTGTGCCAGCATT	624–643 723–705	Lopez-Jimena et al. 2011
oPVP111 oPVP88	RNA2	AF245003 ; AF245004 ; AF281657 ; AF499774 ; AJ245641 ; AJ608266 ; D30814 ; U39876 ; EF433468 ; AY549548 ; EU236149	TCCTGCCTGAYCCAACTGAC TGGTCATCMACGATACGCAC	381-400[Þ] 1058-1039[Þ]	Bigarré et al. 2010
Q-RdRP-1 Q-RdRP-2	RNA1	D38636	GTGTCCGGAGAGGTTAAGGATG CTTGAATTGATCAACGGTGAACA	589-610 861-839	Dalla Valle et al. 2005
RG-RNA1-F: RG-RNA1-R:	RNA1	AY369136	GGCTCAGATCTGGTAATGTTTCAA CAAAGCCAAGGGAAGAAGCA	2144– 2167 2206–2187	Lopez-Jimena et al. 2011
oPVP154 oPVP155 Taqman-Probe	RNA1	AJ401165; EF617335; EU826137 ; AB025018 ; AB056571 ; AF319555 ; GQ402010 ; GQ402012 ; AY690597	TCCAAGCCGGTCCTAGTCAA CACGAACGTKCGCATCTCGT CGATCGATCAGCACCTSGTC*	2717-2736[¥] 2884-2865[¥]	Baud et al. 2015

[§]sequences from wich the primers or probes have been designed; *label position on probes; [ǂ]the primers and probe design was achieved on an isolate obtained from a infected barramundi sampled but not reported in GenBank (Hick & Whittington, 2010); [d]the position of the primers and probe are based on SJNNV genome (AB056572); [Þ]the position of the primers and probe are based on BFNNV genome (AY549548); [¥]the position of the primers and probe are based on BFNNV genome (AJ401165).

Detection of different NNV species coexisting in the same host is still complex and may require a combination of approaches (Lopez-Jimena et al. 2010). An ubiquitous assay detecting all species would be desirable, but because of the high genetic diversity of betanodavirus, selection of specific and wide spectrum primers allowing the detection of all possible variants still remains a big challenge (Hodneland et al. 2011).

2.4.3. Indirect serological methods:

Serological investigations have been developed for several viral fish diseases but only few of them are used for routine surveillance, despite the fact that diseases survivors often become latent carriers with significant antibody response. The major reasons for this are poor knowledge on the kinetics of the antibody response in fish at various water temperatures and lack of validation data. Nevertheless, several ELISA or serum neutralization tests described and improved over time proved their efficiency to detect antibodies specific to VNN (Watanabe et al. 1998; Huang et al. 2001; Fenner et al. 2006b; Scapigliati et al. 2010; Choi et al. 2014; Jaramillo et al. 2016b). For ELISA tests, the determination of the cut-off point is critical to make the distinction between virus free status and viral infection. These indirect methods are routinely used by several fish farms to regularly screen breeders. They have the advantage to be no-lethal and safe for fish, and allow a regular screening of the VNN serological status of a population at an individual level (Breuil & Romestand 1999; Watanabe et al. 2000; Breuil et al. 2002; Breuil et al. 2001; Jaramillo et al. 2016a; Nuñez-Ortiz et al. 2016).

2.5. Control procedures

There are no simple and effective procedures to treat the viral disease in fish once established. Therefore, efforts were concentrated on the means and tools to prevent entry, diffusion and persistence of the virus, mostly strict hygiene, vaccination and eradication of infected populations (Gomez-Casado et al. 2011; Shetty et al. 2012).

In hatcheries, an important route of virus entry is infected asymptomatic breeders (Mushiake et al. 1994; Watanabe et al. 1998). Although ozonation can seemingly prevent NNV transmission from infected broodstock, it is not fully efficient because betanodavirus is not only present on the surface of the eggs but also inside the eggs, and can also penetrate the egg via spermatozoa (Kuo et al. 2012). A positive point is that vertical transmission can be controlled effectively in hatcheries by combining detection via serological tests (ELISA) to detect anti-VNN specific antibodies (in the blood serum of broodstock) or/and sensitive RT-PCR assays to recognize viral RNA (in the eggs or genital fluids), combined with the elimination of positive individuals

(Mushiake et al. 1994; Breuil & Romestand 1999; Watanabe et al. 2000; Breuil et al. 2002; Hodneland et al. 2011). Ozonation and ultra-violet light are also used to clean fertilized eggs and control water quality during rearing larval and juvenile stages. Even if treatment of larvae requires complicated procedures, these treatments appear effective to prevent horizontal transmission (Arimoto et al. 1996; Watanabe et al. 1998; Grotmoll & Totlandl 2000). Although betanodaviruses can be prevent effectively in hatchery based on manage betanodavirus-free broodstock and disinfect hatchery water, the fish can be infected betanodaviruses from the environment when they are cultured at grow-out stages.

Vaccination has been considered as an effective procedure for controlling VNN disease. A number of vaccines made with inactivated NNV, virus-like particles (VLPs), recombinant C protein and synthetic peptides from the C protein have been tested (Gomez-Casado et al. 2011). Recombinant betanodavirus coat proteins expressed in *Escherichia coli* was firstly proposed in different fish species like sevenband grouper *Epinephelus septemfasciatus* and humpback grouper *Cromileptes altivelis* (Tanaka et al. 2001; Yuasa et al. 2002), turbot and Atlantic halibut (Húsgarð et al. 2001; Sommerset et al. 2005). More recent constructions combined to artermia or *Vibrio anguillarum* induced significant levels of protection in larvae of orange-spotted grouper *Epinephelus coioides* (Lin et al. 2007; Chen et al. 2011), and enhanced virus-neutralizing antibody response was observed after immunisation at grow-out stages with recombinant C protein (Sommerset et al. 2005). Virus-like particles have also been developed to create a more effective procedure to control VNN disease (Thiéry et al. 2006; Liu et al. 2006b). To date, the efficiency of the pFNCPE42-DNA vaccine, which has been developed using the capsid protein gene of an Indian isolate of fish nodavirus has been illustrated in Asian seabass with a high relative percent survival of 77.33% (Vimal et al. 2016). All these systems require going through an injection. Consequently, they are only really effective on grow-out size fish or to prevent vertical transmission in breeding, while the VNN disease often occurs in early larval and juvenile stages at which the size of fish is too small to allow vaccination by injection (Sommerset et al. 2005; Kai & Chi 2008; Brudeseth et al. 2013). A water-delivery strategy (immersion) could represent a more interesting way of control (Kai & Chi 2008) but still needs to be improved.

Table 2.4: The different types of NNV vaccine tested in fish

Type of Vaccinations	Species	Method	Results/RPS	Key Ref.
Inactivated vaccines				
- BEI-inactivated HGNNV vaccine - Formalin-inactivated vaccines	Orange-spotted grouper *Epinephelus coioides* (early larval stage-40 dph with average body weight (BW) of 0.2 g and TBL of 2.4cm)	immersion	- RPS = 79% (BEI-inactivated NNV vaccines) - 39% (Formalin-inactivated NNV vaccines)	Kai & Chi 2008
Formalin-inactivated vaccine (RGNNV)	Sevenband grouper *Epinephelus septemfasciatus* (juvenile-25.4 g)	injection	60% in fish groups immunized with $10^{7.5}$ $TCID_{50}$ per fish or higher doses.	Yamashita et al. 2009
BEI-inactivated HGNNV vaccine	Adult Orange-spotted grouper *Epinephelus coioides* (mean body weight of 1.35kg)	injection	High efficiency	Kai et al. 2010
Formalin-inactivated vaccine (RGNNV type)	Brown-marbled grouper *Epinephelus fuscogutattus* (5g)	injection	86 – 100%	Pakingking et al. 2010
Recombinant vaccines				
Recombinant capsid protein vaccine (Artemia-encapsulated recombinant *E. coli* expressing the NNV capsid protein gene)	Orange-spotted grouper *Epinephelus coioides* (Larvae-35dph)	oral	64.5%.	Lin et al. 2007
Recombinant capsid protein (*Vibrio anguillarum*-based oral vaccine)	Orange-spotted grouper *Epinephelus coioides* (fry)	oral	78.3%	Chen et al. 2011
Recombinant capsid protein (rT2 vaccine)	Turbot *Scophthalmus maximus* (weighing from 1 to 3 g (mean 1.8 g))	injection	82%	Húsgarð et al. 2001
Recombinant capsid protein vaccine (recAHNV-C) & vaccine plasmid (called pAHNV-C)	Turbot *Scophthalmus maximus* (Juvenile-mean weight 2.2 g)	injection	- 50%% in fish groups immunized with recAHNV-C (10 mg) + pAHNV-C (5 mg) - 57% in fish groups immunized with recAHNV-C (10 mg)	Sommerset et al. 2005
Recombinant protein vaccine-*E.coli* BL21 (DE3)	Sevenband grouper *Epinephelus septemfasciatus* (28g)	injection	88% in fish groups immunized with $10^{3.4}$ $TCID_{50}$ per fish	Tanaka et al. 2001
VLPs vaccines				
Virus-like particles (VLPs) of GNNV	- Dragon grouper *Epinephelus lanceolatus* (20g) - Malabar grouper *Epinephelus malabaricus* (20g)	injection	Significant efficiency	Liu et al. 2006b
Virus-like particles (VLPs) - MGNNV VLPs (trial 1) - SB2 VLPs (trial 2)	European seabass *Dicentrarchus larbrax* - 66g - 22g	injection	- 71.7 – 89.4% - 27.4 – 88.9%	Thiéry et al. 2006
DNA vaccines				
pFNCPE42-DNA vaccine	Asian seabass *Lates calcarifier* (juvenile stage)	injection	77.33%	Vimal et al. 2016

The viral diversity of betanodavirus with at least four different species described is another challenge to overcome for which DNA vaccines have numerous advantages compared to traditional antigen vaccines (Gomez-Casado et al. 2011). However, no license has been delivered to date for potential applications in commercial fish farms in some areas such as Europe (Gomez-Casado et al. 2011; Brudeseth et al. 2013). The vaccine application is usually expensive in fish and the protection generated often lasts for in short time because of the low immune reactivity in early stages of life (Sommerset et al. 2005). For these disadvantages, although a variety of vaccinations for NNV have been experienced (table 2.4), only one inactivated RGNNV vaccine against NNV of sevenband grouper was commercialized in Japan (Brudeseth et al. 2013). Nevertheless, work in progress to better understand the immune mechanisms involved during NNV infection (Costa & Thompson 2016; Carballo et al. 2016; Wu et al. 2016) will likely result in the near future in the improvement of the prophylactic strategies, like the use of preventive administration of interferons at the larval stage (Kuo et al. 2016) or of ribavirin as antiviral agent (Huang et al. 2016).

2.6. Selective breeding to VNN resistance: Prospective procedures

While selective breeding programs have been mostly targeting productivity traits like e.g. growth and carcass quality (Gjedrem & Thodesen 2005), disease resistance remains a major goal for breeding programs, as mortality caused by diseases is a major threat to aquaculture. Selecting fish with increased resistance to specific diseases seem to be feasible for most diseases (reviewed by Gjedrem 2015). Moreover, it provides cumulative and permanent improvement of resistance over generations at the population level, thus providing unique benefits when compared to other methods. Due to its cost however, the selective breeding strategy toward resistant cultured fish is particularly interesting when other prevention methods are inefficient. The use of resistant populations would not only reduce outbreaks, but also lower the cost of fish production (Ødegård et al. 2011; Yáñez et al. 2014a).

2.6.1. Disease resistance heritability in fish

Improving a trait by artificial selection basically requires sufficient genetic variation for this trait in the population. Genetic variation in disease resistance has been observed for many diseases, and most likely variation would be seen for all diseases (Bishop & Woolliams 2014). While heritability for resistance to viral diseases have been estimated in many species, it remains that most studies have been conducted in salmonids.

The heritability of resistance to viral diseases has been shown to be moderate to high in fish (table 2.5). In the first place, resistance to VHS virus (VHSV) was found highly heritable (h^2=0.57-0.63) in rainbow trout (*Oncorhynchus mykiss*) when assessed by mortality (Dorson et al. 1995; Henryon et al. 2005), while it was little heritable (h^2=0.11±0.10 and 0.13) when resistance was assessed as the time until death following challenge (Henryon et al. 2002; Henryon et al. 2005). Moderate to high heritabilities have been estimated for Infectious Salmon Anaemia Virus (ISAV), ranging from 0.13 to 0.26 on the observable scale and from 0.19 to 0.40 on the liability scale (Gjøen et al. 1997; Ødegård et al. 2007a; Olesen et al., 2007; Kjøglum et al. 2008; Gjerde et al. 2009), while the heritability of Infectious Pancreatic Necrosis Virus (IPNV) resistance was also found moderate to high, ranging between 0.16 and 0.55 (Guy et al. 2006; Guy et al. 2009; Wetten et al. 2007a; Kjøglum et al. 2008). Other viral diseases in fish also display moderate to high heritability, such as resistance to salmon pancreases disease virus (SPDV) in Atlantic salmon (*Salmo salar*) with a liability scale estimate of 0.21±0.05 (Norris et al. 2008), and koi herpesvirus (KHV) resistance (h^2=0.79±0.14) in common carp (*Cyprinus carpio*) (Ødegård et al. 2010a).

To date, a high heritability for NNV has been demonstrated, but only in Atlantic cod (Ødegård et al. 2010b; Bangera et al. 2011; Bangera et al. 2013). Ødegård et al. (2010b) compared the NNV resistance of three different groups of Atlantic cod including Norwegian coastal cod (CC), Northeast Atlantic cod (NEAC) and their F1 crossbreds. They showned that the highest survival was observed in CC (56%), followed by crosses (31%), whereas the survival rate of NEAC was only 10%. The estimated heritability for NNV resistance was high on the observed scale (0.43±0.07) and very high on the underlying scale (0.75±0.11) (Ødegård et al. 2010b). Besides that, a high heritability for NNV resistance was also recorded (0.68±0.14) by Bangera et al. (2011) who later on reported an extremely high heritability (0.91 using a cure model) for NNV resistance in the same species (Bangera et al., 2013). In addition, the genetic correlation between resistance to NNV and to a bacterial disease (Vibriosis) was shown not to significantly differ from zero (Bangera et al. 2011). This lack of correlation is similar to other studies in salmonids which estimated the genetic correlation between resistance against ISAV and furuculosis (Gjøen et al. 1997; Ødegård et al. 2007b; Kjøglum et al. 2008) or VHSV and enteric red-mouth disease as well as rainbow trout fry syndrome (Henryon et al. 2005).

The heritability of resistance to viral disease is moderate to high in almost exsisting studies, indicating viral disease resistance can be improve significantly based on selective breeding in

farmed fish – and the prospects for NNV resistance are specially good, due to the high to very high heritability estimate (only in Atlantic cod for the moment).

Table 2.5: Recent heritability estimates of resistance to viral diseases in farmed fish species

Pathogen	Species (host)	Heritability: h^2 (±S.E.)		Notes	Key ref.
		Binary traits	Time until death		
VNNV	Atlantic cod (*Gadus morhua*)	h^2=0.75 (±0.11)		Threshold model (on the underlying scale)	Ødegård et al. 2010[b]
		h^2= 0.68 (±0.14)		Threshold model (on the underlying scale)	Bangera et al. 2011
		h^2= 0.91		CURE model	Bangera et al. 2013
VHSV	Rainbow trout (*Oncorhynchus mykiss*)	h^2= 0.63 (±0.26)		Linear model (angular transformation)	Dorson et al. 1995
			h^2= 0.13	On the logarithmic-time scale	Henryon et al. 2002
		h^2= 0.57	h^2= 0.11 (±0.10)	Survival, liability scale	Henryon et al. 2005
ISAV	Atlantic salmon (*Salmo salar*)		h^2= 0.13 (±0.03) (O.S.) h^2= 0.19 (U.S.)	Linear model (Observable scale)/ On the underlying liability scale	Gjøen et al. 1997
		h^2=0.24 (±0.03)		Threshold model using cross-sectional data	Olesen et al. 2007
		h^2=0.318(±0.022)		Threshold model (on the underlying scale)	Ødegård et al. 2007[a]
		h^2=0.319(±0.022)		Threshold model (on the underlying scale)	Ødegård et al. 2007[b]
		h^2=0.37		On the underlying liability scale	Kjøglum et al. 2008
		h^2=0.40 (±0.04)		On the underlying liability scale	Gjerde et al. 2009
IPNV	Atlantic salmon (*Salmo salar*)	h^2=0.43	h^2=0.16	transformed to the liability scale/Observed	Guy et al. 2006
		h^2= 0.31		Linear model (Observable scale)	Wetten et al. 2007
		h^2=0.55		On the underlying liability scale	Kjøglum et al. 2008
		h^2=0.38 (±0.017)		On the underlying liability scale	Guy et al. 2009
SPDV	Atlantic salmon (*Salmo salar*)		h^2= 0.21 (±0.005)	transformed to the liability scale/ Linear model (Observable scale)/	Norris et al. 2008
KHV	Common carp (*Cyprinus carpio*)	h^2=0.79 (±0.14)		On the underlying liability scale	Ødegård et al. 2010[a]

2.6.2. Genetic Selection to Viral Disease Resistance in Fish

Following promising heritability estimates, experimental selective breeding for disease resistance has been undertaken and shown to be an effective solution to prevent the outbreak of viral diseases in farmed fish. In the end of the1980s, selective breeding for resistance to VHSV in rainbow trout was successfully tried in France, resulting in an improved resistance in the

second generation, with 0 to 10% mortality, compared to 70 to 90% in the control group (Dorson et al. 1995). In Denmark, relatively VHSV-resistant broodstock were selected from a challenge test, and used to produce first and second generation gynogenetic offspring (Bishop & Woolliams 2014). Salmon commercial breeding programs have included resistance to furunculosis, ISAV and IPNV since 1993 in Norway (Gjøen et al. 1997; Moen et al. 2009; Yáñez et al. 2014). The effective of selective breeding for IPNV resistance in Atlantic salmon was illustrated by Storset et al. (2007), where the fish belonging to low and high resistant families were challenged in both freshwater and seawater and obtaining significant differences in mortalities, which ranged from 29-32% in high resistance families to 66-79% in low resistance families in both freshwater and seawater.

2.6.2.1. Quantitative Trait Loci mapping for resistance to viral diseases

Identifying portions of the genome called Quantitative Trait Loci (QTLs) linked to the disease resistance phenotype is expected to speed up the selection process by using Marker-Assisted selection (Massault, Bovenhuis, Haley & D.-J. de Koning 2008; Bishop & Woolliams 2014).

Most of the QTLs identified for resistance to viral diseases in cultured fish have been identified in Salmonids, the most successful example being the IPNV resistance QTL. Three highly significant QTLs were first identified using microsatellite and AFLP markers in a backcross of rainbow trout strains displaying high and low resistance to IPNV, each explaining 13-15% of the phenotypic variance of the total phenotypic variance (Ozaki et al. 2001; Ozaki et al. 2007). For IPNV resistance in Atlantic salmon, even more significant QTLs have been identified (Houston et al. 2008; Houston et al. 2010; Moen et al. 2009; Gheyas et al. 2010), leading to a breakthrough with respect to the implementation of QTL in salmon breeding. A first QTL, producing a 75% difference in IPNV mortality between the alternative homozygotes, was mapped to linkage group 21 (LG21) (Houston et al. 2008). The same QTL was independently reported in 2009 in Norwegian population, where it explained 29% of the phenotypic variance (Moen et al. 2009). Gheyas et al. (2010) confirmed the resistance effect of the QTL from LG21 at the fry stage in freshwater, with a QTL heritability of 0.45 ± 0.07 on the liability scale and 0.25 ± 0.05 on the observed scale. In one family, 100% of the offspring homozygous for the susceptible QTL alleles died, whereas 100% of the offspring homozygous for the resistant QTL alleles survived (Gheyas et al. 2010).

QTLs for resistance to other viral diseases in Salmonids include QTLs for IHNV resistance (Palti et al. 1999; Palti et al. 2001; Miller et al. 2004; Rodriguez et al. 2004; Barroso et al. 2008),

ISAV resistance (Moen et al. 2004; Moen et al. 2007), VHSV resistance (Verrier et al. 2013) and Salmonid Alphavirus (SAV) resistance (Gonen et al. 2015). Like for IPNV, the IHNV QTLs explained a high part of the phenotypic variance (up to 32.5% according to Barroso et al. 2008), while it was more limited for the ISAV QTL (6% of the phenotypic variance, Moen et al. 2007). In both cases, a significant association with MHC alleles was later demonstrated (Palti et al. 2001; Miller et al. 2004 for IHNV; Kjøglum et al. 2006 for ISAV).

About NNV resistance, five genome-wide significant QTLs, explaining 68% of the phenotypic variance for resistance, detected based on 161 microsatellite markers in Atlantic cod (Baranski et al. 2010), a very high amount, which can be paralleled to the very high heritability of NNV resistance reported earlier. A later analysis with a 12K SNP array confirmed both the high proportion of variance explained by genomic markers, and the location of three of these QTLs (Yu et al. 2014). The latest QTLs related to NNV resistance identified based on 146 microsatellite markers in Asian seabass. In that study, Liu et al. 2016 detected multiple QTLs for NNV resistance and survival time. However, a few proportion of the phenotypic variation were explained by those QTLs (2.2-4.1% for resistance and 2.2-3.3% for survival time).

Taken altogether, these information about the QTLs for resistance to viral diseases in fish are very promising for increasing the rate of resistance through selective breeding, especially as in many cases QTLs seem to be of large effect, which gives good prospects to improve genetic resistance in a relatively short term, by direct marker–assisted selection or by introgression of QTLs from different populations (Bishop & Woolliams 2014). This possibility may especially develop as SNP markers become more and more available and affordable, due to their abundance and to fast technological developments, making both detection and selection of QTLs more economically realistic.

2.6.2.2. Markers-assisted Selection (MAS) and Genomic Selection (GS) for Viral Disease in Fish

Breeding resistant fish based on survivors of challenge trials, although sometimes done, is generally undesirable due to the risk of vertical transmission of the pathogen. The usual way to overcome this limitation in conventional breeding is to perform sib selection. In sib selection, breeding candidates are kept in a pathogen-free environment, and selected using family-wise estimated breeding values obtained from the survival of fish from the same families challenged with the disease. Another possible way to select resistant fish without exposing them to the pathogen is the identification of relevant QTL and the application of molecular markers for

Marker Assisted Selection (MAS), or the direct use of genotype data to perform Genomic Selection (GS). With both methods, fish are selected based only on their genotype, either at specific QTL-linked markers in the case of MAS, or at many markers, which may not all be linked to the resistance in the case of GS. This allows to avoid any contact between the breeding candidate and the pathogen. In terms of efficiency, the advantage of MAS compared to conventional selection is expected to be largest when the trait under selection has a low heritability – which is not generally the case for viral disease resistance in fish - or when the trait is not measured on the breeding candidates – which conversely is typically the case for disease resistance (Gjedrem 2015). With simulated traits and populations, the accuracy of selection was improved significantly by using MAS, compared to non-MAS in selective breeding in aquaculture (Sonesson 2007). Practical application of MAS in aquaculture breeding has been implemented for IPNV resistance Atlantic salmon in both Norwegian (Moen et al. 2009) and Scottish populations (Houston et al. 2010). Still, the limitation of MAS is that it requires prior knowledge of alleles that are associated with the traits of interest, which moreover have to be validated in the specific populations or even families under selection. Furthermore, MAS exploits only a limited part of the genetic differences between individuals, as it does not exploit the polygenic background variation, which may account for a large part of the genetic variance (Meuwissen et al. 2016).

An alternative approach for more polygenic traits is genomic selection. In this approach, genetic markers are used to cover the whole genome so that all QTL, even non statistically significant, are in linkage disequilibrium (LD) with at least one marker and selection is based on genetic values predicted from all the markers (Meuwissen et al. 2001; Goddard & Hayes 2007; Meuwissen et al. 2016). The availability of high density SNP arrays in livestock and now increasingly in aquaculture species is making both genomic selection and genome-wide association studies (GWAS) feasible. GWAS approaches allow studies of the genetic architecture of quantitative traits, while genomic selection will improve the accuracy of selection in breeding programs (Houston et al. 2014). In terms of present realization of these approaches, GWAS showed highly significant association of several SNPs with resistance to IPNV, as well as population level linkage-disequilibrium in salmon commercial populations (Houston et al. 2012). The implementation of such approaches is dependent on the development of SNP genotyping arrays, which for the time being have mostly been developed in salmonids, like a 130K array for farmed and wild Atlantic salmon in Scotland (Houston et al. 2014), 160 K SNP markers were validated based on 200 K SNPs applied to different wild and farmed

populations of Atlantic salmon (Europe population, North America population and Chile population) (Yáñez et al. 2014b), and a 57 K SNP chip which is now available for rainbow trout (Palti et al. 2014). A 12K SNP array has been also developed in Atlantic cod, containing markers distributed across all 23 chromosomes (Yu et al. 2014). It was already used in a GWAS analysis for NNV resistance which revealed 29 genome-wide significant SNPs for binary survival, and 36 genome-wide significant SNPs for number of days fish survived, as well as high genomic heritability of 0.49 and 0.81 for the same traits, respectively (Bangera et al. 2014). Identification of SNPs is being done in other species for which NNV resistance is a key issue, such as European seabass (Tine et al. 2014; Palaiokostas et al. 2015) or Asian seabass (Wang et al, 2015), which is promising for the development of GWAS or GS for NNV resistance in those species.

2.7. Conclusion

Viral encephalopathy and retinopathy is widespread all over the world except in South America. While many of the main marine species in aquaculture are affected by this disease, no simple and effective procedures are available to treat it. Even though VNN can be prevented in hatcheries based on efficient diagnostic methods to monitor the breeders and biosecurity measures during hatchery rearing, this disease still occurs on grow-out sites. Vaccination may be an efficient way to prevent disease occurrence, but because of the specific drawbacks of present vaccination methods and the difficulty to efficiently protect early larval stages, this tool is not fully effective in the case of VNN. Selective breeding has been demonstrated as an effective solution to select resistant aquaculture populations for several diseases, and new genomics based methods allow to foresee even higher efficiency of selective breeding for disease resistance in the near future. However, to reach the expectations of a practical genomic selection, more genetic resources and more advanced studies are required for the vast majority of aquaculture species affected by NNV.

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Chapter 3: Genetic variation of resistance to Viral Nervous Necrosis and genetic correlations with production traits in wild populations of the European seabass (*Dicentrarchus labrax*)

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Abstract

Viral Nervous Necrosis (VNN) disease is considered as one of the most serious threats for European seabass 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 seabass (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 seabass (h^2_u = 0.26±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 seabass for improved resistance to VNN disease.

Key words: VNN disease, VNN resistance, heritability, genetic correlation.

3.1. Introduction

European seabass (*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 seabass 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 seabass 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 seabass 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 seabass culture, because the mass mortality caused in seabass 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 seabass 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).

Improving a trait by artificial selection basically requires sufficient genetic variation 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 seabass would be worth investigating. European seabass 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.

3.2. Materials and methods

3.2.1. The origin of broodstock

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.

3.2.2. 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°C and 37‰ salinity. At 48 h post-fertilization (hpf), sinking (unfertilized or dead) eggs were removed, and 72,000 ready-to-hatch eggs were dispatched into two tanks for 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.

3.2.3. 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, 493 fish and 486 fish, respectively) after betanodavirus injection at 202 dph. All individuals had been tagged, measured and weighed three weeks before, at a mean BW of 16.3 g (4.9 – 44.6 g, CV= 37%) 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 (10^9virus/ml), diluted 100-fold in physiologic solution, thus with a virus dose of $2x10^7$ 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.

3.2.4. Genotyping and parentage assignment

Fin clips from the 2100 offspring and their 69 parents were sent to LABOGENA (Jouy-en-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.

3.2.5. 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:

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

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).

3.2.6. 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):

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

Where y_{ijklm} is the observed trait (VNN resistance as a binary trait - 1 for survivors, 0 for dead, body weight (BW_{180}), daily growth coefficient ($DGC_{180\text{-}431}$), 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 comparisons 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:

$$h_o^2 = \frac{4\sigma_{sire}^2}{(\sigma_{sire}^2 + \sigma_e^2)}$$

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,

$$h_u^2 = \frac{h_o^2 \times p(1-p)}{z^2}$$ (Dempster & Lerner 1950; Lynch & Walsh 1998)

Where h^2_u is the estimation of heritability on the liability scale, h^2_o is the heritability 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.

3.2.7. 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.

3.3. Results

3.3.1. 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).

3.3.2. ELISA results

High levels of betanodavirus were detected in the brains of the dead fish by ELISA, with 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 showed 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).

3.3.3. 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 (Figure 3.1). 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 3.1). 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 3.1). 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 3.2).

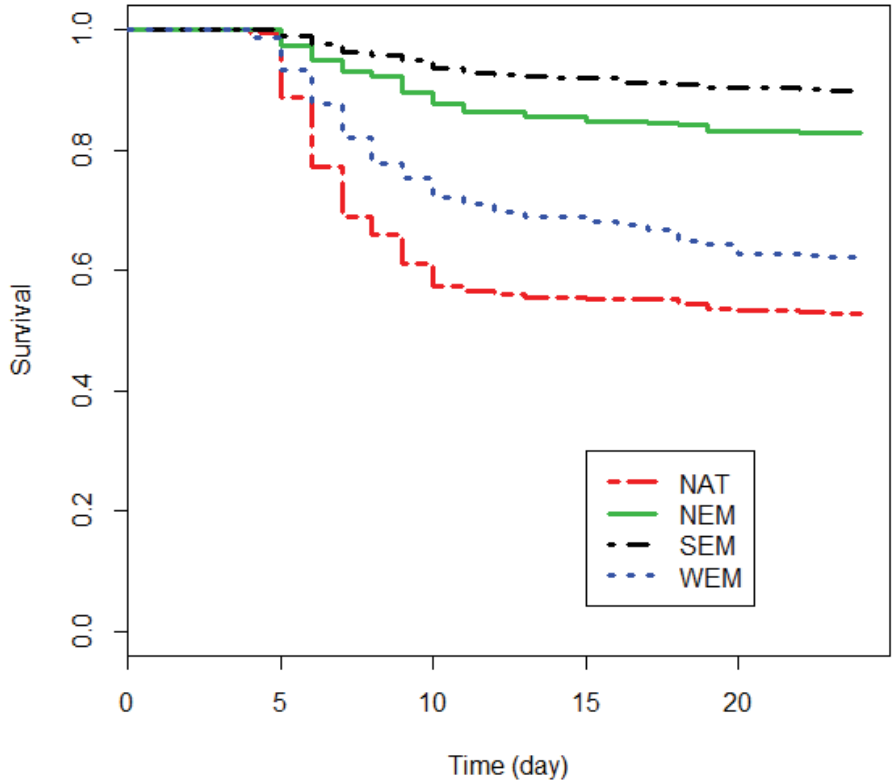

Figure 3.1: Evolution of cumulated survival in the offspring derived from 4 populations of European seabass sires (NAT: North Atlantic, NEM: North-East Mediterranean; SEM: South- East Mediterranean; WEM: West Mediterranean), mated with WEM dams, following experimental infection by NNV.

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 survival probability in all pure strains were computed under the hypothesis of additive and 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.

Table 3.1: Differences in survival and production traits in the offspring of European seabass 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 $DGC_{180-431}$ 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)**

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 3.1). Meanwhile, significant differences in DGC was only experienced between populations belonging to NAT sires and WEM sires ($P<0.05$).

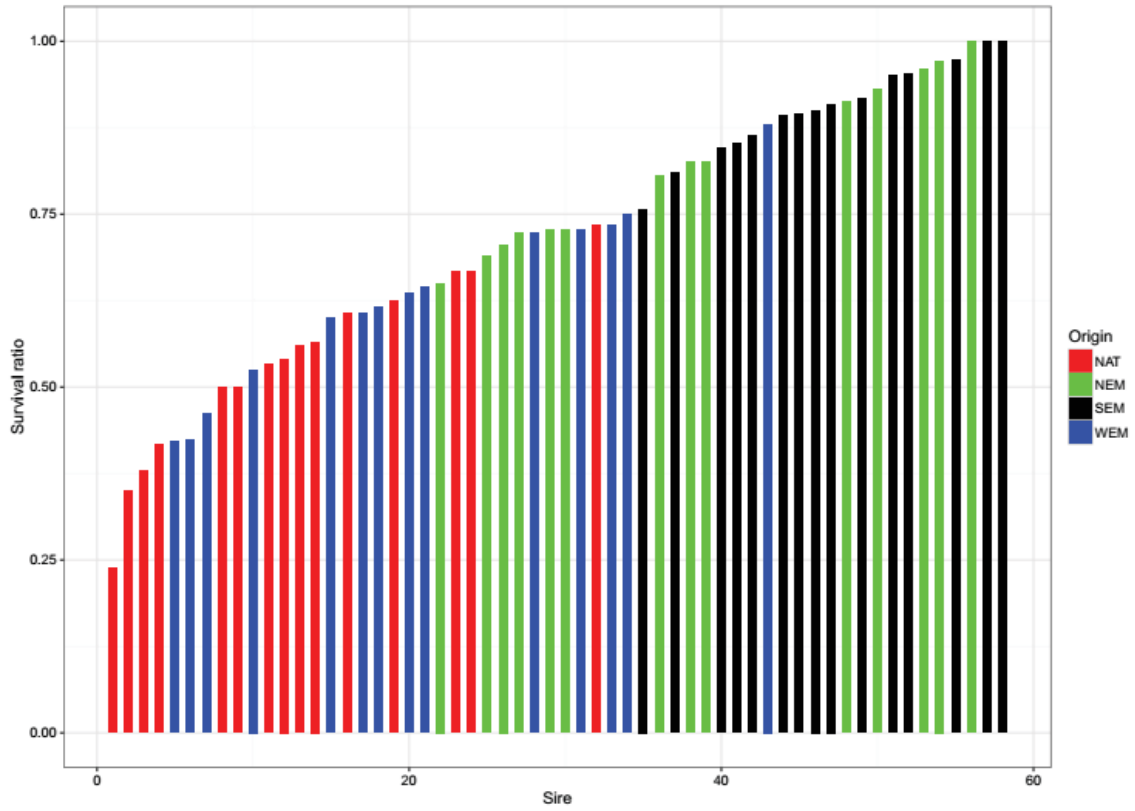

Figure 3.2: The variations of survival of sire families within and between populations during NNV test. North Atlantic in red, North-Eastern Mediterranean in green, South-Eastern Mediterranean in blue and West Mediterranean in yellow. For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.

3.3.4. Genetic parameters

3.3.4.1. 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 3.2). 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 3.2). 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.

Table 3.2: Heritability (intra- and inter-populations) of resistance against VNN and growth related traits. $\mathbf{h_o^2}$ is the heritability estimated on the observed scale, $\mathbf{h_u^2}$ 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				
Traits	**Intra-population**		**Inter-populations**	
	h_o^2	h_u^2 **± SE**	h_o^2	h_u^2 **± SE**
VNN resistance	0.14	0.26 ± 0.10	0.61	1.09±0.20
DGC$_{180\text{-}431}$		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				
Trait	**Intra-population**		**Inter-populations**	
	h_o^2	h_u^2 **± SE**	h_o^2	h_u^2 **± SE**
VNN resistance	0.14	0.26 ± 0.11	0.61	1.09±0.22
DGC$_{180\text{-}431}$		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

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 3.3). To be more specific, the genetic correlation between VNN resistance and BW (-0.35±0.14) and between VNN resistance and DGC (-0.28±0.20) were moderate while that 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 3.3).

Table 3.3: Genetic (above the diagonal) and phenotypic (below the diagonal) correlations 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	

3.4. Discussion

In this experiment, the first major result is the demonstration of substantial differences in resistance to VNN between four wild European seabass 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 3.1). 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.

A second major result of this study is the first demonstration of within population 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 seabass 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 population was not enough to reliably estimate genetic parameters (range 326-407 individuals per population). Heritability for resistance to RGNNV in European seabass 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 seabass. Taken altogether, selective breeding for resistance to VNN in European seabass 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 genetic correlations between them have also been reported, such as between VHSV and BW 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 seabass (Liu, Wang, Wan, et al. 2016a). Identification of such QTLs VNN resistance in European seabass may help to perform Marker-Assisted (Odegård et al. 2009)(Odegård et al. 2009)(Odegård et al. 2009)(Odegård et al. 2009)(Odegård et al. 2009)(Odegård et al. 2009)introgression (Odegård et el. 2009) and is therefore of particular relevance for the future development of selection for VNN resistance in European seabass.

3.5. Conclusion

The results showed survivals between the pure wild populations (SEM, NEM, WEM, NAT) 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 seabass. The large and significant variations in survival, the moderate heritability estimated on liability scale give good prospects of selective breeding of European seabass for improved resistance to VNN disease.

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Chapter 4: Construction of a medium-density SNP linkage map and mapping of QTL for resistance against viral nervous necrosis disease in European seabass (*Dicentrarchus labrax*)

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(MS)

Abstract

Detection of significant QTLs is an essential prerequisite to the development of Marker Assisted Selection (MAS). Regarding European seabass, previous studies identified QTLs linked to production traits (morphology, growth and stress response) and sex-determination. However, no QTLs linked to VNN resistance which has been published in this species. We firstly constructed a medium-density linkage map starting from 1274 SNPs using 1650 individuals derived from 397 full-sib families. In total, 1174 SNPs markers were successfully mapped into 24 linkage groups on the male map, the female map as well as on a sex-averaged map. The average number of markers per individual LG was 49 markers (ranging from 27 for LG24 to 65 for LG4). The length of the genetic male map, female map and sex-averaged map were 1287.8, 1609.7 and 1409.3 cM, respectively. Female map was totally 1.25 fold longer than that of male map. Besides that, the IM of female map (1.40 cM) was also much longer than that of male map (1.13 cM). Their signs illustrate that more frequent of recombination events during meiosis in females than in males. Origin specific linkage maps were also constructed based on separated sire populations (North Atlantic NAT, West Mediterranean WEM, North-Eastern Mediterranean NEM, and South-Eastern Mediterranean SEM). To specify, the total size of the linkage maps Atlantic was the longest (1091.4 cM) compared to the others, meaning that more recombination process were observed in the NAT sires. Secondly, QTL mapping was conducted based on the sex-averaged linkage map. Unfortunately, no significant QTLs linked to VNN resistance could be identified.

4.1. Introduction

Linkage maps are an essential prerequisite for QTL (Quantitative Trait Loci) mapping and for molecular breeding of animal and plant species. Beside the interest for QTL mapping (Yue 2014), such genetic maps also allow characterization of recombination hotspots along individual chromosomes (Gonen et al. 2014), comparative genomic analysis to study chromosomal organization and evolution (Gonen et al. 2014), and facilitate de novo genome assembly at chromosomal level (Shao et al. 2015).

In the past two decades, the linkage maps have been constructed in many commercially important aquaculture species using dominant (amplified-fragment length polymorphism markers (AFLP), random amplified polymorphic DNA (RAPD)) and co-dominant markers (microsatellites, SNPs). Most of them were low-density linkage maps constructed for some major economical aquaculture species using AFLP, microsatellites markers or combined between AFLP and microsatellites and some SNPs (Yue 2014). Several of the medium-density linkage map were also constructed based on microsatellites or using both microsatellites and SNPs in some important species, such as Atlantic salmon (*Salmo salar*) (Moen et al. 2004 & 2008), Japanese flounder (*Paralichthys olivaceus*) (Castaño-Sánchez et al. 2010), European seabass (*Dicentrarchus labrax*) (Chistiakov et al. 2008), Asian seabass (Wang et al. 2011 & Liu et al. 2016) and channel catfish (*Ictalurus punctatus*) (Liu et al. 2003). Recently, with the development of Next Generation Sequencing (NGS) technologies, high-density linkage maps have been constructed based on SNPs in several commercial important species, such as Atlantic salmon (*Salmo solar*) (Gonen et al. 2014), Tilapia (Kocher et al. 1997), channel catfish (Li et al. 2015), Japanese flounder (Shao et al. 2015), European seabass (Palaiokostas et al. 2015), Large Yellow Croaker (*Larimichthys crocea*) (Ao et al. 2015), Asian seabass (Wang et al. 2016) & (Liu 2016), Atlantic Salmon (*Salmo salar*) (Tsai et al. 2016) and ninespine stickleback (*Pungitius pungitius*) (Rastas et al. 2016). A recent review showed linkage maps had been published in at least 45 aquaculture species (Yue 2014). Compared to low-density genetic maps, high-density genetic maps have shown significant advantages in fine mapping of QTLs.

The availability of linkage maps makes possible to identify quantitative trait loci (QTL) for important traits of species of interest to assist the selection of the desired traits. Disease resistance is one of the most frequent traits in QTL studies, as diseases represent one of the major challenges and bottlenecks in aquaculture (Gjedrem 2015). To quantify the resistance of

each individual to pathogens, disease challenge experiments are conducted. Usually, survival, death and the time of survival/time to death are the traits recorded. Identifying portions of the genome (QTLs) linked to the disease resistance phenotype is expected to speed up the selection process by using Marker-Assisted Selection (Massault, Bovenhuis, Haley & Koning 2008; Bishop & Woolliams 2014). QTLs for resistance to most serious viral diseases (such as IPNV, IHNV, ISAV, VHSV, NNV) have been mapped in several important cultured species, such as IPNV in Atlantic salmon (Houston et al. 2008, Moen et al. 2009, Gheyas et al. 2010) and Rainbow trout (Ozaki et al. 2001; Ozaki et al. 2007), IHNV in Atlantic salmon (Miller et al. 2004), and rainbow trout (Palti et al. 1999; Palti et al. 2001; Rodriguez et al. 2004 ; Khoo et al. 2004; Barroso et al. 2008), ISAV resistance and Salmonid Alphavirus (SAV) resistance in Atlantic salmon (Moen et al. 2004; Moen et al. 2007; Gonen et al. 2015), VHSV resistance in rainbow trout (Verrier et al. 2013), Lymphocystis disease in Japanese flounder (Fuji et al. 2006) and bonamiosis in European flat oysters (Lallias et al. 2009), NNV in Atlantic cod and Asian seabass (Yu et al. 2014, Liu et al. 2016). However, Most of the QTLs identified for resistance to viral diseases in cultured fish have been identified in Salmonids, the most successful example being the IPNV resistance QTL.

In European seabass (*Dicentrarchus labrax*), a major aquaculture species cultured in the Mediterranean Sea, a first linkage map was constructed based on 174 microsatellite markers (Chistiakov et al. 2005). An updated linkage map was published in 2008 using 190 microsatellites, 176 AFLP and two SNPs (Chistiakov et al. 2008). One QTL for morphometric traits was identified based on the first linkage map (Chatziplis et al. 2007). Later, QTLs for growth trait, morphometric traits and stress response were identified (Massault et al. 2010). Recently, a high-density linkage map was constructed using 6706 SNPs obtained by RAD-sequencing. Based on this high-density linkage map, three sex-related QTLs were identified (Palaiokostas et al. 2015). Besides that, a gene-centromere map was constructed based on 804 female-informative SNPs in 24 linkage groups using F_2 generation, their F_1 parents and F_0 female (Oral et al. 2017). To date, no studies have investigated QTLs related to disease resistance in European seabass.

In this study, a medium-density linkage map was constructed using 1274 SNPs in order to identify QTLs for VNN resistance in European seabass.

4.2. Materials and methods

4.2.1. Mapping population

The mapping population used in this study is that (challenged and unchallenged fish with NNV) described in Chapter 2. Therefore, 1650 individuals (including 1308 challenged fish and 342 unchallenged fish) and their 67 parents (58 sires and 9 dams) were selected for SNP genotyping, resulting in 397 full-sib families, 58 sire half-sib families and 9 dam half-sib families genotyped. The number of fish per full-sib family ranged from 1 to 20 individuals (4 offspring per family in average). The number offspring ranged from 7 to 53 individuals in sire half-sib families while it ranged from 2 to 424 individuals in dam half-sib families.

4.2.2. SNP genotyping

Genomic DNA was extracted from 1650 offspring and their parents. Genotyping was performed with an iSelect Custom Infinium Illumina® European seabass 3K SNP array (manuscript in preparation) at LABOGENA in France. Quality control of genotype data was performed and SNPs with minor allele frequency lower than 5% and those deviating from Mendelian segregation rules were discarded, resulting in a total of 1274 SNPs distributed across the genome that were used for further analysis. This large drop between the 2722 markers spotted on the chip and the 1274 ones with valid genotypes is largely due to a technical bioinformatics issue during the SNPChip design that truncated the sequence of the markers spotted from the reverse submitted sequences (nearly half of the SNP array).

4.2.3. Construction of a medium-density SNP-based linkage map

The linkage map was constructed based on Lep-Map2 (Rastas et al. 2013). The 'SeparateChromosomes' module was applied to cluster markers into linkage groups. Linkage groups were formed with a minimum LOD value of 8, allowing a maximum distance between consecutive SNPs of 50 centiMorgans (cM). SNPs within each linkage group were ordered by applying the OrderMarkers module, where the likelihood of marker order is computed by using a hidden Markov chain model. Map distances were calculated in cM using the Kosambi mapping function (Rastas et al. 2013). In total, sex-averaged and sex-specific maps were computed from the global data set but also by origin and visualized using on MapChart-version 2.3 (Voorrips R.E. 2002).

4.2.4. QTL mapping

QTL detection for resistance to VNN was performed for both traits: time to death and binary survival. This was done using composite interval mapping (CIM), interactive QTL mapping (iQTLm) and iQTLm genome-wide (iQTLm-GW) methods implemented in the Spell-qtl v.0.2 (Leroux & Jasson 2017). For QTL detection, we used the sex-averaged map. Spell-qtl performs QTL mapping using a linear regression model and allows automatic identification of multiple QTL (Leroux & Jasson S. 2017). This method assumes that the QTL locations are the same in all families, that QTL genotypic effect are linked through multiple families' relationship and two different alleles are assumed at the QTL. The QTL significance is determined based on the Spell-qtl test, which equal to –log10 of the Fisher test *P* value thresholds corresponding to a type I error rate of 0.05 at the genome-wide level and at the chromosome level. Significance levels were determined using 1000 intra-family permutations of each trait data. The model assumes both additive and dominance genetic effects as describe below:

$$y = \mu_{c_i} + \sum_{h \in \mathrm{H}} \sum_{g \in \mathrm{G}(h)} P(i,g,h)\, \xi_{g,h,c_i} + \varepsilon_i$$

Where:

y is the value of the VNN resistance for individual i;

μ_{c_i} is the estimated mean value of the trait for this cross;

H is the set of genetic explicative factors;

G(h) is the set of possible genotypes for factor h (if h is the unit set h = {1} that is we address a single QTL, the G(h) = G(l) is the set of possible genotypes at locus l);

$P(i, g,h)$ is the probability that individual i has genotype g on h;

ξ_{g,h,c_i} is the phenotypic value of genotype g on h in genetic background c_i;

ε_i is the modeling error for individual i.

4.3. Results

4.3.1. Linkage map

In total, 1174 SNPs markers (92% of the SNPs) were successfully mapped onto 24 linkage groups for the male map, the female map as well as for the sex-averaged map (figure 4.1, 4.2 and table 4.1). There were on average 49 markers per individual LG (ranging from 27 for LG24

to 65 for LG4). The total length of the genetic male map, female map and sex-average map were 1287.8 cM, 1609.7 cM and 1409.3 cM, respectively. Regarding the sex-averaged map, the genetic length of LGs ranged from 31.13 cM (LG3) to 70.67 cM (LG6) with an average length of 58.72cM. The average marker interval (MI) was 1.24 cM (ranging from 0.83 for LG16 to 1.78 for LG24).

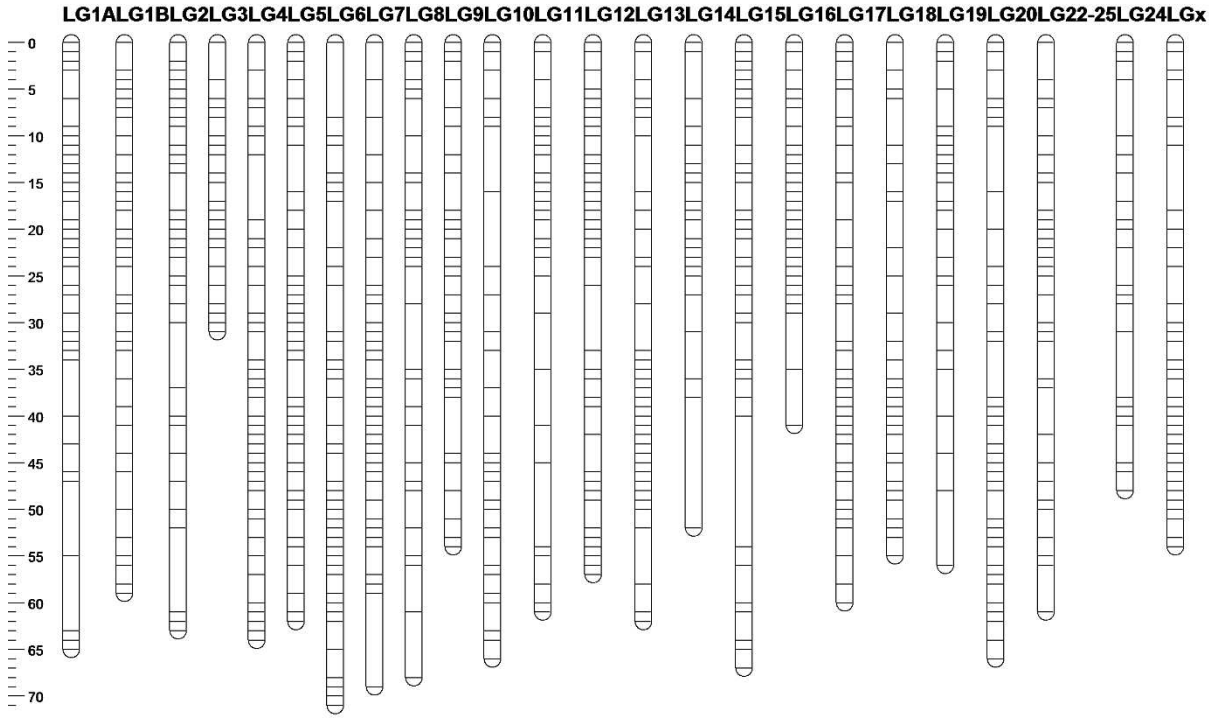

Figure 4.1: Genetic lengths and marker distribution of 24 linkage groups in the sex-averaged linkage map of European seabass

Origin specific linkage maps were obtain by computing males linkage maps on separated sire populations (four different origin linkage maps were constructed, namely NAT sire map, WEM sire map, NEM sire map and SEM sire map, figure 4.3). The total number of markers that assigned to LGs were varied between NAT and the others. The assigned marker numbers of NAT, WEM, NEM and SEM maps were 853, 916, 919 and 930, respectively. However, the total length of NAT was similar to that of SEM (1091.35 cM for NAT map, compared to 1057.68 cM for SEM map). Meanwhile those of WEM and NEM maps were close to each other (907.56 cM and 915.12 cM, respectively). In addition, the variations of them according to specific LG were very large. For instance, the length of WEM map for LG11 (11.04 cM) was equal to ½ the length of NEM map (23.84 cM) and equal to ¼ the length of NAT (48.0 cM) and SEM (52.82 cM) maps (figure 4.3).

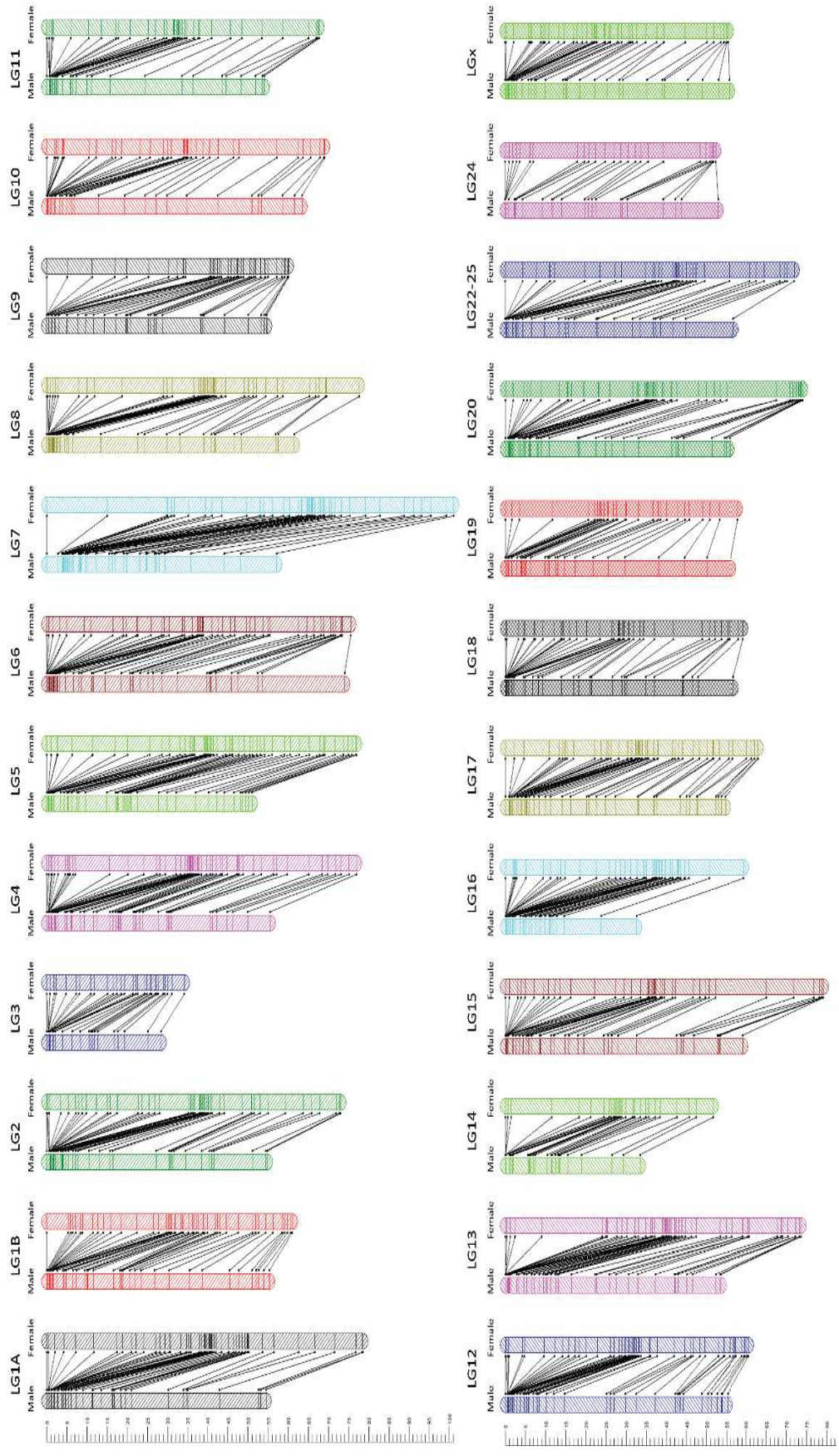

Figure 4.2: Comparative male and female linkage map of European seabass

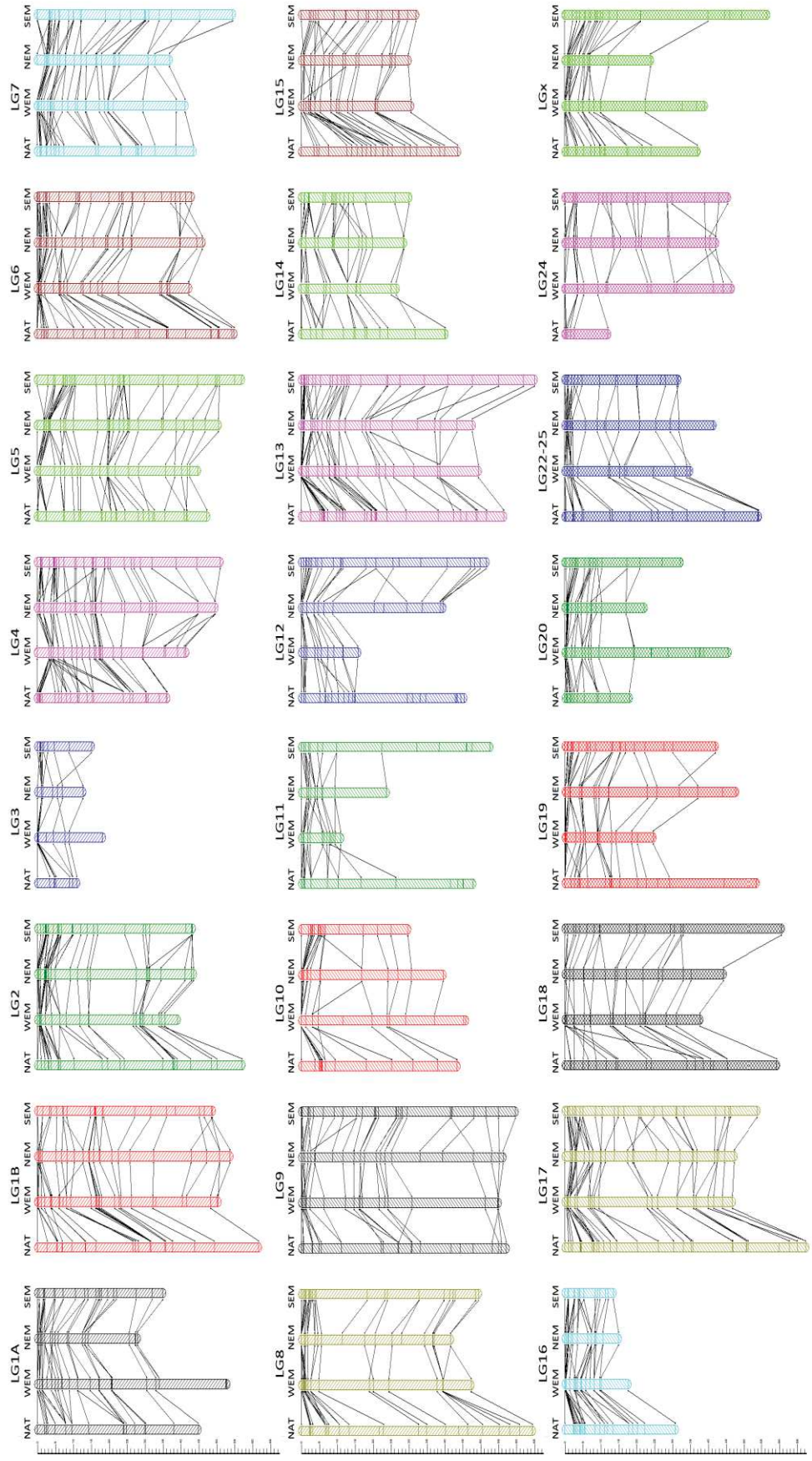

Figure 4.3: Comparative population-specific linkage maps in European seabass

Table 4.1: Genetic lengths and marker distribution of 24 linkage groups in the linkage maps of European seabass

LG	Mapped markers	Male map		Female map		Sex-averaged map	
		Genetic length (cM)	Marker interval (cM)	Genetic length (cM)	Marker interval (cM)	Genetic length (cM)	Marker interval (cM)
LG1A	**53**	**54.53**	**1.01**	**78.47**	**1.48**	**64.53**	**1.22**
LG1B	51	55.26	1.08	60.96	1.2	58.68	1.15
LG2	**60**	**54.82**	**0.91**	**72.99**	**1.22**	**62.77**	**1.05**
LG3	33	28.39	0.86	34.18	1.04	31.13	0.94
LG4	**65**	**55.49**	**0.85**	**76.88**	**1.18**	**64.28**	**0.99**
LG5	61	51.01	0.84	76.86	1.26	62.07	1.12
LG6	**60**	**73.98**	**1.23**	**75.52**	**1.26**	**70.67**	**1.18**
LG7	54	57.07	1.06	101.15	1.87	68.8	1.27
LG8	**44**	**61.5**	**1.40**	**77.57**	**1.76**	**67.69**	**1.54**
LG9	42	54.7	1.30	60.13	1.43	53.88	1.28
LG10	**44**	**63.54**	**1.44**	**69.1**	**1.57**	**65.64**	**1.49**
LG11	44	54.06	1.23	67.7	1.54	61.4	1.4
LG12	**58**	**55.34**	**0.95**	**60.59**	**1.04**	**57.45**	**0.99**
LG13	61	53.62	0.88	73.55	1.21	62.37	1.02
LG14	**33**	**33.45**	**1.01**	**51.6**	**1.56**	**51.56**	**1.56**
LG15	52	59.24	1.14	79.12	1.52	65.51	1.28
LG16	**50**	**32.64**	**0.65**	**59.34**	**1.19**	**41.4**	**0.83**
LG17	56	54.7	0.98	63.04	1.13	59.82	1.07
LG18-21	**46**	**56.77**	**1.23**	**59.21**	**1.29**	**55.28**	**1.2**
LG19	36	56.16	1.56	57.89	1.61	55.7	1.55
LG20	**53**	**55.84**	**1.05**	**74.02**	**1.4**	**65.73**	**1.24**
LG22-25	45	56.75	1.26	71.88	1.6	61.14	1.36
LG24	**27**	**53.05**	**1.96**	**52.38**	**1.94**	**48.1**	**1.78**
LGx	46	55.84	1.21	55.53	1.21	53.72	1.17
MEAN	49	53.66	1.13	67.07	1.40	58.72	1.24
Total	**1174**	**1287.75**		**1609.66**		**1409.32**	

4.3.2. Mapping QTLs for resistance against VNN

Genome scans of QTLs were performed in different methods (CIM, iQTLm, iQTLm-GW) implemented in Spell-qtl-0.2. Unfortunately, no QTL was found for any trait (time to death or binary survival).

4.4. Discussion

4.4.1. Linkage map:

The main function of the construction of a linkage map is to identify chromosome regions and/or genes that are responsible for the trait of interest by determining the association between phenotype and genotype of markers through QTL mapping. Therefore, it is an essential step for QTL mapping as well as for genomic studies (Collard et al. 2005; Gjedrem & Baranski 2010; Boopathi 2012). Density linkage maps, especially those constructed using sequence-based SNP markers, are useful in genetic studies including comparative genomics, fine mapping of interesting genes, positional cloning of candidate genes and facilitating assembly of genome sequences.

Linkage maps has been constructed for at least 45 main aquaculture species over the world, such as salmonid, European seabass, Asian seabass (Yue 2014). However, many of them were constructed based on a single family, leading to potential lack of generality for breeding programs (Rastas et al. 2013). Several linkage maps have also been constructed using multi-cross families and were used to identified QTLs for body weight, morphometric traits, stress response and sex-determination in the case of European seabass (Chatziplis et al. 2007; Massault et al. 2010; Palaiokostas et al. 2015). In our study, we constructed a medium-density linkage map of European seabass using 1274 SNP markers using multi-cross families with the hypothesis it would be more generic for genomic studies of European seabass populations.

Several different linkage maps have been constructed for European seabass using microsatellite markers, SNPs markers or combining microsatellite markers and AFLP markers and SNPs markers. There were large different lengths of those linkage maps, which can be explained by the different number of markers using to compile linkage map. To specify, the length of first sex-average linkage map of European seabass is 815 cM, which was constructed based on 162 microsatellite markers and using a single family with 50 full-sib progeny derived of wild

biparental diploids originated from Northern Adriatic Sea (Chistiakov et al. 2005), while the second sex-average linkage map using the same parents and offspring as Chistiakov et al. 2005 is 1373 cM long with 368 markers (including 190 microsatellite markers, 176 AFLP markers and two SNPs markers) (Chistiakov et al. 2008). Meanwhile, the high-density linkage map constructed using 6706 SNPs by Palaiokostas et al. is 4816 cM long (but only 2676 unique positions), which was constructed based on 175 F_2 individuals, six F_1 parents and the F_0 female (Palaiokostas et al. 2015). To date, a gene-centromere map was constructed based on 804 female-informative SNPs in 24 LGs but only 764 SNPs cover 24 LGs (only 448 unique positions) with total length of 1251 cM using a single meiotic gynogenetic family with 79 progeny (Oral et al. 2017).

In our linkage maps, the sex-averaged linkage map with 1174 SNP markers (with 1152 unique positions) is 1409.32 cM long with an averaged IM of 1.24 cM. While these maps were more accurate than Chistiakov's (IM of 5.03 cM in Chistiakov et al. 2005 & and 3.67 cM in Chistiakov et al. 2008), it is less precise than Palaiokostas' map with an averaged IM of 0.72 cM (Palaiokostas et al. 2015). However, from 199 SNPs genotyped from the "unknown" chromosome of the physical map, 152 (76% in total) were fitting succefully into 24 LGs. This new information could be useful to rearrange unadressed scaffolds of the physical map to their chromosome. Last but not least, the different length of linkage maps according to different populations were firstly experienced in our linkage maps (figure 4.3). The number of SNPs in the NAT sire population map was found the lowest (853 SNPs). However, this does not necessarily reflect a lower diversity in Atlantic population, because this may be due to the SNPChip design protocol. Indeed, SNPs spotted onto the beadchip were selected among SNPs revealed after whole genome sequencing of different individuals. But more effort has been done on Western Mediterranean animals. Therefore it is likely that SNPs spotted were more polymorphic in Mediterranean than in Atlantic. Moreover, comparing the total size of the linkage maps Atlantic was the longest (1091.4 cM) compared to the others, meaning that more recombination process were observed in the NAT sires. This result is in contradiction with potential lower diversity in Atlantic population as in the European genome study (Tine et al 2014), authors show that variation in local recombination rates strongly influences the genomic diversity by accelerating the diversification of the genome after the post-glacial secondary contact between Atlantic and Mediterranean lineages.

Another originality brang by our study is the comparison of the sex-specific maps to identify potential sex-mediated rates and patterns of recombination. In our study, male map was much shorter than that of female map (1287.75 cM for male map, compared to 1609.66 cM for female map). This situation was true for all LGs, exception of LG24 and LGx for which LG length were equal. Therefore, the IM of female map (1.40 cM) was also much longer than that of male map (1.13 cM). Their signs illustrate that more frequent of recombination events during meiosis in females than in males. It was also experienced for the first generation linkage map in European seabass where the female map is 905.9 cM long, whereas in contrast, the male map only covers 567.4 cM (Chistiakov et al. 2005). In addition, it was still true for the second generation linkage map of European seabass with 1380 cM in female map, compared to 1046.9 cM in male map (Chistiakov et al. 2008). Such sexual dimorphism in recombination landscape, called heterochiasmy (i.e. fact that recombination rate varies between sex), was often observed in fish (Sakamoto et al. 2000; Tsai et al. 2016; Rastas et al. 2016; Gonen et al. 2014) and has been hypothesized due to a possible male-female dimorphism in haploid selection (Lenormand & Dutheil 2005). The fact that in animals at least some genes are expressed and under selection during the male haploid phase, would tend to bias towards tighter linkage in males. In addition, from the comparison of the sex-specific map, we noticed that the recombination pattern is different between male and female, for almost for each LG. Indeed, in the male map, the IM is shorter at the upper position of the LGs, revealing lower recombination rate in these zones. In contrast, in the female map, MIs are high at the both extrimities and lower in the middle. This has to be highlighted by the recently published centromere mapping in European seabass (Oral et al. 2017). In this study, the authors conclude in acrocentric chromosomes (mono-arm) for all LGs except for LG14, LG17 and LG24. Such centromere mapping suggests that contrary to female, male's recombination rate reduces at the chromosome centromere. In summary, the result reveals a genome-wide reduction in male recombination, but also a sexual dimorphism in the recombination pattern, with higher telomeric recombination in males.

4.4.2. QTL mapping

Identifying quantitative trait loci (QTL) for viral disease resistance is of particular importance in selective breeding programs of fish species. In general, the relevant QTL for that trait must be identified in order to make MAS for any trait. The purpose of mapping QTL is to reveal the number and effect of portions of chromosomes carrying genes that affect a trait so that it assists

selective breeding to accelerate genetic improvement of important traits (Naish and Hard 2008). Based on identification of major QTL affecting VNN resistance in European seabass, genes or marker information may provide individual information on unchallenged selection candidates. Therefore, it is of particular relevance in fish breeding schemes for improved VNN disease resistance.

Several major QTLs have been identified in salmon and successfully applied for selective breeding in Scotland (Houston et al. 2008) and in Norway (Moen et al. 2009). Regarding VNN disease, several minor and major QTLs were published in Asian seabass and in Atlantic cod. To be more specific, five genome-wide significant QTLs, explaining 68% of the phenotypic variance for resistance, were detected based on 161 microsatellite markers in Atlantic cod (Baranski et al. 2010). A later analysis with a 12K SNP array confirmed both the high proportion of variance explained by genomic markers, and the location of three of these QTLs (Yu et al. 2014). Several minor QTLs (explaining a small proportion of phenotypic variance, 2.2-4.1% for resistance and 2.2-3.3% for survival time) responsible for VNN resistance and survival time were identified based on 146 microsatellite markers in Asian seabass (Liu et al. 2016). A major QTL was later published by the same authors, explaining 11.0% of the total phenotypic variations for resistance against VNN as well as for time to death in Asian seabass (Liu et al. 2017).

QTL mapping are usually achieved based on F_2 populations or backcross populations, it has been also constructed using F_1 hybrid populations derived from crossing genetically diverse individuals (Yue 2014). In the present study, unfortunately, no QTLs for VNN resistance were found. The power of mapping QTL can be influenced by a number of factors, such as genetic properties of QTL, experiment design, environmental effects, marker density and informativeness, genotyping errors and precision of trait measurement. In our case, it can be explained by the genetic diversity of the four wild different populations used in our study (Yue 2014). In such case, two adjacent markers may be linked to various underlying genetic variation due to ancient and repeated recombination processes. Therefore, SNPs with similar alleles may be identity by descent (in a single family) but also by state (between families and origins), in which case their association with polymorphisms causative of resistance may differ. Another reason that may explain the absence of identified QTLs in our study is the small number of progeny per full-sib family. The optimum family size in the QTL mapping population strongly

influences the intrinsic power of the experiment. Usually, large family sizes (>300 individuals) are required for detecting QTL of small effects (Massault et al. 2008). In our experiment, despite the fact that there were 1650 individuals in total, the number of offspring per full-sib family ranged from 1 to 20. Furthermore, as explained by Martinez, at least 50 individual per full-sib family is required for a reasonable-size QTL mapping design in outbred populations if the QTL explains about 10% of the phenotypic variance (Guimarães et al. 2007, Chapter 17 (Martinez)). Last but not least, another possible explanation may be the structure of the population used for the QTL search, which in our case are F_1 populations, instead of F_2 or backcross population. In a F_1 population, where there is large difference in resistance among origins, as in our case (see chapter 3), it is likely that potential QTLs would be homozygous in the resistant fish, which would result in the absence of segregation of the resistance in the F_1 population, if all F_1 fish share then heterozygous for the QTL. However, although this situation is far from optimal, even some QTL mapping studies have been successfully conducted using F_1 populations (Ozaki et al. 2007; Chatziplis et al. 2007).

In European seabass, several QTLs have been determined using multiple cross families, such as QTLs for body weight, morphometric traits, stress response and sex-determination in European seabass (Chatziplis et al. 2007; Massault et al. 2010; Palaiokostas et al. 2015). To be more specific, one QTLs for length and depth was identified using a F_1 population consisted of 26 full-sib families including a common dam and several sires (Chatziplis et al. 2007). QTL mapping performed using five family with the number of offspring per family range from 93 to 143 individuals. As the results, two QTLs for body weight, six QTLs for morphometric traits and three suggestive QTLs for stress response, explaining between 8 and 38% of phenotypic variance (Masaultet al. 2010).

4.5. Conclusion

In this study, we show that female map was globally 1.25 fold longer than that of male map. Besides that, the IM of female map (1.40 cM) was also much longer than that of male map (1.13 cM). Their signs illustrate that more frequent of recombination events during meiosis in females than in males. In addition, 152 SNPs (76% in total) according to unknown LG were fitting succefully into 24 LGs. This new information could be useful to rearrange unadressed scaffolds of the physical map to their chromosome. However, QTL mapping was not successful.

Chapter 5: Genome-wide association study and genomic evaluations for resistance to VNN in European seabass (*Dicentrachus labrax*)

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Abstract

Genomic selection is currently spreading over the world, in terrestrial livestock but also for salmonids breeding. In European seabass, it is still not employed in practice, and its performance needs to be assessed. In this chapter, two-steps unweighted then weighted Genome-Wide Association Study (GWAS & wGWAS) was performed in the BLUPF90 software based on a single trait linear mixed models using the same SNPs as for QTL mapping. One individual significant SNP was identified post-wGWAS, which explains 3.11% of the resistance belonging to LG9. Finally, the potential for genomics prediction for VNN resistance using the different genomic models was performed and extensively presented. Genomic evaluation was done with GBLUP and SNP-BLUP models fitted in BLUPF90 and GS3, respectively, in order to assess the accuracy of genomic prediction, compared to pedigree-based (PBLUP) prediction. The accuracy of breeding values predicted based on genomic models were similar to those of traditional model (PBLUP) even though the genomic heritability (0.14±0.05 for binary trait and 0.08±0.03 for time to death) we estimated only equals half of the polygenic heritability (0.23±0.08 and 0.14±0.05 for time to death). This may suggest that our experimental setup was not optimal to account for the variation in VNN resistance using genomic information, and that VNN resistance may be more efficiently improved with genetic evaluations incorporating dense SNP genotype information in appropriate designs using larger families within one population.

5.1. Introduction

Genetic markers associated with quantitative trait loci (QTL) alleles for disease resistance are an essential tool for Marker-assisted Selection (MAS) of breeding candidates based on their genotypes, even in the absence of phenotypes, to accelerate genetic progress (Meuwissen et al. 2016). To date, the carriers of favorable QTL alleles and its effects are usually identified through dense panels of Single Nucleotide Polymorphisms (SNP) using linkage and association mapping (Yue 2014). Several major QTLs related to disease resistance have been identified in some main aquaculture species. For instance, a major QTL related to resistance to IPNV has been applied successfully for MAS in salmon commercial populations (Houston et al. 2008; Moen et al. 2009). However, the limitation of MAS is that it requires prior knowledge of alleles that are associated with the traits of interest, which moreover have to be validated in the specific populations or even families under selection. Furthermore, MAS exploits only a limited part of the genetic differences between individuals, as it does not exploit the polygenic background variation, which may account for a large part of the genetic variance (Meuwissen, Hayes & Goddard 2016).

An alternative approach for more polygenic traits is genomic selection (GS). In this approach, genetic markers are used to cover the whole genome so that all QTLs, even non-statistically significant, are in linkage disequilibrium (LD) with at least one marker, and selection is based on genetic values predicted from all the markers (Meuwissen, Hayes & Goddard 2001; Goddard & Hayes 2007; Meuwissen et al. 2016). The availability of high-density SNP arrays in livestock and now increasingly in aquaculture species is making both GS and genome-wide association studies (GWAS) feasible. GWAS approaches allow studies of the genetic architecture of quantitative traits, while GS will improve the accuracy of selection in breeding programs (Houston et al. 2014). In terms of present realization of these approaches, GWAS showed highly significant association of several SNPs with resistance to IPNV in Atlantic salmon (Houston et al. 2012) and to VNN in Asian seabass (Wang et al. 2017).

In GS, sibs of the selection candidates with both phenotype and genotype are used to estimate each marker effect which are later used to predict GEBV for the selection candidates using only genotypic information (Meuwissen 2001). In aquaculture, studies using simulated (Nielsen et al. 2009; Sonesson & Meuwissen 2009; Lillehammer et al. 2013) and real data (Tsai et al. 2015; Correa et al. 2017; Bangera et al. 2017) have shown the superior performance of GS methods

in terms of increased genetic gain, accuracy of selection and lower rate of inbreeding. The accuracy of genomic prediction is considered as the key parameter to evaluate the ability of genomic selection to improve the traits of interest (Houston et al. 2014). Many GS methodologies varying with respect to assumptions about marker effects have been proposed for the genome-enabled prediction of EBV, such as GBLUP, rr-BLUP, SNP-BLUP, Bayesian methods (Bayes A, Bayes B, Bayes C (C_π), Bayesian LASSO). The results of the different models using high-density SNP genotypes are however rather similar to each other in practical fish experiments (Bangera et al. 2017; Correa et al. 2017). The most widely used GS methods are the genomic best linear unbiased prediction (GBLUP) approach using realized genomic relationship matrix calculated from the dense genome-wide SNP markers and Bayesian methods (Meuwissen et al. 2001; Habier et al. 2009).

The accuracy of genomic-based predicted breeding values is often substantially higher than that of pedigree-based breeding values. Therefore, GS approaches have been tested in several diseases in aquaculture species. However, most of them were carried out in salmonids. Odegard et al. have illustrated genomic prediction using GBLUP and IBD-GS models with different density of SNPs (ranging from 1K to 220K) for salmon lice resistance in Atlantic salmon, showing a systematically higher accuracy than pedigree-based prediction (Ødegård et al. 2014). Similarly, Tsai et al. tested genomic models for sea lice resistance in Atlantic salmon with different SNP densities, showing that the accuracy of genomic prediction was highest when the training and validation sets were close to each other (Tsai et al. 2016). To date, a higher accuracy of various genomic models using diffent density of SNPs, compared to pedigree-based model has been reported for resistance against sea louse, salmon rickettsial syndrome in Atlantic salmon (Correa et al. 2017; Bangera et al. 2017), and for BCWD resistance in rainbow trout (Vallejo et al. 2017). Last but not least, though no significant individual SNPs linked to resistance to pasteurellosis in gilthead sea bream was identified, the accuracy of genomic-based prediction was sharply higher than that of pedigree-based model (Palaiokostas et al. 2016). However, until now, no genomic evaluations for disease resistance have been published in European seabass.

The first objective of this study was to perform GWAS in order to identify any significant genome wide association of markers linked to resistance to VNN. The second objective was to

compare the reliability of commonly used genomic prediction methods for genomic selection and pedigree based BLUP for VNN resistance in European seabass.

5.2. Materials and methods

5.2.1. The populations and SNP genotypes

The populations used in this study were originally derived from the previously described populations challenged and unchallenged with NNV (chapter 3), together with the SNP genotypes which were described in chapter 4.

5.2.2. Principal component analysis

To describe briefly the genetic structure among individuals genotyped, we performed a principal component analysis (PCA) using the R package SNPRelate (Zheng et al 2012). PCA was conducted with minor allele frequency (MAF) ≥ 0.05 missing rate criteria ≤ 0.15 from snpgdsPCA function of SNPRelate package. For the four first principal components, the percentage of variation explained was computed and two-dimension scatter plots were drawn.

5.2.3. Genome-wide association study

GWAS data was performed using the BLUPF90 family of programs (Misztal et al. 2015). The data (including 1391 individuals genotyped for 2722 SNPs) were firstly filtered by quality control procedures to exclude SNPs with Mendelian errors, SNPs with a minor allele frequency (MAF) lower than 0.05 and SNPs with a proportion of missing genotypes greater than 0.1. Individuals with conflicts between the pedigree and the SNP genotype were also discarded in this process. As the result, 16 individuals and 1515 SNPs were discarded on this basis, leaving 67 parents and their 1308 challenged offspring and 1207 SNPs which were retained for the analysis.

The Genome-wide association study (GWAS) was carried out in two steps as advised by (Zhang et al. 2016; Wang et al. 2012). To do so a first GWAS was done based on a single trait linear mixed model as below:

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Wa} + \boldsymbol{e} \qquad \textbf{(1)}$$

Where y is the vector of VNN resistance phenotypes, $\boldsymbol{b}$ is a vector of fixed effects (overall mean of Origin, Tank and BAC effects), $\boldsymbol{a}$ is a vector of (additive genetic) animal effects with

distribution ~N(0,$\boldsymbol{G}\sigma^2_a$), $\boldsymbol{e}$ is the vector of random error effects with a distribution ~N(0,$\boldsymbol{I}\sigma^2_e$), $\boldsymbol{X}$ and $\boldsymbol{W}$ are the incidence matrices, and $\boldsymbol{I}$ is the identity matrix.

SNP effects ($\boldsymbol{u}$) were estimated by POSTGSF90 using the model $\boldsymbol{a}=\boldsymbol{Zu}$ where $\boldsymbol{Z}$ is a matrix relating genotypes of each locus and $\boldsymbol{u}$ is a vector of SNP marker effects. A first round of unweighted GWAS was run using similar weights for all SNPs, giving initial estimates of $\boldsymbol{u}=\boldsymbol{DZ'[ZDZ']}^{-1}\hat{\boldsymbol{a}}_g$, with $\boldsymbol{D}$ a diagonal matrix of weights which was the identity matrix in this first run (Wang et al. 2012).

Then, as proposed by Wang et al (2012), a new run of GWAS was performed by weighting SNPs based on their effect in the first run. The weight for SNP i was calculated as u_i^2 and implemented in the $\boldsymbol{D}$ matrix. The results of this second run were used as estimates of SNP effects.

5.2.4. Prediction of phenotype for VNN resistance based on (genomic/pedigree) breeding values

5.2.4.1. Breeding value estimation

The two resistance traits time to death (all survival fish were set as the same value that is day 30) and binary survival were analyzed separately as a linear trait (in BLUPF90) and threshold trait (in GS3), respectively in univariate models using 1391 fish (comprising 1308 challenged fish their 67 parents) with 1207 SNP markers. The EBV were estimated using polygenic pedigree based BLUP (PBLUP) (Misztal et al. 2009). The SNP effects and GEBV were estimated using SNP genotype information on genomic BLUP (GBLUP) (Misztal et al. 2009) and SNPBLUP (Legarra et al. 2016).

Pedigree-based BLUP

The conventional pedigree-based variance components and EBV were estimated using PBLUP as the model as below:

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Wa} + \boldsymbol{e} \qquad (2)$$

Where $\boldsymbol{y}$ is the vector of VNN resistance phenotypes, $\boldsymbol{b}$ is a vector of fixed effects (overall mean of Origin, Tank and BAC effects), $\boldsymbol{a}$ is a vector of random additive genetic polygenic effects with a distribution ~N(0,$\boldsymbol{A}\sigma^2_a$), $\boldsymbol{e}$ is the vector of random error effects with a distribution

~N(0,$\boldsymbol{I}\sigma^2_e$), $\boldsymbol{X}$ and $\boldsymbol{W}$ are the incidence matrices, $\boldsymbol{A}$ is the pedigree-based additive genetic relationship matrix (Lynch & Walsh 1998) And $\boldsymbol{I}$ is the identity matrix.

The time to death and binary survival traits were firstly fitted by AIREMLF90 uses Average-Information REML for estimating variance components. Besides that, they were also fitted by Bayesian analyses via Gibbs sampler using THRGIBBS1F90 for binary survival and GIBBS1F90 for linear trait (time to death) implemented in BLUPF90 family programs (Misztal et al. 2015). For Bayesian analysis, the Gibbs sampler was run for 120 000 iterations with a burn in of 20 000 iterations, and samples from every 100th sample were saved.

Genomic BLUP (GBLUP)

The SNP based variance components and GEBV were estimated using GBLUP, similar to the PBLUP model (2) described above. However, in GBLUP, the relationship matrix $\boldsymbol{A}$ based on the pedigree information in mixed model equations is replaced by matrix $\boldsymbol{G}$ ($\boldsymbol{G}$ is created as describe by VanRaden (VanRaden 2008), and $\boldsymbol{a}$ is the vector of random additive genetic polygenic effects with distribution ~N(0,$\boldsymbol{G}\sigma^2_a$). All other parameters and details of the analysis for time to death and binary survival are the same as for PBLUP (2).

SNPs-based BLUP

This method was similar to GBLUP , where *a priori* distribution of additive marker locus effects was considered to be normal (Meuwissen et al. 2001; VanRaden 2008; Legarra 2014).

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Zu} + \boldsymbol{Ta} + \boldsymbol{e} \qquad \textbf{(3)}$$

Where $\boldsymbol{y}$ is the vector of VNN resistant phenotypes, $\boldsymbol{u}$ is the additive marker locus effect, $\boldsymbol{Z}$ is the incidence matrix relating to marker genotype and all other parameters are the same as PBLUP. It was assumed that $\boldsymbol{u}$ follows *a priori* a normal distribution ~N(0,$\boldsymbol{I}\sigma^2_u$), where I is an identity matrix. This model is often called ridge-regression best linear unbiased prediction (rrBLUP) with a normal distribution of marker effects (Meuwissen et al. 2001; VanRaden 2008; Legarra 2014).

The known variances were set for all random effects, the binary survival was analyzed as a threshold (probit) model and random effects were estimated via Gibbs sampler by running "MCMCBLUP" command while the time to death trait was analyzed as a linear-mixed model in the context of Henderson's BLUP with the command "BLUP" in the GS3 software (Legarra

et al. 2016) (a single chain with a length of 120000 iterations was run. The burn-in period and the thinning interval used were 20000 and 100 iterations, respectively).

For both traits, the initial genetic variance σ_u^2 and residual variance σ_e^2 estimated from the model PBLUP were used to estimate additive marker variance $\sigma_u^2 = \sigma_a^2 / 2\sum piqi$ (Legarra et al. 2016).

5.2.4.2. Genetic parameters and GEBV

The total additive genetic variance (σ_u^2) computed based on AIREML and threshold model in PBLUP and GBLUP models as calculated using relationship matrix ***A*** and ***G***, respectively. Heritability was estimated as follows:

$$h^2 = \frac{\sigma_u^2}{(\sigma_u^2 + \sigma_e^2)}$$

The observed heritability for binary survival trait was estimated by AIREML then transformed to liability heritability using Dempster & Lerner correction (Dempster & Lerner 1950; Lynch & Walsh 1998) as described in materials and methods part of Chapter 3.

5.2.4.3. Cross validation scheme for model comparison

Predictive abilities of models are assessed through five-fold cross validation (CV) scheme (for all models) or Leave-one-out model (Loo-model – for models applied in BLUPF90).

For the Five-fold model, all the fish with both phenotypes and genotypes were randomly chosen into five validation sets. The GEBVs of the validation data sets were predicted one at a time where the phenotype of the validation fish (20% of the each population) was masked (set to missing values) and all remaining fish with phenotype and genotype (80% individuals of each population) were used as training data. The process was repeated 5 times, each time with a different set of individuals as the validation portion, until all individuals had their phenotype predicted. Regarding Loo-model, it was replicated N times with N-1 training sets and a single validated individual for each time. Predictive ability was presented as reliability estimated as:

$$R_{EBV,BV} = \frac{R_{EBV,y}}{h}$$

Where $R_{EBV,y}$ is the correlation between predicted (G)EBV for fish in the validation data in a given model (predicted from the training data) and the recorded phenotype (y), while h is the square-root of heritability (h^2) of the trait estimated based-on PBLUP with full-dataset and without marker information.

The Pearson's correlation coefficients between the (G)EBV, which were reported by the different models, was used to measure the degree of similarity between the rankings of fish. Besides that, the slope of regression of recorded phenotype (either time to death or binary survival) on (G)EBV was also calculated for all models. Then they were used as a measure to indicate the bias of the (G)EBV. To specify, a slope of less than 1 or greater than 1 indicates a biased underestimation or overestimation in the (G)EBV prediction, respectively , whereas in contrast, a slope of regression coefficient close to 1 indicates no bias in the model and breeding values are equal in magnitude (Resende et al. 2012). The reliability, Spearman's rank correlation and slope of regression for each model, were reported as the average of the cross validation schemes used.

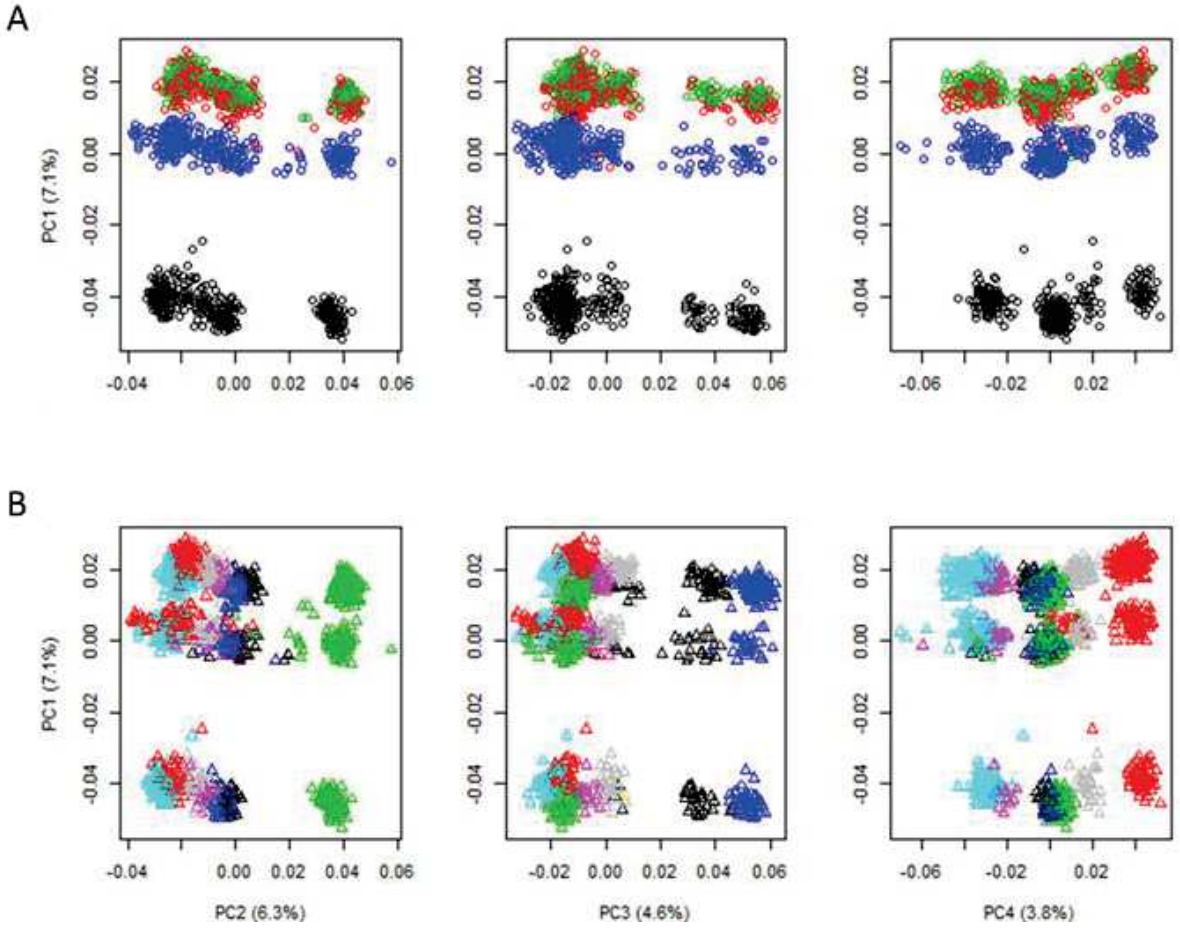

Figure 5.1: Scatterplots showing the first four principal components of the principal component analyses (DAPC). A: colors represents sires origins: NAT in black; WEM in blue; NEM in red and SEM in green. B: colors represents the different dam progenies. Variance explained by PCs are in brackets.

5.3. Results

5.3.1. Principal component analysis

Principal components were generated from the SNP genotyped on the 1650 offspring. The figure 5.1 shows the first principal component plotted against the second, third and fourth to illustrate genotype grouping patterns. The four first components explained respectively 7.1%, 6.3%, 4.6% and 3.8% of the variance. From figure 5.1.A the first component clearly distinguish three groups consistant with Atlantic, Western Mediterranean or Eastern Mediterranean sires origin, confirming Origin of sires as the major cause of population stratification (to be taken as a fixed effect to account for population structure in the GWAS). However, we notice secondary structure from other components linked to dams as depicted in figure 5.1.B.

5.3.2. Genome-wide association study

Approximately 3K SNPs were genotyped. However, 1515 SNPs were eliminated essentially due to a bioinformatics error during the beadarray design (see 4.2.2 for more explanation), but also after minor allele frequency filtering (MAF > 0.05), after Mendelian error assessment and low call rate. Therefore, only 1207 SNPs were kept for association study. After the first step of unweighted GWAS, the LOD values of all SNPs were below the genome-wide significant threshold after Bonferroni correction (LOD value = 5.08= $-\log_{10}(0.01/N)$ (Bush & Moore 2012; Clarke et al. 2011), where N is the number of SNPs post-quality control) (figure 5.2). However, we notice one SNP in LG9, which has a LOD value of 4.21, closed to the significance threshold, and which explains 1.98% of the resistance (figure 5.2.A1 & A2). Interestingly, by using a sliding windows of 5 SNPs, we observe some groups of SNPs belonging to LG9, LG12 and LGx explaining from 1.7% to 3.16% of VNN resistance variance (figure 5.2.A3). Similar results of GWAS (data not shown) were observed using *--assoc* function of PLINK when adding sire Origin as a clustering constraints (Purcell et al. 2007). From this results, individual weights of SNPs were calculated and used to run a wGWAS. From this wGWAS we observe that one SNP surpassed the threshold of significance with a variance explained of 3.11% (figure 5.2B1 & B2). Moreover, by using the sliding windows computation of variance explained by 5 adjacent SNPs, we notice 3 remarkable locations in LG9, LG12 and LGx as already suggested from the first unweighted GWAS (figure 5.2B3). Sliding windows with noticeable variance represent

regions of 1.69 Mbp for LG9 (LG size = 22 Mbp), 3.29 Mbp for LG12 (LG size = 23 Mbp) and 1.82 Mbp for LGx (LG size = 17 Mbp).

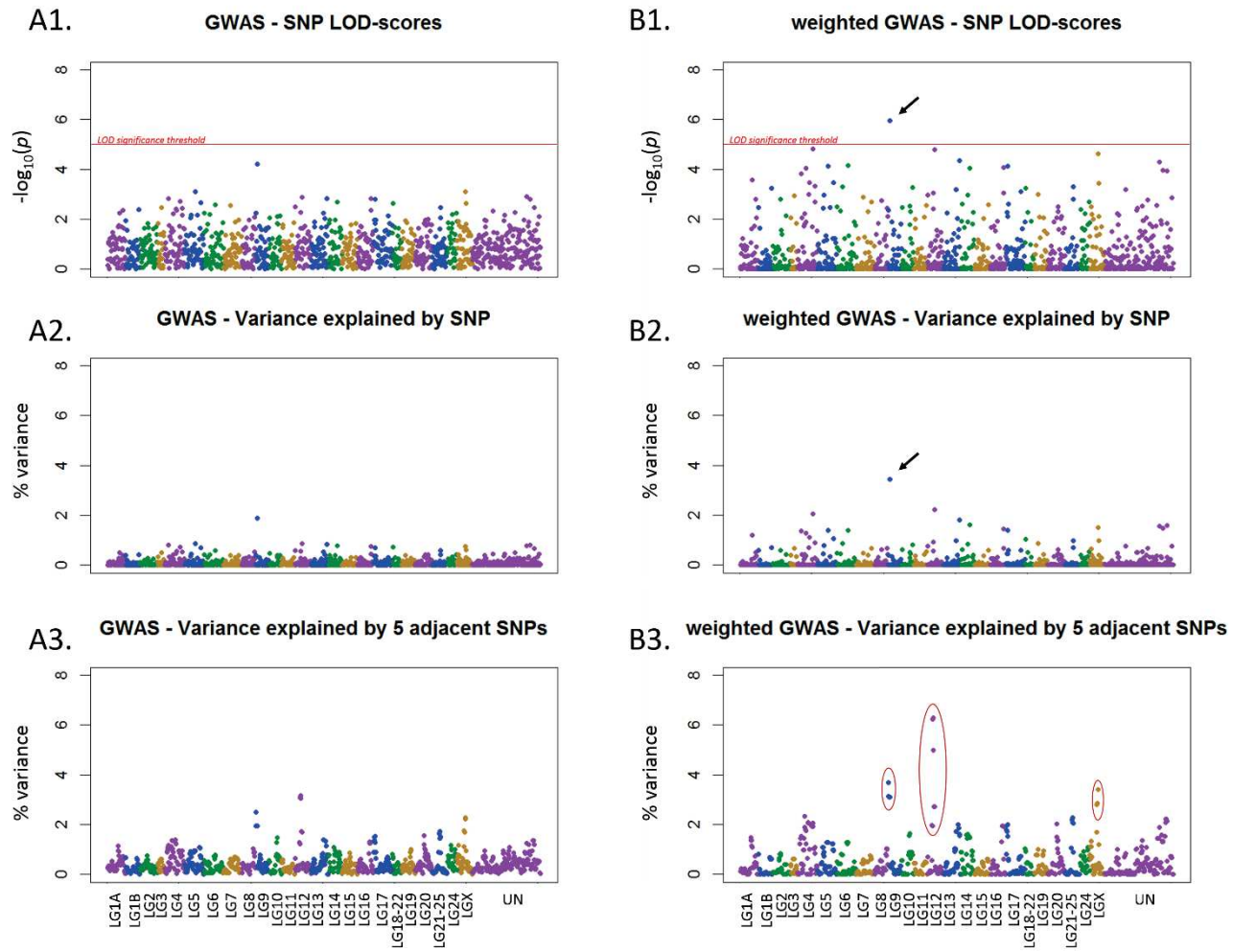

Figure 5.2: Manhattan plots for the significance and variance explained by SNP taken individually or in a sliding window of 5 SNPs for NNV resistance measured as a binary survival. Figures A1 to A3 present results of unweighted GWAS. Figures B1 to B3 present results of weighted GWAS. The horizontal red line in A1 and B1 represent the genome-wide threshold significance.

5.3.3. Genomic evaluations

5.3.3.1. Estimated variance components

The heritability estimated using the different models for both traits (binary survival and time to death) are reported in table 5.1. The heritability estimated for time to death was always lower than that of binary survival. Furthermore, as can be seen from table 5.1, heritability estimated by genomic models was lower than heritability estimated by pedigree-based models in both methods (REML and Bayesian): the genomic heritability for time to death and binary survival,

estimated by REML method, were 0.08±0.03 and 0.14±0.05, respectively while these of pedigree-based BLUP models were 0.14±0.05 and 0.23±0.08, respectively (similar to heritability estimated in VCE6.0, Chapter 3). Heritability estimated by REML was slightly lower than that estimated by Bayesian methods.

Table 5.1: Estimates variance components and heritability with standard errors for resistance against VNN (time to death and binary survival) using different models.

Model	Trait						
	time to death			binary survival			
	σ^2_e	σ^2_u	h^2±SE	σ^2_e	σ^2_u	h_o^2	h_u^2±SE
Estimated via AIRELM							
PBLUP	71.211	11.503	0.14±0.05	0.154	0.023	0.13±0.05	0.23±0.08
GBLUP	75.867	6.398	0.08±0.03	0.162	0.014	0.08±0.03	0.14±0.05
Estimated via Gibbs sampling							
PBLUP	70.42	13.72	0.16±0.05	1	0.46147		0.32±0.08
GBLUP	76.101	6.803	0.08±0.03	1	0.20773		0.17±0.05

5.3.3.2. Correlation between predicted breeding values

As can be seen from table 5.2, there were strong Pearson's correlations among estimated breeding values for resistance against VNN of different models. It means the ranking of the fish in different models were globally similar to each other in estimated breeding value prediction. To specify, these correlations ranged from 0.89 between SNP-based model and pedigree-based model to 0.99 between genomic model and pedigree-based model for binary survival. Regarding time to death, the correlation coefficient between SNP-based model and genomic model was 0.91 while that was 0.98 between genomic model and pedigree-based model.

All of the Spearman's ranking coefficients were closed to 1. This coefficient of genomic models were the highest in both binary survival and time to death traits (0.99), whereas in contrast, these of SNP-based models were the lowest for both traits (0.97 for binary survival and 0.94 for time to death).

Table 5.2: Correlations between estimated breeding values for VNN resistance phenotype (time to death above diagonal and binary survival below diagonal) estimated based on different models

Models	GBLUP	PBLUP	SNP-BLUP	Spearman' ranking
GBLUP	1	0.98	0.91	**0.99 (1)**
PBLUP	0.98	1	0.96	**0.98 (2)**
SNP-BLUP	0.98	0.89	1	**0.94 (3)**
Spearman' ranking	**0.99 (1)**	**0.97** (2)	**0.97 (2)**	

5.3.3.3. Reliability and bias of different models

The reliability of all models were medium and close to each other for both traits (table 5.3). For binary survival, the reliability of the pedigree-based model was the highest (78%), followed by the reliability of single-step genomic model with 76%. The reliability of SNP-based model was the lowest (74%). In contrast, the reliability of SNP-based model was the highest for the time to death (84%) while the reliability of single-step genomic model and pedigree-based model were 78% and 81%, respectively. In the figures 5.3.A and B we can observe that in the prediction model poorly predict the resistance of fish, the largest part of the breeding value estimation being linked to the origin of the sires.

Bias coefficients of genomic model and pedigree-based model for both traits were close to one (0.95 for binary survival and 0.96 for time to death), indicating no bias in those models and breeding values are equal in magnitude. This coefficient for the SNP-based model for binary survival was 0.26, suggesting an underestimation of the breeding value, whereas in contrast, for the same model for time to death overestimation bias for GEBVs was reported by the slope of the regression (1.23).

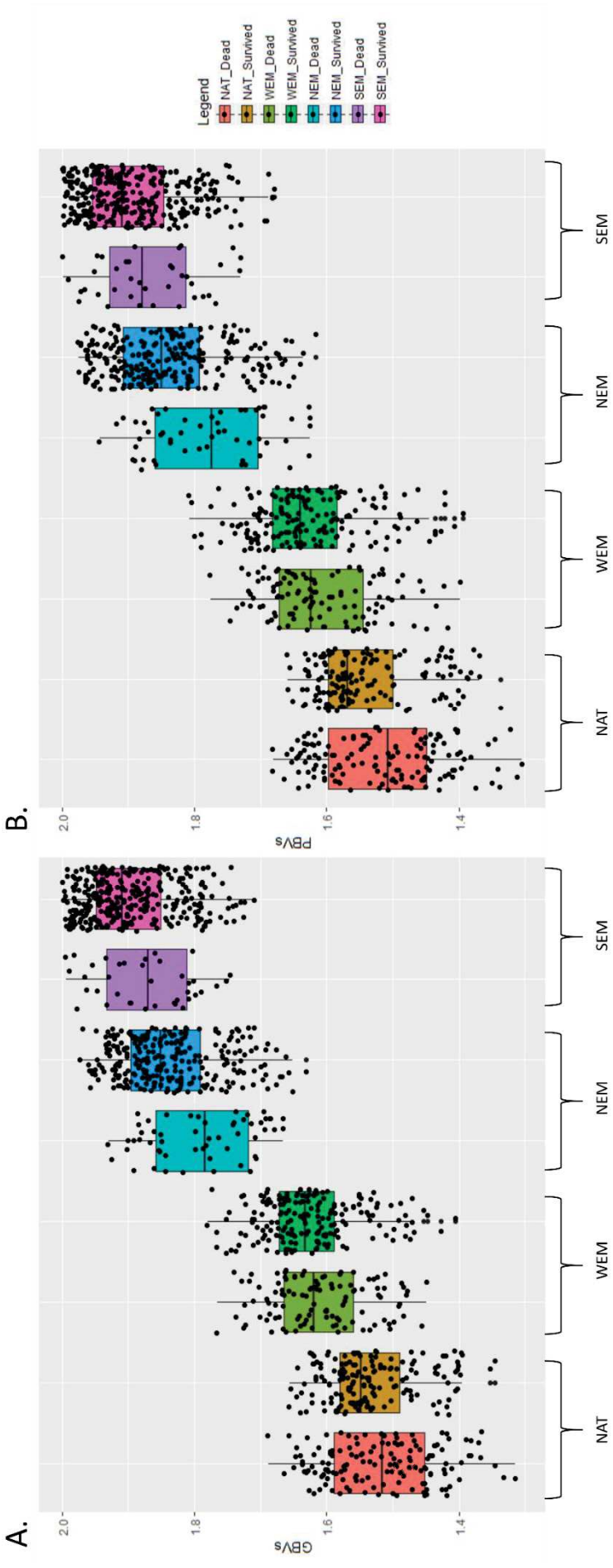

Figure 5.3: The plots of maker-based estimated breeding values from genomic (A) or pedigree (B) according to actual VNN resistance phenotypes in different population.

Table 5.3: Mean reliability and bias of estimated breeding value (EBV) and genomic EBV (GEBV) for VNN survival Continuous and Binary using pedigree based and genomic models

Models	Traits					
	Binary survival			Time to death		
	Slope (bias)	R^2	Reliability	Slope (bias)	R^2	Reliability
GBLUP	0.95	0.1313	0.76	0.96	0.1407	0.78
PBLUP	0.95	0.139	0.78	0.96	0.1514	0.81
SNP-BLUP	0.26	0.1261	0.74	1.23	0.1614	0.84

5.4. Discussion

5.4.1. Genome-wide association study

With the advance in sequencing and genotyping technologies, GWAS has gained increased feasibility, leading to an increasing number of genome-wide association study in animals, including the aquaculture sector. GWAS approaches allow studies of the genetic architecture of quantitative traits, which are complex traits not always easy to evaluate in traditional breeding programs (such as disease resistance, fillets, carcass quality) because of some major limitations. Firstly, traditional breeding is time consuming, not convenient for early stage selection and is not easy to conduct for traits of low heritability. Furthermore, in traditional breeding, the performance of the candidates in case of "sib selection" (see section 1.2 and 2.6.2.2 for detail) is the mean performance of the family, while in the case of GWAS, individual performance can be estimated even for not phenotyped animals, including breeders and their progeny. Last but not least, because accuracy of traditional selection depends on the number of fish phenotyped, it is less effective in case of traits expensive or difficult to measure (Gjedrem & Robinson 2014).

GWAS has been successfully tested especially for the improvement of disease resistance in several important aquaculture species. In terms of present realization of these approaches, GWAS showed highly significant association of several SNPs with resistance to IPNV, as well as population-level LD in salmon commercial populations (Houston et al. 2012). The implementation of such approaches is dependent on the development of SNP genotyping arrays,

which for the time being have mostly been developed in salmonids, like a 130 K array for farmed and wild Atlantic salmon in Scotland (Houston et al. 2014), 160 K SNP markers were validated based on 200 K SNPs applied to different wild and farmed populations of Atlantic salmon (Europe population, North America population and Chile population) (Yanez et al. 2014b), and a 57 K SNP chip which is now available for rainbow trout (Palti et al. 2014). A 12 K SNP array has been also developed in Atlantic cod, containing markers distributed across all 23 chromosomes (Yu et al. 2014). It was already used in a GWAS analysis for NNV resistance which revealed 29 genome-wide significant SNPs for binary survival and 36 genome-wide significant SNPs for number of days fish survived, as well as high genomic heritability of 0.49 and 0.81 for the same traits, respectively (Bangera, Baranski & Lien 2014). To date, Wang et al. used high-density SNP (44498 bi-allelic genetic variants using GBS) in order to perform GWAS for VNN resistance in Asian seabass, as the results, three genome-wide significant loci were identified on chromosomes 16, 19 and 20. (Wang et al. 2017). Regarding present work, we performed GWAS using low-density SNPs. Strikingly, based on wGWAS one significant individual SNP linked to VNN resistance according to LG9 was detected and it explained 3.11% of VNN resistant phenotypes of seabass.

Identification of significant SNPs which are responsible for NNV resistance in European seabass are desirable for breeding programs aiming to improve NNV resistance, which is of special importance to support sustainable aquaculture development in the Mediterranean. In this study, while no QTLs were reported in previous chapter, one significant SNP linked to resistance against VNN phenotypes was identified in LG9 (figure 5.2.B1) and explaining nearly 3.11% of the variance after the weighting process (figure 5.2.B2). However, this SNP raised the significance level only in the case of the weighted GWAS, where SNPs are weighted by mean of their effects on the breeding values obtained from GEBV predictions. This iterative method (default weight GBLUP) has been validated by (Wang et al. 2012; Zhang et al 2016), and was reported to retrieve better QTLs in simulated data. Other weighting methods exist but as they involve groupment of several SNPs in the calculation, we preferred not to use them regarding the low number of SNPs in our dataset. From this weighting method we also observed a valuable proportion of variance explained in several locations (in LG9, LG12 and LGx) when computing variance explained in sliding windows of 5 adjacent SNPs (figure 5.2.B3). The regions concerned in LG9, LG12 and LGx are however quite large as they comprise 1.69 to 3.29 Mbp. Implementing fine QTL mapping to better locate potential QTLs would be needed,

to verify the veracity of those potential QTLs. To permit such validation, we would recommend a more appropriate population structure. A valuable mating design would be to backcross supposedly heterozygous sibs for the resistance (i.e. from a sire that produced a highly resistant progeny and a female that gave a poor resistance) with a sensitive parent (i.e. the same female of poor resistance in the progeny). We could therefore expect to follow the segregation of the resistance allele in the progeny challenged (figure 5.4). Another option would be to improve the density of SNP with the same mating design as our study so that to elucidate differences between methods based on Identity by State like in the present paper and Identirty by Descent (that we could not perform here).

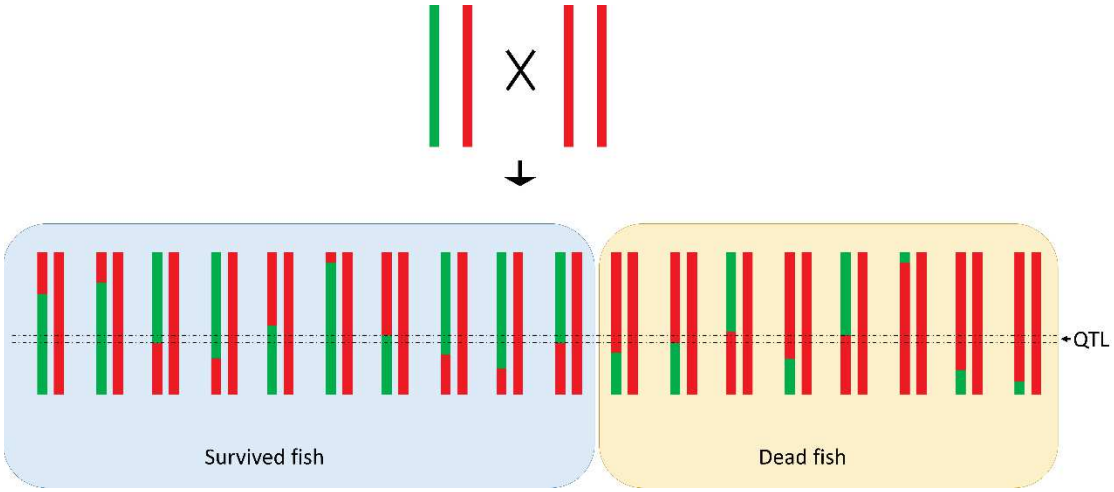

Figure 5.4: Segregation of a QTL of resistance in a progeny derived from the backcross mating of a heterozygous resistant fish and his homozygous sensitive parent. In green, the chromosome carrying the resistant allele at the QTL and in red the chromosome with the sensitive allel at the QTL.

5.4.2. Genomic evaluations

Because of their expected higher accuracy, compared to the traditional pedigree-based model, genomic models with high-density SNP markers are more and more used in order to estimate breeding values. The advantage of genomic model using high-density markers has been illustrated for disease resistant trait in some economical species in aquaculture (Ødegård et al. 2014; Bangera et al. 2017; Correa et al. 2017; Tsai et al. 2016).

In this study, the breeding values were estimated based on PBLUP, GBLUP and SNP-BLUP. The Pearson's correlations between models were high and closed to 1 for both time to death

and binary status. These results suggested that predicted EBV with different models were not different predictors of genetic merit of fish and the ranking of the fish were similar to each other in these models for VNN resistance in those populations.

The reliability of GBLUP was in our case not better than that of of pedigree-based models for both traits (time to death and binary status) (table 5.3, figure 5.3). In contrast, the reliability of SNP-BLUP was slightly higher than that of PBLUP or GBLUP. However, there was an overestimation bias in this model.

In general, the reliability of genomic model is affected by different effects. First and foremost, in this study, there was a high but highly structured genetic diversity (four different wild populations with very different resistance to VNN, see chapter 3). This led to few resistance variance in NEM and SEM sires offspring (83% and 90% of survival respectively) and to the fact that due to the high effect of Origin in the model EBV was overestimated in dead fish (figure 5.3 A & B). In other studies on fish, the higher reliability of genomic evaluations was illustrated for salmon lice (*Lepeophtheirus salmonis*) resistance of Atlantic salmon (Ødegård et al. 2014). However, in that study, there was just one population, and the number of fish per family ranged from 30 to 40 individuals while this number ranged from 1 to 20 in our study. Another reason that may lead to a lesser reliability of genomic evaluation in our case may be the low genomic heritability (equal to a half of that estimated in the pedigree-based model). This phenomenon has already been observed in published data, where genomic heritability was lower than pedigree-based heritability, but the reliability of GBLUP remained higher than that that of PBLUP (Vallejo et al. 2017).

Last but not least, in our data we have a mixture of F1 hybrid populations (NAT x WEM, NEM x WEM, SEMLxWEM) and a pure population (WEM x WEM), which were all mixed for analyses. In order to try to control this phenomenon, we tried to use only hybrid data for the genomic model. Even though the reliability of all models increased slightly, the reliability of the genomic model was still not better than that of the pedigree-based model (figure 5.5).

The inefficiency of genomic models in our case could be explained by the above-mentioned phenomena. However, the main issue here may be the confusion between IBD and IBS between genotypes in our mating scheme design. Indeed, due to repeated recombination processes since there last common ancestor, two individual may have a similar genotype for two adjacent

markers but with completely diverging underlying genes in between. In addition, QTLs of resistance may exist in some populations but not in others, or may not be the same in all populations, and thus a given SNP could be predictive of resistance in one population and not in another, leading to insignificance of individual SNPs inter-populations. Unfortunately, there were not enough fish from each population in our sample to obtain meaningful results population by population.

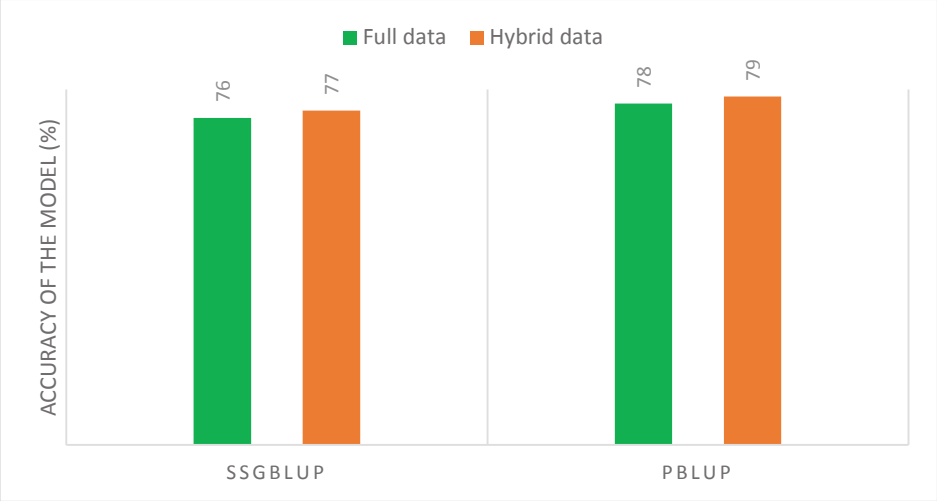

Figure 5.5: Comparison the reliability of ssGBLUP and PBLUP between full data and only hybrid data used for estimation breeding values.

A final possibility is that the number of SNPs used in the present study is too low. Several studies have shown that while estimated breeding values for disease resistance are strongly improved with high-density SNPs, compared to traditional model (PBLUP), their reliability is also frequently higher than that of PBLUP even using low density SNP genotyping (Ødegård et al. 2014; Correa et al. 2017). However this is not always the case, and with low density SNP genotyping (0.5 and 1.0 K), Bangera et al. showed that the genomic breeding values estimated by Bayesian method (Bayes C and Bayesian LASSO) were more accurate than those estimated by PBLUP based on 1K SNPs, while they were nearly the same between GBLUP and PBLUP (Bangera et al. 2017). In our study, the Bayes C_{π} and Bayesian LASSO have been also tested in GS3 software in order to compare the reliability of these models. However, the convergence during the estimation of variance components was not reached, probably due to parameterization problems and maybe to insufficient sample size.

5.5. Conclusion

In this chapter, we explored the genomic prediction ability of resistance to VNN resistance in our experimental progeny. Be using the weighted GWAS strategy, one significant SNP belonging to LG9 showed significant linkage to the phenotype, explaining 3.11% of the variance of resistance. While the variance explained remains moderate, it is likely that a QTL of resistance to VNN located in LG9 exists in European seabass. This will require further work, and we discussed the mating strategy and the genotyping effort to improve and validate potential QTLs. In addition, we tried to predict binary survival as well as time to death by genoimic methods, but highlighted that in our experiment breeding values predicted based on genomic models were not improved, compared to traditional model (PBLUP). We discuss this discrepancy as may be due to the genetic diversity, parameterization, population structure and small amount of SNPs in our experimental design. In total the present study cannot conclude on strong QTLs but gives valuable clews for further improvement of VNN resistance by mean of genomic methods with either GS or MAS.

Chapter 6:
General discussion

6.1. Summary of the main results

As presented in **Chapter 2**, VNN is the one of the most important threats to the aquaculture industry of European sea bass, and no simple and effective procedures are available to treat this disease. Therefore, selective breeding for VNN resistance improvement is considered as an important perspective for the sustainable aquaculture of European seabass. However, sufficient heritability and phenotypic variation are required for any artificial breeding programs. In the present work, we firstly illustrated in **Chapter 3** the large variation for VNN resistance among four different wild populations of European seabass, which are representative of the major genetic units identified so far in the wild. Sires from the four populations were crossed with West Mediterranean females. We showed that the highest resistance to VNN was found in crosses involving South-Eastern Mediterranean (SEM) sires (90% survival). In contrast, crosses with Northern Atlantic (NAT) sires were the worst ones (53% survival). Survival rates of the Western Mediterranean (WEM) population and of crosses involving North-Eastern Mediterranean (NEM) sires were intermediate (62% and 83%, respectively). Then, the VNN resistance in pure strains was computed based on these results under the hypothesis of a purely additive genetic determinism of VNN resistance at the population level. The resulting estimates of survival among pure populations showed even wider variation: 44% of estimated survival in pure NAT, 62% in WEM, 94% in NEM, and 99% in SEM. We also investigated the within population variation of resistance to VNN, and showed a moderate heritability for resistance to VNN (0.26±0.11 on the underlying liability scale). This heritability estimate is the first one for VNN resistance in European seabass. We also estimated correlations between VNN resistance and other production traits. A medium negative genetic correlation between VNN resistance and daily growth coefficient (-0.28±0.20) was observed, as well as a weak negative genetic correlation between VNN resistance and fillet adiposity (-13±0.19). We also observed a significant but moderate negative correlation between VNN resistance and body weight (-0.35±0.14).

Based on 1274 SNPs genotyped on 1650 fish, we developed in **Chapter 4** a medium density multi-family linkage map which comprised 1174 SNP and 1152 unique positions across all populations. The map comprised 24 Linkage groups, and had a total length of 1409.3 cM. The sex-specific maps reveal a heterochiasmy in European seabass, the female map being 1.25 fold longer than the male map, illustrating that more recombination events occur during female meiosis than in male meiosis. The map was developed in order to enable the search for VNN

disease resistance QTLs. Unfortunately, no significant QTLs responsible for VNN resistance could be identified based on this linkage map

Finally, in **Chapter 5**, using the same SNPs as for QTL mapping, a two steps Genome Wide Association Study (GWAS) was carried out, where a first unweighted GWAS allowed to calculate individual SNP weights, based on their effect in the GEBV predictions, then used in second weighted GWAS (wGWAS). From this analysis one SNP significant at a genome wide threshold explaining 3.11% of the resistance and belonging to LG9 was identified in our study. In addition, in the same set of analyses, we also evaluated the genomic heritability of VNN resistance, which we found to be lower (0.14±0.05 or 0.17±0.05 on the underlying scale with REML and Gibbs Sampling, respectively) than the pedigree-based heritability estimate (0.23±0.08 and 0.32±0.08 with the same estimation methods). We also evaluated the reliability of genomic breeding value evaluation against that of pedigree-based evaluation. The reliability was rather similar, but slightly higher of Pedigree-PBLUP than for GBLUP (0.78 vs. 0.76, respectively, for binary survival).

In this study, we provided a species-wide evaluation of the genetic variation of resistance to VNN in European sea bass, from differences between populations to genetic and genomic evaluation within populations, using the latest genomic tools. Although the initially expected QTLs for VNN resistance were not found, this work is a major step towards the development of selective breeding strategies for VNN resistance in European seabass.

6.2. Practical implications of the results for selective breeding

If no QTL was identified by mapping strategies, significant clues on a putative QTL was suggested by a significant association with one SNP in LG9. Even those results need to be validate, there are already excellent prospects to improve selective breeding of European seabass for resistance to VNN disease within or between populations by both traditional selective breeding programs as well as by genomic selection.

The first important observation is that there is a major difference in VNN resistance among seabass populations. To date, only half of the seabass cultured originate from those breeding programs (Jannsen et al., 2017) and some hatcheries still use unselected broosdstock which are replaced using fish caught from the wild populations. For such hatcheries, in case they would like to start new breeding programs for sea bass, it would certainly be a major asset to start from

Eastern Mediterranean populations, due to their very high level of resistance to VNN. This option is even more worth considering, taking into account the fact that there are only limited differences for other production traits between the eastern Mediterranean populations and the Western Mediterranean or Atlantic populations (Vandeputte et al. 2009a; Guinand et al. 2017)

The situation would be different for hatcheries which already have selective breeding programs (mostly on production traits) operating. In their case, they are likely to have achieved high genetic gains for growth, either by mass or by family-based selection, due to the high heritability of growth (Dupont-Nivet et al. 2008; Vandeputte et al. 2009). Moreover, due to the negative genetic correlation we evidenced between growth and VNN resistance, it is likely that fish strongly selected for growth would be less resistant to VNN. In already established breeding programs, the reasonable option is probably to setup a breeding objective combining growth and VNN resistance improvement in a single design where only families with high growth fish as well as high VNN resistance are chosen as the candidates for VNN resistant improvement. VNN resistance can be improved within populations, as shown by our significant heritability estimate, and the challenge would be to improve it without degrading the high level of performance for production traits already reached.

An alternative procedure for introducing VNN resistance in already established breeding programs could be the introgression of resistance from (resistant) eastern Mediterranean populations to (more sensitive) Atlantic or Western Mediterranean domesticated populations selected population with good performance for production traits. The traditional way to introgress traits is to create a hybrid population, and then to backcross it to the recipient population while selecting for the trait of interest. However, there are several limitations to this traditional procedure, and especially a low accuracy when using sib selection to identify resistant families, as sib selection does not permit to distinguish between individuals within family. A possible strategy to alter and improve the classical introgression sheme is to combine introgression and QTL identification in one step (Yazdi et al. 2010) or to combine introgression with a genomic selection program where individuals are selected based on estimated marker effects distributed over the entire genome (Meuwissen et al. 2001). Indeed, Ødegård et al. showed that the traditional selection was less efficient in preserving the target QTL through the backcrossing phase, compared to genomic selection, and this was true even without specific knowledge of the target QTL (Ødegård et al. 2009).

A positive point is that despite of the fact that the genomic heritability for VNN resistance was only half of pedigree-based heritability, the reliability of the genomic model was hardly less than that of pedigree-based BLUP. It is potentially interesting for future genomic evaluation for resistance against VNN in European seabass. In the case where a breeding design would be based on NAT or WEM population with a large number of offspring per full-sib family and high-density SNPs, it may thus be expected that genomic models be more accurate than the PBLUP model.

6.3. Limitations of the present study

Our results demonstrated that selective breeding for VNN resistance improvement is an important perspective for improving the sustainability of the culture of European seabass. Several minor limitations should be however considered to improve analyses in further studies.

First, in our design the fish were challenged at a mean body weight of 16.3 g while the highest mortality of fish (up to 100%) is usually experienced in larvae and juvenile stages because of the lack of well-developed adaptive immune system as large fish (Skliris et al. 2001; Øvergård et al. 2012). Still, the highest cost of disease is reached when large fish are dying, because a lot of resources have already been used for their rearing. Thus, challenging them as large jueniles may also be reasonable in this respect.

In addition, the fish were challenged by an injection procedure instead of an immersion or cohabitation challenge which are frequently applied for young fish challenge. Although the injection method is efficient for most of the viral and bacterial diseases and is often preferred for large fish, this is not representative of the natural infection route by NNV in fish farms (Vendramin et al. 2014; Liu, Wang, Wan, et al. 2016b). Indeed, the first contact of the fish with the pathogen is through the mucus on the skin and gills, which may represent a first natural barrier to the infection (Murray et al. 1992). Consequently, VNN resistance estimated by an immersion challenge procedure could be different, compared to an injection challenge procedure. There is very little literature on this subject, but it has been suggested that the differences in individual variation between injection and immersion challenges do exist but are minor (Wargo et al. 2012). Other unpublished results tend to suggest that fish resistant by injection are also resistant when challenged by immersion, while the reverse may not necessarily be true (Quillet, pers. comm.). Thus, from a practical point of view, while we may

not have seen the full range of resistance to VNN expressed in this experiment, selective breeding using an injection challenge can still be reasonably expected to produce fish that will be resistant to VNN by the natural route of infection. Another noticeable remark is that in this study we used, as a viral source, the W80 strain belonging to RGNNV genotype. We ignore if there is a viral strain-based resistance in the wild populations of European seabass. In particular the W80 strain has been isolated in a French seabass farm of the Mediterranean, and it is possible that different performance of resistance would have been observed using strains belonging to alternative NNVgenotypes (SJNNV, RGNNV/SJNNV or SJNNV/RGNNV).

Furthermore, we can also note that the heritability estimated in the different populations seemed rather variable (ranging from 0.19 to 0.42), suggesting that the genetic basis may be different among populations, and thus the global analysis as we performed it may not be the most relevant. However, the standard errors were large because of the relatively small number of fish per population (ranging from 326 to 407 individuals). Investigating this aspect more in details would require the use of larger population samples. Concerning our experimental design, due to optimization of the budget towards the "new" trait VNN resistance, the fish kept for the evaluation of production traits were in even smaller numbers, leading to large standard errors of the correlations between VNN resistance and DGC and FA. These correlations may then not be representative of the true correlations.

Next, the sex-determination for the dead fish was not done in the NNV challenge experiment. Therefore, we could not analyze the difference between male and female for resistance against VNN. In juvenile sea bass, females are larger than males (Saillant et al. 2001), so we cannot rule out a sex-specific mortality. In that case, not taking it into account in the model may artificially decrease the heritability estimate by increasing environmental noise. The fact that, the big fish were less resistant than the small fish (there was the moderate negative correlation between VNN resistance and BW) may be indicative of such bias.

Concerning the genomic analysis, no QTLs responsible for VNN resistance were identified based on the medium-density linkage map. A first possible cause would be an inaccurate linkage map, a situation that may be caused by various effects such as the genetic diversity of the different populations used for the construction of the linkage map, and a small number of progenies per full-sib family. However, the software we used for the construction of the map (LEP-Map) is normally well adapted to the use of multiple families (Rastas et al. 2013; Rastas

et al. 2016; Shao et al. 2015). A second level hypothesis to explain the absence of detected QTLs is more linked to the true genetic architecture of VNN resistance. We used all populations together, to look for QTLs, and this implicitly assumes that there are specific QTLs responsible for VNN resistance which are the same across all populations. However, if this is not the case, it may be likely that no QTL is detected, even if specific QTLs exist within populations. It is also possible that SNPs in a similar genotypic state but from different populations are not linked to the same inter-SNP genetic polymorphisms. In this case, working at the population level may be more relevant, but here again the relatively low number of offspring per population is not favourable in our case. A third hypothesis, especially for the East Mediterranenan populations which are very resistant, is that possible resistance QTLs would be at a homozygous stage in most families, therefore precluding the possibility to reveal their co-segregation with SNPs in the progeny.

This absence of QTL detection was not expected, first because of the large differences in VNN resistance across populations, and second because VNN QTL studies in other marine species have been successful. Indeed, several major QTLs have been identified in Atlantic cod based on 161 microsatellite markers (Baranski et al. 2010) and by high-density SNP markers (Yu et al. 2014). To date, several major QTLs related to VNN resistance were also reported in Asian seabass based on high-density SNP markers (Liu et al. 2016). These cases where major QTLs can be identified seem relatively common for viral diseases in fish (e.g. see also IPN in salmon (Houston et al. 2008; Moen et al. 2009)). However, it may also be that VNN resistance in sea bass is a complex trait with highly polygenic determinism. In such specific case, the individual significant SNP would not be identified even with higher density SNP markers. For instance, Palaiokostas et al. performed GWAS for resistance to pasteurellosis in gilthead sea bream using 12,085 SNPs. However, no significant individual SNP at genome wide threshold was identified (Palaiokostas et al. 2016). To ascertain the existence or not of QTLs, some more refined studies may be needed, which will be highlighted in the next section. Making sure that there are or not large QTLs is very important, as large QTLs have allowed the practical application of MAS in Atlantic salmon for IPNV resistance in commercial population in Norway and Scotland (Houston et al. 2008; Moen et al. 2009). This has been so successful that the incidence of IPN outbreaks has sharply decreased in Norway as “QTL eggs” were released on the market (Moen et al. 2015).

In the present study, we also performed GWAS and tested the possibility to estimate (genomic) breeding values using SNP genotypes. In our case, the accuracy of genomic models was not better than that of classical pedigree-based models. The accuracy of genomic prediction is considered as the key parameter to evaluate the potential benefits of this approach. The accuracy of genomic prediction can been improved a lot using different genomic models and it can sometimes reach 100% with a very large amount of data (including phenotypic and genotypic data) (Goddard 2009). In general, the accuracy of genomic-based predicted breeding values is expected to be substantially higher than pedigree-based breeding values. However, it is still influenced by many major variables (Ødegård et al. 2014; Wolc et al. 2016). Firstly, it depends on the number of training individuals. The accuracy is higher with a large training set (Calus et al. 2008; Pszczola et al. 2014; Castillo-Juárez et al. 2015; Wang et al. 2017; Vallejo et al. 2016). In addition, the reliability of genomic breeding values is influenced by the degree of relationship between the training and validation sets. If this relationship is close, the accuracy of genomic prediction usually increases (Sonesson & Meuwissen 2009; Tsai et al. 2016; Palaiokostas et al. 2016; Robledo et al. 2017). Tsai et al. 2016 compared the accuracy of genomic prediction for lice resistance in Atlantic salmon which the genomic prediction was performed by cross-validation analyses based on various scenarios such as: random selection, sibling, non-sibling and across two populations. They illustrated that the accuracy of genomic prediction was highest when the training and validation sets were chosen under full-siblings from each family, whereas in contrast, the predictive ability was less when no full-siblings were used in both training and validation sets (Tsai et al. 2016). Similar results were experienced in chickens where the training and testing were set under the different generations (Calus et al. 2014; Weng et al. 2014; and de los Campos et al. 2012).

Besides that, the family structure also has an effect on the accuracy of genomic prediction. Nielsen et al demonstrated that the accuracy of GEBV increased when the number of sibs in the test group used for GEBV scheme rose from 20 to 40 sibs (Nielsen et al. 2009). Furthermore, the density of markers has a major effect on the accuracy of genomic breeding values (Goddard 2009; Sonesson & Meuwissen 2009; Bangera et al. 2017; Correa et al. 2017; Tsai et al. 2016). Despite of the fact that the accuracy of GEBV can be higher than that of pedigree-based breeding values with a few thousand SNPs, the most accurate GEBVs are always obtained with high-density SNP (Ødegård et al. 2014; Correa et al. 2017; Bangera et al. 2017). However, the accuracy reaches a plateau at a density of SNPs which varies among traits and species. For

instance, this value reaches a peak with 10k SNPs when GEBV were estimated for sea louse resistance in Atlantic salmon (Correa et al. 2017). However, a plateau of GEBV was only reached at around 20K for salmon lice resistance or for *Piscirickettsia salmonis* in the same species (Ødegård et al. 2014; Bangera et al. 2017). Last but not least, the accuracy of the traditional or genomic prediction is affected by the heritability of the traits. Genomic selection is considerably more accurate than traditional selection, particularly for a low-heritability trait (Calus et al. 2008). The different of accuracy between high and low heritability based genomic prediction has been experienced in simulated data as well as empirical data (Calus et al. 2008; Sonesson & Meuwissen 2009; Villanueva et al. 2011).

Regarding above considerations, a limitation appears in our design to meet the standards required for accurate genomic prediction. Firstly, we faced a very large genetic diversity combining allele frequencies derived from various wild populations with confusion between identity-by-descent and identity-by-state of SNP genotyped. Second, the number of progenies per full-sib family evaluated was low (ranging from 1 to 20 individuals - 4 offspring per family in average). Finaly, only 1207 usable SNPs were available, far from the minimum used in successful studies (3K SNPs at least). All this leds to unefficiency of genomic models, compare to pedigree-based model.

6.4. The way forward

We previously highlighted that, due to the diversity of the populations used, SNPs with similar alleles may not be linked to the same causal polymorphisms in the different populations. In this case, it could be useful to genotype parents at a higher density (or even to re-sequence them) in order to impute the genotype of their offspring to the same level of density, using their known genotype for the 3K SNP-Chip, in order to perform GWAS of genomic prediction with a higher accuracy (Hickey et al. 2013; Wolc et al. 2014; Sargolzaei et al. 2014). In particular, this would help clarifying the possible association SNP-phenotype suggested in LG9 by the wGWAS. Most of the parents of our experiment are being re-sequenced in an ongoing project, so this possibility will shortly become a reality.

If we think of additional experimental studies that could be worth performing for identifying potential QTLs, they could be designed based on the Atlantic or Western Mediterranean populations because they show large variations of VNN resistance (sire half-sib family survival

ranging from 24 to 73% in NAT, and from 42 to 88% in WEM). This is in contrast with eastern Mediterranean populations, where there were very few VNN sensitive phenotypes, thus somehow hindering the identification of genetic/genomic variation. A very efficient alternative approach may also be, in order to increase the power of a QTL detection experiment, to use a population derived from a backcross of resistant x sensitive F1 hybrids (such as SEM x WEM) with (sensitive) WEM in order to be able to have a better segregation of VNN resistance alleles in the challenged population studied.

Even without identified, QTLs, genomic selection may be performed, based on genomic breeding values which are computed using genome-wide dense marker maps (Meuwissen et al. 2001). The genomic breeding value estimation uses a reference dataset that includes animals that have both known genotypic and phenotypic information (Calus et al. 2008). In our case, the reference population would be the sibs of the selection candidates, challenged with VNN and genotyped. The higher accuracy of genomic models with high-density SNPs, compared to the traditional model (pedigree-based BLUP), has been illustrated for resistance to different diseases in different economic species in aquaculture (Palaiokostas et al. 2016; Correa et al. 2017; Ødegård et al. 2014; Bangera et al. 2017). However, this has not been the case in European seabass for the time being. Traditional selection (based first on phenotypes, and in some cases on family relationships) has been performed since the 90's in European seabass, with several breeding programs having been performed on this species. Nevertheless, the drawback of the traditional selection is that it is the decrease of genetic diversity and increase of inbreeding in farmed stocks (Hillen et al. 2015), especially when mas selection is used, without any pedigree control. The use of molecular tools is now common in all programs, mainly for pedigree traceability, but genomic selection remains to be implemented in seabass selective breeding (Chavanne et al. 2016).

6.5. Concluding remarks

In this study, we investigate the VNN resistance variability in seabass wild populations and evaluate the possibilities for traditional and genomic selective breeding. A large genetic variation for VNN resistance of European seabass was first recorded, South-Eastern Mediterranean sires' offspring were the most resistant (90% of survival), followed by offspring from North-Eastern (83%) and Western Mediterranean sires (62%), and Northern Atlantic sire's offspring being the most sensitive (53%). This resistance has a moderate but significant

heritability and negative correlations on production traits. Then, first assumptions on the location of potential QTLs were made by identifying one SNP in LG9 explaining a reduced by significant part of variance (3.11%). This finding however requires to be validate, claiming for a fine QTL mapping. Once validate, such QTL could represent an interesting add-value in potential marker-assisted selection to VNN resistance in European seabass. In addition, from the linkage mapping we report a heterochiasmy (a sex-biased recombination ratio) in favor to female. Finaly, we pointed the limitations of our experiment that did not allow us to make a reliable interval mapping, and which are probably responsible of the weak performances of genomic predictions compare to pedigree-based breeding values.

COMBINING VITASSIGN AND COLONY: AN EFFICIENT PRACTICAL PROCEDURE FOR PARENTAL ASSIGNMENT WITH MISSING PARENTS

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The contribution of parentage assignment in selective breeding of aquaculture species is undeniable. However, breeding programs often face practical management problems and it is not uncommon that some broodstock genotypes miss because of premature death, trace ability problems or sample quality problems. This may lead to unexpectedly low parentage assignment and decrease markedly the potential of genetic improvement. In this study, we explored the potential of combining two softwares, VITASSIGN and COLONY, for obtaining parentage assignment in the case of a few missing parental genotypes in a full factorial mating design. Nine dams were crossed to 60 sires in a full factorial mating scheme and 2000 offspring were reared in a single batch. Biological samples of offspring and parents (caudal fin or sperm) were genotyped for 12 microsatellite markers at Labogena (Jouy-en-Josas, France). Due to low sample quality, 2 dams, 2 sires and 9 offspring could not be genotyped. First pedigree assignment trials were run with this partial dataset (1991 offspring, 7 dams and 58 sires). Using VITASSIGN, an exclusion-based parentage assignment software, 40.8% of offspring were assigned to single parent pair (55.8% allowing up to 2 mismatches). Using Colony, a maximum likelihood parentage software, highly probable pedigree was obtained for 52.6% of the offspring. The average posterior probabilities of 259 additional potential dams genotypes generated by Colony were collected over 7 plausible configurations, and 2 dams showing posterior probabilities higher than 0.95 were identified. The next pedigree assignment included those two inferred dam genotypes, and resulted in 78.0% perfect match in VITASSIGN (92.4% allowing up to 2 mismatches) and in 77.1% assignment in Colony. Finally, candidate sires and dams with missing loci or genotyping errors were corrected based on the genotypes inferred by Colony (12 more parent genotypes were corrected or completed, for a total of 29 corrected alleles). In the end, using VITASSIGN, 96.4% of the offspring were uniquely assigned (86.1% with perfect match and 96.4% with up to 2 mismatches allowed), and only 3.4% of the offspring could not be assigned.

Keywords: pedigree assignment, VITASSIGN, Colony, missing genotypes

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COMBINING VITASSIGN AND COLONY: AN EFFICIENT PRACTICAL PROCEDURE FOR PARENTAL ASSIGNMENT WITH MISSING PARENTS

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INTRODUCTION

The contribution of parentage assignment in selective breeding of aquaculture species is undeniable [1, 3, 7]. Parentage assignment is based on two computation methods, exclusion-based methods and likelihood-based methods [3]. Exclusion is very simple and makes no hypotheses other than Mendelian segregation of alleles, but is very sensitive to genotyping errors while likelihood methods use a different approach, with probabilities. Likelihood methods generally give higher assignment rates than exclusion with low power marker sets, but sometimes give inconsistent results. However, breeding programs often face practical management problems and it is not uncommon that some broodstock genotypes miss because of premature death, traceability problems or sample quality problems [5, 6, 7]. This may lead to unexpectedly low parentage assignment [5, 6] and decrease markedly the potential of genetic improvement. In this study, we explored the potential of combining two softwares, VITASSIGN (exclusion) and COLONY (likelihood), for obtaining parentage assignment in the case of a few missing parental genotypes in a full factorial mating design.

MATERIALS AND METHODS (figure 1)

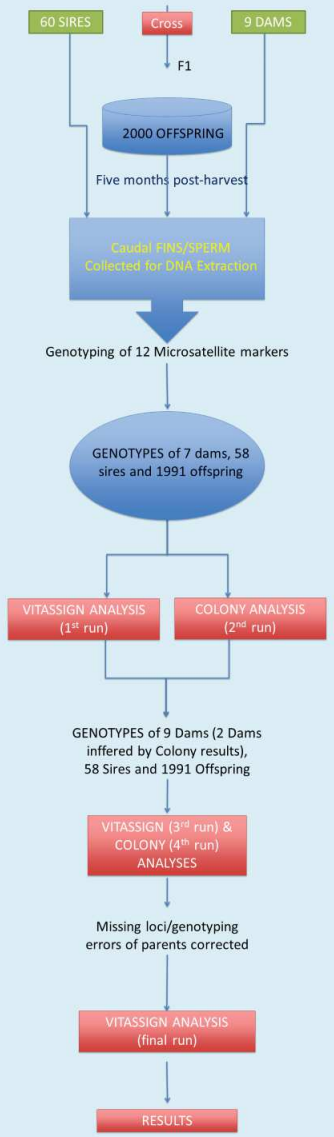

Figure 1: *The studied process*

❖ **Materials:** 60 wild sea bass sires were crossed to 9 wild sea bass dams in a full factorial mating scheme and 2000 offspring were reared in a single batch. The caudal fins or sperm of parents were collected directly at mating design processing while the caudal fins of 2000 offspring were collected at five months post-harvest. All were sent to LABOGENA (Jouy-en-Josas, France) for DNA extraction and genotyping with 12 microsatellite markers.

❖ **VITASSIGN:** running as described by Vandeputte et al. 2006 [5], allowing for up to two allelic mismatches between parents and offspring.

❖ **COLONY:** running as described by Jones and Wang, 2010 [2, 9] without data for known as well as excluded parentalship and indication of the putative number of parents (60 sires , 9 dams).

❖ **Reconstructing missing genotypes and correcting genotyping errors:** Lying on the genotypes inferred by COLONY, 2 dams showing posterior probabilities higher than 0.95 were chosen to alternative for two dams of missing genotypes. In addition, candidate sires and dams with missing loci or genotyping errors were also corrected using genotypes inferred by COLONY with posterior probabilities equal 1 (12 parent genotypes were corrected or completed, for a total of 29 corrected alleles).

RESULTS AND DISCUSSION

All samples (60 sires, 9 dams and 2000 offspring) were genotyped for 12 microsatellite loci. However, because of low sample quality, 2 dams, 2 sires and 9 offspring could not be genotyped [3, 7]. Therefore, only seven dams, 58 sires and 1991 offspring were used for first pedigree assignment trials, using VITASSIGN, an exclusion-based parentage assignment software [5]. However, because of the genotyping errors as well as missing genotypes, only 40.8% of offspring were assigned to a single parent pair with perfect match (55.8% allowing up to 2 mismatches) (figure 2).

In order to identity the missing genotypes and genotyping errors, the same data set was processed with COLONY, a maximum likelihood parentage software [2, 9]. Highly probable pedigree was obtained for only 52.6% of the offspring (figure 2). However, 259 potential dam genotypes were inferred and the probabilities of 9 dam genotypes were over 0.95 including the 7 original dam genotypes validated above with VITASSIGN and two new dam genotypes which may be two missing dam genotypes. The probabilities of the other dams was under 0.95, only a few offspring were assigned to them and always in cases of multiple possible parent pairs. Furthermore, most of dams in this group were consistent with only one offspring.

The next pedigree assignment included those two dam genotypes inferred by COLONY (later genotyping of alternative samples of the missing dams confirmed that the genotypes inferred by COLONY for those two missing fish were the exact genotypes), and resulted in 78.0% perfect match in VITASSIGN (92.4% allowing up to 2 mismatches) and in 77.1% assignment in Colony (figure 2). Nevertheless, because of missing loci or genotyping errors of some sires as well as several dams, the proportion of parental assignment with perfect match remains lower than expected by VITASSIGN simulations [5]. These candidate sires and dams were corrected based on the genotypes inferred by COLONY (1 dam and 11 sire genotypes were corrected or completed, for a total of 29 corrected alleles). Finally, using VITASSIGN, 96.4% of the offspring were uniquely assigned with assignment power >0.99 (86.1% with perfect match and 96.4% with up to 2 mismatches allowed), and only 3.4% of the offspring could not be assigned (figure 2).

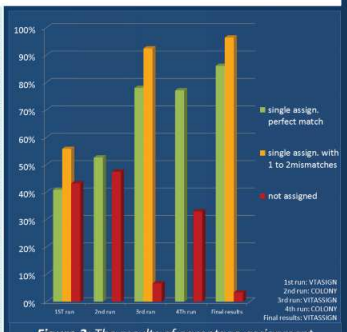

Figure 2: *The results of parentage assignment*

CONCLUSION

The proportion of parentage assignment was significantly improved when both softwares VITASSIGN and COLONY were combined to perform pedigree assignment. Strikingly, the results of assignment of VITASSIGN is extremely high if missing genotypes and genotyping errors were reconstructed and corrected based on genotypes which were inferred by COLONY. The results also illustrated that the missing genotypes as well as genotyping errors could be reconstructed or corrected by genotypes inferred by COLONY. The combining of these softwares is an efficient procedure for parentage assignment with missing parental genotypes.

REFERENCE

1. Garcia de Leon et al. (1998). *Aquaculture* **159**: 303–316
2. Jones, O. and Wang, J. (2010) *Mol. Ecol. Re.* **10**: 551-555.
3. Vandeputte and Haffray . (2014) *Front. Genet.* **5**:432
4. Vandeputte et al. (2011) *Aquaculture* **314**: 80-86.
5. Vandeputte et al. (2006) *Mol. Ecol. Res.* **6:** 265–267
6. Villanueva et al. (2002) *Anim. Genet.* **33**, 33-41.
7. Wang, J. (2004) *Genetics* **166**: 1963-1979.
8. Wang, J. (2012) *Genetics* **191**: 183-194.
9. Wang, J. (2013) *Mol. Ecol. Res.* **13**: 734-739.

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References

Adachi K., Ichinose T., Watanabe K., Kitazato K. & Kobayashi N. (2008) Potential for the replication of the betanodavirus red-spotted grouper nervous necrosis virus in human cell lines. *Archives of Virology* **153**, 15–24.

Aquaculture Europe 14, October 14th-17th, 2014, Spain.

Arimoto M., Sato J., Maruyama K., Mimura G. & Furusawa I. (1996) Effect of chemical and physical treatments on the inactivation of striped jack nervous necrosis virus (SJNNV). *Aquaculture* **143**, 15–22.

Antonello, J., Massault, C., Franch, R., Haley, C., Pellizzari, C., Bovo, G., Patarnello, T., de Koning, D-J. & Bargelloni, L (2009) Estimates of heritability and genetic correlation for body length and resistance to fish pasteurellosis in the gilthead sea bream (Sparus aurata L.). *Aquaculture*, 298(1–2), pp.29–35.

Ao, J., Li, J., You, X., Mu, Y., Ding, Y., Mao, K., Bian, C., Mu, P., Shi, Q. & Chen, X. (2015) Construction of the high-density genetic linkage map and chromosome map of large yellow croaker (Larimichthys crocea). *International Journal of Molecular Sciences*, **16**, 26237–26248.

Argue, B.J., Arce, S.M. & Lotz, 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.

Arimoto, M., Sato, J., Maruyama, K., Mimura, G. & Furusawa, I (1996) Effect of chemical and physical treatments on the inactivation of striped jack nervous necrosis virus (SJNNV). *Aquaculture*, **143**, 15–22.

Arimoto M., Mori K., Nakai T., Muroga K. & Furusawa, I. (1993) Pathogenicity of the causative agent of viral nervous necrosis disease in striped jack (*Pseudocaranx dentex* (Bloch & Schneider)). *Journal of Fish Diseases* **16***,* 461–469.

Aspehaug V., Devold M. & Nylund A. (1999) The phylogenetic relationtionship of nervous necrosis virus from halibut (*Hippoglossus hippoglossus*). *Fish Pathology* **19**, 196-202.

Athanassopoulou F., Billinis C. & Prapas T. (2004) Important disease conditions of newly cultured species in intensive freshwater farms in Greece: first incidence of nodavirus infection in *Acipenser sp*. *Diseases of Aquatic Organisms* **60**, 247–52.

Ball L.A. & Johnson K.L. (1999) Reverse genetics of nodavirus. *Advanced in Virus Research* **53**, 229-244.

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., Correa, K., Lhorente, J.P., Figueroa, R. & Yáñez, J.M. (2017) Genomic predictions can accelerate selection for resistance against Piscirickettsia salmonis in Atlantic salmon (Salmo salar). *BMC Genomics*, **18**, 121.

Bangera R., Baranski M. & Lien S. (2014) A genome-wide association study for resistance to viral nervous necrosis in Atlantic cod using a 12K single nucleotide polymorphism array. *Proceedings, 10th World Congress of Genetics Applied to Livestock Production,* August 17th-22th, 2014, Vancouver, BC, Canada.

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.

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.

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.). *Proceedings, 9th World Congress of Genetics Applied to Livestock Production,* August 1th-6th, 2010, Leipzig, Germany.

Barroso R.M., Wheeler P.A., LaPatra S.E., Drew R.E. & Thorgaard G.H. (2008) QTL for IHNV resistance and growth identified in a rainbow (*Oncorhynchus mykiss*) × Yellowstone cutthroat (*Oncorhynchus clarki bouvieri*) trout cross. *Aquaculture* **277**, 156–163.

Bates, D.M. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, **67**, 1–48.

Baud M., Cabon J., Salomoni A., Toffan A., Panzarin V. & Bigarré L. (2015) First generic one step real-time Taqman RT-PCR targeting the RNA1 of betanodaviruses. *Journal of Virological Methods* **211**, 1–7.

Bigarré L., Baud M., Cabon J., Crenn K. & Castric J. (2010) New PCR probes for detection and genotyping of piscine betanodaviruses. *Journal of Fish Diseases* **33**, 907–912.

Bigarré L., Cabon J., Baud M., Heimann M., Body A, Lieffrig F. & Castric J. (2009) Outbreak of betanodavirus infection in tilapia (*Oreochromis niloticus* L.), in fresh water. *Journal of Fish Diseases* **32**, 667–73.

Binesh C.P. (2013) Mortality due to viral nervous necrosis in zebrafish (*Danio rerio*) and goldfish (*Carassius auratus*). *Diseases of Aquatic Organisms* **104**, 257–260.

Bishop S.C. & Woolliams J.A. (2014). Genomics and disease resistance studies in livestock. *Livestock Science* **166**, 190–198.

Bloch B., Gravningen K. & Larsen J.L. (1991) Encephalomyelitis among turbot associated with a picornavirus-like agent. *Diseases of Aquatic Organisms* **10**, 65-70.

Bonami J.R. & Widada S.J. (2011) Viral diseases of the giant fresh water prawn *Macrobrachium rosenbergii: a review*. *Journal of invertebrate pathology* **106**, 131–42.

Bovo G., Gustinelli A., Quaglio F., Gobbo F., Panzarin V., Fusaro A. & Fioravanti M.L. (2011) Viral encephalopathy and retinopathy outbreak in freshwater fish farmed in Italy. *Diseases of*

Aquatic Organisms **96**, 45–54.

Boonyaratpalin, S., Supamattaya, K., Kasornchandra, J. & Hoffmann, R.W. (1996) Picorna-like virus associated with mortality and a spongious encephalopathy in grouper Epinephelus malabaricus., **26**, 75–80.

Bovo G., Nishizawa T., Maltese C., Borghesan F., Mutinelli F., Montesi F. & De Mas S. (1999) Viral encephalopathy and retinopathy of farmed marine fish species in Italy. *Virus Research*, *63*(1-2), 143–146.

Breuil, G., Pepin, J. F. P., Boscher, S. & Thiery, R. (2002). Experimental vertical transmission of nodavirus from broodfish to eggs and larvae of the sea bass (*Dicentrarchus labrax* L.). *Journal of Fish Diseases* **25**, 697–702.

Breuil G. & Romestand B. (1999) A rapid ELISA method for detecting specific antibody level against nodavirus in the serum of the sea bass, (*Dicentrarchus labrax* L.): application to the screening of spawners in a sea bass hatchery. *Journal of Fish Diseases* **22**, 45-52.

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*) larvae and juveniles. *Aquaculture* **97***,* 109-l 16.

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.

Calus, M.P.L. & Veerkamp, R. F. (2008) Accuracy of genomic selection using different methods to define haplotypes. *Genetics*, **178**, 553–561.

Calus, M.P.L., H. Huang, Y.C.J. Wientjes, J. ten Napel, J.W.M. Bas- tiaansen, M. Price, R.F. Veerkamp, A. Vereijken, and J.J. Windig (2014) (A)cross-breed genomic prediction. In: Proc 10th World Congress of Genetics Applied to Livestock Production (WC- GALP). http://bit.ly/1HZD6lj.

Carballo C., Garcia-Rosado E., Borrego J.J. & Carmen Alonso M. (2016) SJNNV down-regulates RGNNV replication in European sea bass by the induction of the type i interferon system Viruses infecting fish Alexandra Adams; Carlos P Dopazo. *Veterinary Research* **47**:6.

Castaño-Sánchez, C., Fuji, K., Ozaki, A., Hasegawa, O., Sakamoto, T., Morishima, K., Nakayama, I., Fujiwara, A., Masaoka, T., Okamoto, H., Hayashida, K., Tagami, M., Kawai, J., Hayashizaki, Y. & Okamoto, N. (2010) A second generation genetic linkage map of Japanese flounder (Paralichthys olivaceus). *BMC genomics*, 11, 554.

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

Castillo-Juárez, H., Campos-Montes, G.R., Caballero-Zamora, A. & Montaldo, H.H. (2015) Genetic improvement of Pacific white shrimp [Penaeus (Litopenaeus) vannamei]: perspectives for genomic selection. *Frontiers in genetics*, 6, 93.

Castric, J., Thiéry, R., Jeffroy, J., Kinkelin, P.D. & Raymond, J.C. (2001) Sea bream Sparus aurata, an asymptomatic contagious fish host for nodavirus. *Dis Aqual Org,* **47**, 33–38.

Chatziplis, D., Batargias, C., Tsigenopoulos, C.S., Magoulas, A., Kollias, S., Kotoulas, G., Volckaert, F.A.M. & Haley, C.S. (2007) Mapping quantitative trait loci in European sea bass (Dicentrarchus labrax): The BASSMAP pilot study. *Aquaculture*, **272**, 172–182.

Chen Y.M., Shih C.H., Liu H.C., Wu C.L., Lin C.C., Wang H.C. & Lin J.H.Y. (2011) An oral nervous necrosis virus vaccine using *Vibrio anguillarum* as an expression host provides early protection. *Aquaculture* **321**, 26–33.

Chi S.C., Shieh J.R. & Lin S.J. (2003) Genetic and antigenic analysis of betanodaviruses isolated from aquatic organisms in Taiwan. *Diseases of Aquatic Organisms* **55**, 221–228.

Chi S.C., Lo C.F., Kou G.H., Chang P.S., Peng S.E. & Chen S.N. (1997) Mass mortalities associated with viral nervous necrosis (VNN) disease in two species of hatchery-reared grouper (*Epinephelus fuscogutatus* and *Epinephelus akaara* (Temminck & Schlegel)). *Journal of Fish Diseases* **20**, 185–193.

Chi, S.C., Hu, W.W. & Lo, B.J. (1999) Establishment and characterization of a continuous cell line (GF-1) derived from grouper , Epinephelus coioides (Hamil- ton): a cell line susceptible to grouper nervous necrosis virus (GNNV). *Journal of Fish Diseases*, **22**, 173–182.

Chi, S.C., Wu, Y.C. & Cheng, T.M. (2005) Persistent infection of betanodavirus in a novel cell line derived from the brain tissue of barramundi Lates calcarifer. *Diseases of aquatic organisms*, **65**, 91–8.

Chistiakov, D.A., Tsigenopoulos, C.S., Lagnel, J., Hellemans, B., Haley, C.S., Volckaert, F.A.M. & Kotoulas, G. (2008) A combined AFLP and microsatellite linkage map and pilot comparative genomic analysis of European sea bass Dicentrarchus. *Animal Genentic*, **39**, 623–634.

Chistiakov, D.A., Hellemans, B., Haley, C.S., Law, A.S., Tsigenopoulos, C.S., Kotoulas, G., Bertotto, D., Libertini, A. & Volckaert, F.A.M. (2005) A Microsatellite Linkage Map of the European Sea Bass. *Genetic*, **170**, 1821-1826.

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.

Choi B., Gye H.J., Oh M.J. & Nishizawa T. (2014) Cell culture medium inhibits antigen binding used in an ELISA for detection of antibodies against nervous necrosis virus. *Journal of Aquatic Animal Health* **26**,168–172.

Ciulli S., Galletti E., Grodzki M., Alessi A., Battilani M. & Prosperi S. (2007) Isolation and genetic characterization of Betanodavirus from wild marine fish from the Adriatic sea. *Veterinary Research Communications* **31**, 221–224.

Cochrane K., De Young C., Soto D. & Bahri T. (2009) Climate change implications for fisheries and aquaculture: overview of current scientific knowledge. *FAO Fisheries and Aquaculture Technical Paper. No. 530. Food and Agriculture Organization of the United Nations, Rome.*

Correa, K., Bangera, R., Figueroa, R., Lhorente, J.P. & Yáñez, J.M. (2017) The use of genomic information increases the accuracy of breeding value predictions for sea louse (*Caligus rogercresseyi*) resistance in Atlantic salmon (*Salmo salar*). *Genetics Selection Evolution*, **49**, 15.

Costa J.Z. & Thompson K.D. (2016) Understanding the interaction between Betanodavirus and its host for the development of prophylactic measures for viral encephalopathy and retinopathy. *Fish & shellfish immunology* **53**, 35–49.

Crane M. & Hyatt A. (2011) Viruses of fish: an overview of significant pathogens. *Viruses*, 3(11), 2025–46.

Curtis P.A., Drawbridge M., Iwamoto T., Nakai T., Hedrick R.P. & Gendron A.P. (2001) Nodavirus infection of juvenile white sea-bass (*Atractoscion nobilis*) cultured in southern California: First record of viral nervous necrosis (VNN) in North America. *Journal of Fish Diseases* **24,** 263-271.

Cutrín J.M., Dopazo C.P., Thiéry R., Leao P., Olveira J.G., Barja J.L. & Bandín I. (2007) Emergence of pathogenic betanodaviruses belonging to the SJNNV genogroup in farmed fish species from the Iberian Peninsula. *Journal of Fish Diseases* **30**, 225–32.

Daetwyler, H.D., Villanueva, B., Bijma, P. & Woolliams, J.A. (2007) Inbreeding in genome-wide selection. *J. Anim. Breed. Genet*. **124**, 369-376.

Dalla Valle L., Toffolo V., Lamprecht M., Maltese C., Bovo G., Belvedere P. & Colombo L. (2005) Development of a sensitive and quantitative diagnostic assay for fish nervous necrosis virus based on two-target real-time PCR. *Veterinary Microbiology,* **110,** 167–179.

Dalla Valle L., Negrisolo E., Patarnello P., Zanella L., Maltese C., Bovo G. & Agripolis P.T. (2001) Sequence comparison and phylogenetic analysis of fish nodaviruses based on the coat protein gene. *Archives of Virology* **146**, 1125–1137.

Dalla Valle L., Zanella L., Patarnello P., Paolucci L., Belvedere P. & Colombo L. (2000) Development of a sensitive diagnostic assay for fish nervous necrosis virus based on RT-PCR plus nested PCR. *Journal of Fish Diseases* **23**, 321–327.

de los Campos, G., A.I. Vazquez, A. Kranis, K. Watson, S. Aven- daño, and M.P.L. Calus. 2012. Factors affecting prediction accu- racy of genome-enabled prediction: Lessons learnt from empirical studies and implications for breeding programs. In: 2012 Poultry Breeders' Roundtable, St. Louis, MO.

Dempster, E.R. & Lerner, I.M. (1950) Heritability o f threshold characters*i. *Bliss*, (March), 212–236.

Doan, Q.K. et al., 2017. Viral encephalopathy and retinopathy in aquaculture: a review. *Journal of Fish Diseases*, 40(5), 717–742.

Dorson M., Quillet E., Hollebecq M.G., Torhy C., Chevassus B. & Selection B.C. (1995) Selection of rainbow trout resistant to viral haemorrhagic septicaemia virus and transmission

of resistance by gynogenesis to cite this version: haemorrhagic septicaemia virus and transmission. *Veterinary Research* **26**, 361-368.

Dupont-Nivet, M., Vandeputte, M., Vergnet, A., Merdy, O., Haffray, P., Chavanne, H. & 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.

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.

Fauvel, C., Savoye, O., Dreanno, C., Cosson, J. & Suquet, M. (1999) Characteristics of sperm of captive seabass in relation to its fertilization potential. *Journal of Fish Biology*, **54**, 356-369.

Food and Agriculture Organization of the United Nations (2016).

Fenner B.J., Thiagarajan R., Chua H.K. & Kwang J. (2006a) Betanodavirus B2 is an RNA interference antagonist that facilitates intracellular viral RNA accumulation. *Journal of Virology* **80**, 85–94.

Fenner B.J., Du Q., Goh W., Thiagarajan R., Chua H.K. & Kwang J. (2006b) Detection of betanodavirus in juvenile barramundi, *Lates calcarifer* (Bloch), by antigen capture ELISA. *Journal of Fish Diseases **29***, 423–432.

Fjalestad, K., Gjedrem, T. & Gjerde (1993) Genetic improvement of disease resistance in fish: an overview. *Aquaculture*, **111**, 65-74.

Frerichs, G.N., Tweedie, A., Starkey, W.G. & Richards, R.H. (2000) Temperature , pH and electrolyte sensitivity , and heat , UV and disinfectant inactivation of sea bass ž Dicentrarchus labrax / neuropathy nodavirus. *Aquaculture*, **185**, 13–24.

Fukuda Y., Nguyen H.D., Furuhashi M. & Nakai T. (1996). Mass Mortality of Cultured Sevenband Grouper (*Epinephelus septemfasciatus*) associated with Viral Nervous Necrosis. *Fish Pathology* **31**, 165–170.

Furusawa R., Okinaka Y. & Nakai T. (2006) Betanodavirus infection in the freshwater model fish medaka (*Oryzias latipes*). *The Journal of General Virology* **87**, 2333–2339.

Gagné N., Johnson S.C., Cook-Versloot M., MacKinnon A.M. & Olivier G. (2004) Molecular detection and characterization of nodavirus in several marine fish species from the northeastern Atlantic. *Diseases of Aquatic Organisms* **62**, 181–189.

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.

Giacopello C., Foti M., Bottari T., Fisichella V. & Barbera G. (2013) Detection of viral encephalopathy and retinopathy virus (VERV) in wild marine fish species of the South Tyrrhenian Sea (Central Mediterranean). *Journal of Fish Diseases* **36**, 819–821.

Gitterle, T., Salte, R., Gjerde, B., Cock, J., Johansen, H., Salazar, M., Lozano, C. & Rye, M. (2005) Genetic (co)variation in resistance to White Spot Syndrome Virus (WSSV) and harvest weight in Penaeus (Litopenaeus) vannamei. *Aquaculture*, 246(1–4), pp.139–149.

Gjedrem T. (2015) Disease resistant fish and shellfish are within reach: A Review. *Journal of Marine Science and Engineering* **3**, 146–153.

Gjedrem T. & Thodesen J. (2005) Selection and breeding programs in aquaculture. *Dordrecht, The Netherlands: Springer* 89–111.

Gjerde B., Evensen O., Bentsen H.B. & Storset A. (2009) Genetic (co)variation of vaccine injuries and innate resistance to furunculosis (*Aeromonas salmonicida*) and infectious salmon anaemia (ISA) in Atlantic salmon (*Salmo salar*). *Aquaculture* **287**, 52–58.

Gjøen H.M., Refstie T., Ulla O. & Gjerde B. (1997) Genetic correlations between survival of Atlantic salmon in challenge and field tests. *Aquaculture* **158**, 277–288.

Glazebrook J.S., Heasman M.P. & Beer S.W. (1990) Picorna-like viral particles associated with mass mortalities in larval barramundi (*Lates calcarifer* Bloch). *Journal of Fish Diseases* **13**, 245–249.

Goddard, M., 2009. Genomic selection: prediction of accuracy and maximisation of long term response. *Genetica*, 136(2), pp.245–57.

Goddard M.E. & Hayes B.J. (2007) Genomic selection. *Journal of Animal Breeding and Genetics* **124**, 323–330.

Gomez D.K., Lim D.J., Baeck G.W., Youn H.J., Shin N.S., Youn H.Y. & Park S.C. (2006) Detection of betanodaviruses in apparently healthy aquarium fishes and invertebrates. *Journal of Vetarinary Science* **7***,* 369–374.

Gomez D.K., Baeck G. W., Kim J.H., Choresca C.H. & Park S.C (2008) Molecular detection of betanodaviruses from apparently healthy wild marine invertebrates. *Journal of Invertebrate Pathology*, 97(3), 197–202.

Gomez D.K., Mori K.I., Okinaka Y., Nakai T. & Park S.C (2010) Trash fish can be a source of betanodaviruses for cultured marine fish. *Aquaculture*, 302(3-4), 158–163.

Gomez D.K., Sato J., Mushiake K., Isshiki T., Okinaka Y. & Nakai T. (2004) PCR-based detection of betanodaviruses from cultured and wild marine fish with no clinical signs. *Journal of Fish Diseases* **27**, 603–608.

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.

Gonen S., Baranski M., Thorland I., Norris A., Grove H., Arnesen P., Bakke H., Lien S. & Bishop S.C. (2015) Mapping and validation of a major QTL affecting resistance to pancreas disease (*salmonid alphavirus*) in Atlantic salmon (*Salmo salar*). *Heredity* **115**, 405–414.

Groeneveld, E., Milena Kovac & Norbert Mielenz (2008) VCE User's Guide and Reference Manual Version 6.0.

Grotmoll S. & Totlandl G.K. (2000) Surface disinfection of Atlantic halibut Hippoglossus hippoglossus eggs with ozonated sea-water inactivates nodavirus and increases survival of the larvae. *Diseases of aquatic organisms* **39**, 89–96.

Grotmoll S., Berghl O. & Totland G.K. (1999) Transmission of viral encephalopathy and retinopathy (VER) to yolk-sac larvae of the Atlantic halibut - *Hippoglossus hippoglossus*: occurrence of nodavirus in various organs and a possible route of infection. *Diseases of Aquatic Organisms* **36**, 95-106.

Grotmol S., Totland G.K., Thorud K. & Hjeltnes B.K. (1997) Vacuolating encephalopathy and retinopathy associated with a nodavirus-like agent: a probable cause of mass mortality of cultured larval and juvenile Atlantic halibut - *Hippoglossus hippoglossus*. *Diseases of Aquatic Organisms* **29**, 85-97.

Grove S., Johansen R., Reitan L.J., Press C.M. & Dannevig B.H. (2006) Quantitative investigation of antigen and immune response in nervous and lymphoid tissues of Atlantic halibut (*Hippoglossus hippoglossus*) challenged with nodavirus. *Fish & Shellfish Immunology* **21**, 525–539.

Gudmundsdóttir, B.K. & Björnsdóttir, B. (2007) Vaccination against atypical furunculosis and winter ulcer disease of fish. *Aquaculture*, **25**, 5512-5523.

Guinand B., **Vandeputte M.**, Dupont-Nivet M., Vergnet A., Haffray P., Chavanne H., Chatain B., 2017. Metapopulation patterns of additive and non-additive genetic variance in the sea bass (*Dicentrarchus labrax*). *Ecology and Evolution* **7**, 2777-2790

Guo Y.X., Wei T., Dallmann K. & Kwang J. (2003) Induction of caspase-dependent apoptosis by betanodaviruses GGNNV and demonstration of protein α as an apoptosis inducer. *Virology* **308**, 74–82.

Guy D.R., Bishop S.C., Woolliams J.A. & Brotherstone S. (2009) Genetic parameters for resistance to Infectious Pancreatic Necrosis in pedigreed Atlantic salmon (*Salmo salar*) post-smolts using a Reduced Animal Model. *Aquaculture* **290**, 229–235.

Guy D.R., Bishop S.C., Brotherstone S., Hamilton A, Roberts R.J., McAndrew B.J. & Woolliams J.A. (2006) Analysis of the incidence of infectious pancreatic necrosis mortality in pedigreed Atlantic salmon, *Salmo salar* L., populations. *Journal of Fish Diseases* **29**, 637–647.

Habier, D., Fernando, R.L. & Dekkers, J.C.M. (2009) Genomic selection using low-density marker panels. *Genetics*, 182(1), pp.343–53.

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(3), 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 GENIMPACT*, (3), pp.40–46.

Hegde A., Teh H.C., Lam T.J. & Sin Y. M. (2003) Nodavirus infection in freshwater ornamental fish, guppy, *Poicelia reticulate* - comparative characterization and pathogenicity studies. *Archives of Virology* **148**, 575–586.

Hegde A., Chen C.L., Qin Q.W., Lam T.J. & Sin Y.M. (2002) Characterization, pathogenicity and neutralization studies of a nervous necrosis virus isolated from grouper, *Epinephelus tauvina*, in Singapore. *Aquaculture* **213,** 55–72.

Henryon M., Berg P., Olesen N.J., Kjær T.E., Slierendrecht W.J., Jokumsen A. & Lund I. (2005) Selective breeding provides an approach to increase resistance of rainbow trout (*Onchorhynchus mykiss*) to the diseases, enteric redmouth disease, rainbow trout fry syndrome, and viral haemorrhagic septicaemia. *Aquaculture* **250**, 621–636.

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.

Heppell, J. & Davis, H.L. (2000) Application of DNA vaccine technology to aquaculture. *Aquaculture*, **43**, 29-43.

Hick P. & Whittington R.J. (2010) Optimisation and validation of a real-time reverse transcriptase-polymerase chain reaction assay for detection of betanodavirus. *Journal of Virological Methods* **163**, 368–377.

Hickey, J.M., Cleveland, M.A., Maltecca, C., Gorjanc, G., Gredler, B. & Kranis A. 2013 Genotype Imputation to Increase Sample Size in Pedigreed Populations. *In: Gondro C., van der Werf J., Hayes B. (eds) Genome-Wide Association Studies and Genomic Prediction*. M*ethods in Molecular Biology (Methods and Protocols)*, vol 1019. Humana Press, Totowa, NJ.

Hillen, J., Carr, A., Hellemans, B., Ogden, R., Taggart, J.B., Vandeputte, M., Vergnet, A., Volckaert, F.A.M. & Coscia, I. (2015) Estimates of genetic variability and inbreeding in selected populations of European sea bass. *Aquaculture*, 479(March), 742–749.

Hodneland K., García R., Balbuena J.A, Zarza C. & Fouz B. (2011) Real-time RT-PCR detection of betanodavirus in naturally and experimentally infected fish from Spain. *Journal of Fish Diseases* **34**, 189–202.

Houston R.D., Taggart J.B., Cézard T., Bekaert M., Lowe N.R., Downing A. & Hamilton A. (2014) Development and validation of a high density SNP genotyping array for Atlantic salmon (*Salmo salar*). *BMC Genomics* **15**:90.

Houston R.D., Davey J.W., Bishop S.C., Lowe N.R., Mota-Velasco J.C., Hamilton A. & Taggart J.B. (2012) Characterization of QTL-linked and genome-wide restriction site-associated DNA (RAD) markers in farmed Atlantic salmon. *BMC Genomics* **13**:244.

Houston R.D., Haley C.S., Hamilton A., Guy D.R., Mota-Velasco J.C., Gheyas A.A. & Bishop S.C. (2010) The susceptibility of Atlantic salmon fry to freshwater infectious pancreatic necrosis is largely explained by a major QTL. *Heredity* **105**, 318–327.

Houston R.D., Haley C.S., Hamilton A., Guy D.R., Tinch A.E., Taggart J.B. & Bishop S.C.

(2008) Major quantitative trait loci affect resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*). *Genetics* **178**, 1109–1115.

Huang B., Tan C., Chang S.F., Munday B., Mathew J.A., Ngoh G.H. & Kwang J. (2001) Detection of nordavirus in barramundi, *Latest calcarifer* (Block), using combinant coat protein-based ELISA and RT-PCR. *Journal of Fish Diseases* **24**, 135-141

Huang Y.-C., Lin T.-S., Peng T., Chan N.-L. & Han Y.-S. (2016) Strong inhibition of betanodavirus replication by ribavirin targeting RNA-dependent RNA polymerase. *Journal of Fish Diseases* **39**, 619–623.

Húsgarð S., Grotmol S., Hjeltnes B.K., Rødseth O.M. & Biering E. (2001) Immune response to a recombinant capsid protein of striped jack nervous necrosis virus (SJNNV) in turbot *Scophthalmus maximus* and Atlantic halibut *Hippoglossus hippoglossus*, and evaluation of a vaccine against SJNNV. *Diseases of Aquatic Organisms,* **45**, 33–44.

Hwang J., Suh S.S., Park M., Oh M.J., Kim J.O., Lee S. & Lee T.K. (2016) Detection of coat protein gene of nervous necrosis virus using loop-mediated isothermal amplification. *Asian Pacific Journal of Tropical Medicine* **9**, 235–240.

Ibañez-Escriche, N. & Gonzalez-Recio, O. (2011) Review.Promises, pitfalls and challenges of genomic selection in breeding programs. *Spanish Journal of Agricultural Research*, **9**, 404-413.

Iwamoto T., Nakai T., Mori K., Arimoto M. & Furusawa I. (2000) Cloning of the fish cell line SSN-1 for piscine nodaviruses. *Diseases of Aquatic Organisms* **43**, 81–89.

Iwamoto T., Okinaka Y., Mise K., Mori K.I., Arimoto M., Okuno T. & Nakai T. (2004) Identification of Host-Specificity Determinants in Betanodaviruses by Using Reassortants between Striped Jack Nervous Necrosis Virus and Sevenband Grouper Nervous Necrosis Virus. *Journal of Virology* **78**, 1256–1262.

Janssen, K., Chavanne, H., Berentsen, P. & Komen, H. (2017) Impact of selective breeding on European aquaculture. *Aquaculture*, **472**, 8-16.

Jaramillo D., Durr S., Hick P. & Whittington R. (2016a) Bayesian estimation of diagnostic sensitivity and specificity of a nervous necrosis virus antibody ELISA. *Preventive Veterinary Medicine* **123**, 138–142.

Jaramillo D., Hick P., Deece K., Tweedie A., Kirkland P., Arzey E. & Whittington R.J. (2016b) Comparison of ELISA formats for detection of antibodies specific for nervous necrosis virus (Betanodavirus) in the serum of immunized barramundi Lates calcarifer and Australian bass Macquaria novemaculeata. *Aquaculture* **451**, 33–38.

Johansen R., Sommerset I., Tørud B., Korsnes K., Hjortaas M. J. & Nilsen F. (2004) Characterization of nodavirus and viral encephalopathy and retinopathy in farmed turbot, *Scophthalmus maximus* (L.). *Journal of Fish Diseases* **27**, 591–601.

Johansen, R., Sommerset, I., Tørud, B., Korsnes, K., Hjortaas, M.J. & Nilsen, F. (2004) Characterization of nodavirus and viral encephalopathy and retinopathy in farmed turbot , Scophthalmus maximus (L .). *Journal of Fish Diseases*, **27**, 591–601.

Johnson S.C., Sperker S.A. & Leggiadro C.T. (2002) Identification and characterization of a piscine neuropathy and nodavirus from juvenile Atlantic cod from the Atlantic Coast of North America. *Journal of Aquatic Animal Health* **14**, 124–133.

Kai Y.H., Su H.M., Tai K.T. & Chi S.C. (2010) Vaccination of grouper broodfish (*Epinephelus tukula*) reduces the risk of vertical transmission by nervous necrosis virus. *Vaccine* **28**, 996–1001.

Kai Y.H. & Chi S.C. (2008) Efficacies of inactivated vaccines against betanodavirus in grouper larvae (*Epinephelus coioides*) by bath immunization. *Vaccine* **26**, 1450–1457.

Kjøglum S., Henryon M., Aasmundstad T. & Korsgaard I. (2008) Selective breeding can increase resistance of Atlantic salmon to furunculosis, infectious salmon anaemia and infectious pancreatic necrosis. *Aquaculture Research* **39**, 498–505.

Kjøglum S., Larsen S., Bakke H.G. & Grimholt U. (2006) How specific MHC class I and class II combinations affect disease resistance against infectious salmon anaemia in Atlantic salmon (*Salmo salar*). *Fish & Shellfish Immunology* **21**, 431–441.

Kuo H.C., Wang T.Y., Hsu H.H., Chen P.P., Lee S.H., Chen M.C., Tsai T.J., Wang C.K., Ku H.T., Lee G.B., Chen T.Y. (2012) Nervous necrosis virus replicates following the embryo development and dual infection with iridovirus at juvenile stage in grouper. *PLoS ONE* **7**, e36183.

Kuo H.-P., Chung C.-L., Hung Y.-F., Lai Y.-S., Chiou P.P., Lu M.-W. & Kong Z.-W. (2016) Comparison of the responses of different recombinant fish type I interferons against betanodavirus infection in grouper. *Fish and Shellfish Immunology* **49**, 143–153.

Lai Y.S., Chiu H.C., Murali S., Guo I.C., Chen S.C., Fang K., Chang C.Y. (2001) Propagation of yellow grouper nervous necrosis virus (YGNNV) in a new nodavirus susceptible cell line from yellow grouper, *Epinephelus awoara* (Temminck & Schlegel), brain tissue. *Journal of Fish Diseases* **24**, 299–309.

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.

Legarra, A. (2014) Bases for Genomic Prediction.

Legarra, A., Ricard, A. & Filangi, O. (2016) GS3.

Legarra, a, Aguilar, I. & Misztal, I. (2009) A relationship matrix including full pedigree and genomic information. *Journal of dairy science*, 92(9), pp.4656–63.

Leroux, D. & Jasson, S. (2017) Spell-QTL User Manual – Version 0.2-alpha.

Li, Y., Liu, S., Qin, Z., Waldbieser, G., Wang, R., Sun, L., Bao, L., Danzmann, R.G., Dunham, R. & Liu, Z. (2015) Construction of a high-density, high-resolution genetic map and its integration with BAC-based physical map in channel catfish. *DNA Research*, 22(1), 39–52.

Lillehammer, M., Meuwissen, T.H. & Sonesson, A. (2013) A low-marker density implementation of genomic selection in aquaculture using within-family genomic breeding values. Genet. Sel. Evol. 45, 39.

Lin C.C., Lin J.H.Y., Chen M.S. & Yang H.L. (2007) An oral nervous necrosis virus vaccine that induces protective immunity in larvae of grouper (*Epinephelus coioides*). *Aquaculture* **268**, 265–273.

Lin C.S., Lu M.W., Tang L., Liu W., Chao C.B., Lin C.J. & Schneemann A. (2001) Characterization of virus-like particles assembled in a recombinant baculovirus system expressing the capsid protein of a fish nodavirus. *Virology* **290**, 50–58.

Liu C., Zhang J., Yi F., Wang J., Wang X., Jiang H., Xu J. & Hu Y. (2006a) Isolation and RNA1 nucleotide sequence determination of a new insect nodavirus from Pieris rapae larvae in Wuhan city, China. *Virus research* **120**, 28–35.

Liu, P., Wang, L., Wong, S.-M. & Yue, G.H. (2016) Fine mapping QTL for resistance to VNN disease using a high-density linkage map in Asian seabass. *Scientific Reports*, 6(April), 32122.

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.

Liu W., Hsu C.H., Chang C.Y., Chen H.H. & Lin C.S. (2006b) Immune response against grouper nervous necrosis virus by vaccination of virus-like particles. *Vaccine* **24**, 6282–6287.

Liu, Z., Karsi, A., Li, P., Cao, D. & Dunham, R. (2003) An AFLP-Based Genetic Linkage Map of Channel Catfish (Ictalurus punctatus) Constructed by Using an Interspecific Hybrid Resource Family. *Genetics*, 165(2), 687–694.

Lopez-Jimena B., Alonso M.D.C., Thompson K.D., Adams A., Infante C., Castro D., Borrego J.J., Garcia-Rosado E. (2011) Tissue distribution of red spotted grouper nervous necrosis virus (RGNNV) genome in experimentally infected juvenile European seabass (*Dicentrarchus labrax*). *Veterinary microbiology* **154**, 86–95.

Lopez-Jimena B., Cherif N., Garcia-Rosado E., Infante C., Cano I., Castro D., Alonso M.C. (2010) A combined RT-PCR and dot-blot hybridization method reveals the coexistence of SJNNV and RGNNV betanodavirus genotypes in wild meagre (*Argyrosomus regius*). *Journal of Applied Microbiology* **109**, 1361–1369.

Lu M.W., Chao Y.M., Guo T.C., Santi N., Evensen O., Kasani S.K., Hong J.R., Wu J.L. (2008) The interferon response is involved in nervous necrosis virus acute and persistent infection in zebrafish infection model. *Molecular immunology* **45**, 1146–1152.

Massault, C., Hellemans, B., Louro, B., Batargias, C., Van Houdt, J.K.J., Canario, A., Volckaert, F.A.M., Bovenhuis, H., Haley, C. & de Koning, D.J. (2010) QTL for body weight, morphometric traits and stress response in European sea bass Dicentrarchus labrax. *Animal Genetics*, 41(4), 337–345.

Massault C., Bovenhuis H., Haley C. & Koning D.J. (2008) QTL mapping designs for aquaculture. *Aquaculture* **285**, 23–29.

Meuwissen T., Hayes B. & Goddard M. (2016) Genomic selection: A paradigm shift in animal breeding. *Animal Frontiers* **6**, 6-14.

Meuwissen T.H.E., Hayes, B.J. & Goddard, M.E., 2001. Prediction of total genetic value using Genome-Wide Dense Marker Maps. *Genetics* **157,** 1819–1829.

Miller K.M., Winton J.R., Schulze A.D., Purcell M.K. & Ming T.J. (2004) Major histocompatibility complex loci are associated with susceptibility of Atlantic salmon to infectious hematopoietic necrosis virus. *Environmental Biology of Fishes* **69**, 307–316.

Misztal, I., Tsuruta, S., Lourenco, D., Aguilar, I., Legarra, A. & Vitezica, Z (2015) Manual for BLUPF90 family of programs.

Moen, T., Torgersen, J., Santi, N., Davidson, W.S., Baranski, M., Ødegård, J., Kjøglum, S., Velle, B., Kent, M., Lubieniecki, K.P., Isdal, E., Lien, S., 2015. Epithelial Cadherin Determines Resistance to Infectious Pancreatic Necrosis Virus in Atlantic Salmon. Genetics 200, 1313–1326.

Moen T., Baranski M., Sonesson A.K. & Kjøglum S. (2009) Confirmation and fine-mapping of a major QTL for resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar*): population-level associations between markers and trait. *BMC Genomics* **10**, 368.

Moen T., Sonesson A.K., Hayes B., Lien S., Munck H. & Meuwissen T.H.E. (2007) Mapping of a quantitative trait locus for resistance against infectious salmon anaemia in Atlantic salmon (*Salmo salar*): comparing survival analysis with analysis on affected/resistant data. *BMC Genetics* **8**, 53.

Moen T., Fjalestad K.T., Munck H. & Gomez-Raya L. (2004) A multistage testing strategy for detection of quantitative trait Loci affecting disease resistance in Atlantic salmon. *Genetics* **167**, 851–858.

Mori K., Mangyoku T., Iwamoto T., Arimoto M., Tanaka S. & Nakai T. (2003) Serological relationships among genotypic variants of betanodavirus. *Diseases of Aquatic Organisms* **57**, 19–26.

Mori K., Mushiake K. & Arimoto M. (1998) Control measures for viral nervous necrosis in striped jack. *Fish Pathology,* **33***,* 443-444.

Mori K., Nakai T., Muroga K., Arimoto M., Mushiake K. & Furusawa I. (1992) Properties of a new virus belonging to nodaviridae found in larval striped jack (*Pseudocaranx dentex*) with nervous necrosis. *Virology* **187**, 368–371.

Munday B.L., Kwang J. & Moody N. (2002) Review article Betanodavirus infections of teleost fish: a review. *Journal of Fish Diseases* **25**, 127–142.

Munday E.L., Langdsnb J.S., Hyaw A. & Humphrey J.D. (1992) Mass mortality associated with vacuolating encephalopat larval and juvenile barramundi. *Aqttaculture* **103**, 197-211.

Murray, C., Evelyn, T., Beacham, T., Bamer, L., Ketcheson, J. & Prosperi-Porta, L. (1992) Experimental induction of bacterial kidney disease in Chinook salmon by immersion and cohabitation challenges. *Dis. Aquat. Org*. **12**, 91-96.

Mushiake K., Nishizawa T., Nakai T., Furusawa I. & Muroga K. (1994) Control of VNN in striped jack: Selection of spawners based on the detection of SJNNV gene by polymerase chain reaction (PCR). *Fish Pathology* **29**, 177–182.

Myhr, A.I. & Dalmo, R.A. (2005) Introduction of genetic engineering in aquaculture: Ecological and ethical implications for science and governance. *Aquaculture*, **250**, 542-554.

Naciri, M., Lemaire, C., Borsa, P. & Bonhomme, F. (1999) Genetic study of the Atlantic/Mediterranean transition in sea bass (Dicentrarchus labrax). *Journal of Heredity*, 90, 591–596.

Nagai T. & Nishizawa T. (1999) Sequence of the non-structural protein gene encoded by RNA1 of striped jack nervous necrosis virus. *Journal of General Virology* **80**, 3019–3022.

Nguyen, H.D., Nakai, T. & Muroga, K. (1996) Progression of striped jack nervous necrosis virus (SJNNV) infection in naturally and experimentally infected striped jack Pseudocaranx dentex larvae. , 24, 99–105.

Nath, M., Woolliams, J.A. & Bishop, S.C. (2004) Identifying critical parameters in the dynamics and control of microparasite infection using a stochastic epidemiological model. *Journal of Animal Science*, **82**, 384-396.

Nielsen, H.M., Sonesson, A.K., Yazdi, H. & Meuwissen, T.H.E. (2009) Comparison of accuracy of genome-wide and BLUP breeding value estimates in sib based aquaculture breeding schemes. *Aquaculture*, **289**, 259-264.

Nishizawa T., Furuhashi M., Nagai T., Nakai T. & Muroga K. (1997) Genomic classification of fish nodaviruses by molecular phylogenetic analysis of the coat protein gene. *Applied and Environmental Microbiology* **63**, 1633–1636.

Nishizawa T., Muroga K. & Arimoto M. (1996) Failure of the polymerase chain reaction (PCR) method to detect striped jack nervous necrosis virus (SJNNV) in striped jack, *Pseudocaranx dentex*, selected as spawners. *Journal of Atfutttic Animal Health* **8**, 332-334.

Nishizawa T., Mori K., Furuhashi M., Nakai T., Furusawa I. & Muroga K. (1995). Comparison of the coat protein genes of five fish nodaviruses, the causative agents of viral nervous necrosis in marine fish. *Journal of General Virology* **76**, 1563-1569.

Nishizawa T., Nakail T. & Muroga K. (1994) Polymerase chain reaction (PCR) amplification of RNA of striped jack nervous necrosis virus (SJNNV). *Diseases of Aquatic Organisms* **18**,

103-107,

Norris A, Foyle L. & Ratcliff J. (2008) Heritability of mortality in response to a natural pancreas disease (SPDV) challenge in Atlantic salmon, *Salmo salar* L., post-smolts on a West of Ireland sea site. *Journal of Fish Diseases* **31**, 913–20.

Nuñez-Ortiz N., Stocchi V., Toffan A., Pascoli F., Sood N., Buonocore F., Picchietti S., Papeschi C., Taddei A.R., Thompson K.D. & Scapigliati G. (2015) Quantitative immunoenzymatic detection of viral encephalopathy and retinopathy virus (betanodavirus) in sea bass Dicentrarchus labrax. *Journal of fish diseases* **39**, 821-831.

Nylund A., Karlsbakk E., Nylund S., Isaksen T.E., Karlsen M., Korsnes K. & Ottem K.F. (2008) New clade of betanodaviruses detected in wild and farmed cod (*Gadus morhua*) in Norway. *Archives of Virology* **153**, 541–547.

Ødegård, J., Moen, T., Santi, N., Korsvoll, S.A., Kjøglum, S. & Meuwisse, T.H.E. (2014) Genomic prediction in an admixed population of Atlantic salmon (Salmo salar). *Frontiers in Genetics*, 5, 1–8.

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., Olesen I., Dixon P., Jeney Z., Nielsen H.M., Way K. & Gjerde B. (2010a) Genetic analysis of common carp (*Cyprinus carpio*) strains. II: Resistance to koi herpesvirus and *Aeromonas hydrophila* and their relationship with pond survival. *Aquaculture* **304**, 7–13.

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

Ødegård, J., Sonesson, A., Yazdi, K., Hossein, M. & Meuwissen, T.H.E. (2009) Introgression of a major QTL from an inferior into a superior population using genomic selection. Genetics Selection Evolution. **41**:38.

Ødegård J., Olesen I., Gjerde B. & Klemetsdal G. (2007a) Evaluation of statistical models for genetic analysis of challenge-test data on ISA resistance in Atlantic salmon (*Salmo salar*): Prediction of progeny survival. *Aquaculture* **266**, 70–76.

Ødegård J., Olesen I., Gjerde B. & Klemetsdal G. (2007b) Positive genetic correlation between resistance to bacterial (furunculosis) and viral (infectious salmon anaemia) diseases in farmed Atlantic salmon (*Salmo salar*). *Aquaculture* **271**,173–177.

OIE, 2013. Viral encephalopathy and retinopathy 1, 1–19.

Okinaka Y. & Nakai T. (2008) Comparisons among the complete genomes of four betanodavirus genotypes. *Diseases of Aquatic Organisms* **80**, 113–21.

Olesen I., Hung D. & Ødegård J. (2007) Genetic analysis of survival in challenge tests of furunculosis and ISA in Atlantic salmon. Genetic parameter estimates and model comparisons. *Aquaculture* **272**, S297-S298.

Olveira J.G., Souto S., Dopazo C.P., Thiéry R., Barja J.L. & Bandín I. (2009) Comparative analysis of both genomic segments of betanodaviruses isolated from epizootic outbreaks in farmed fish species provides evidence for genetic reassortment. *The Journal of general virology* **90**, 2940–2951.

Oral, M., Colléter, J., Bekaert, M., Taggart, J.B., Palaiokostas, C., McAndrew, B.J., Vandeputte, M., Chatain, B., Kuhl, H., Reinhardt, R., Peruzzi, S. & Penman, D.J. (2017) Gene-centromere mapping in meiotic gynogenetic European seabass. BMC Genomics 18, 449. doi:10.1186/s12864-017-3826-z

Øvergård, A.C., Nerland, A.H., Fiksdal, I.U. & Patel, S. (2012) Atlantic halibut experimentally infected with nodavirus shows increased levels of T-cell marker and IFNγ transcripts. *Developmental and Comparative Immunology*, **37**, 139-150.Ozaki A., Khoo S.K., Yoshiura Y., Ototake M., Sakamoto T., Dijkstra J.M. & Okamoto N. (2007) Identification of additional quantitative trait loci (QTL) responsible for susceptibility to infectious pancreatic necrosis virus in rainbow trout. *Fish Pathology* **42**, 131–140.

Ozaki A., Sakamoto T., Khoo S.K., Nakamura K., Coimbra M.R.N., Akutsu T. & Okamoto N. (2001) Quantitative trait loci (QTLs) associated with resistance/susceptibility to infectious pancreatic necrosis virus (IPNV) in rainbow trout (*Oncorhynchus mykiss*). *Molecular Genetics and Genomics* **265**, 23-31.

Pakingking R., Bautista N.B., De Jesus-Ayson E.G. & Reyes O. (2010) Protective immunity against viral nervous necrosis (VNN) in brown-marbled grouper (*Epinephelus fuscogutattus*) following vaccination with inactivated betanodavirus. *Fish & Shellfish Immunology* **28**, 525–533.

Palaiokostas, C., Ferarreso, S., Franch, R., Houston, R.D. & Bargelloni, L. (2016) Genomic prediction of resistance to pasteurellosis in gilthead sea bream (Sparus aurata) using 2b-RAD sequencing. *G3: Genes|Genomes|Genetics*, X, 1–8.

Palaiokostas C., Bekaert M., Taggart J.B., Gharbi K., Mcandrew B.J., Chatain B., Penman D.J., Vandeputte M. (2015) A new SNP-based vision of the genetics of sex determination in European sea bass (*Dicentrarchus labrax*). *Genetics Selection Evolution* **47**:68.

Palti1 Y., Gao G., Moen T., Liu S., Kent M.P., Lien S., Miller M.R. & Rexroad C.E. (2014) The Development and Characterization of a 57K SNP Chip for Rainbow Trout. *Proceedings, 10th World Congress of Genetics Applied to Livestock Production,* August 17th-22th, 2014, Vancouver, BC, Canada.

Palti Y., Nichols K.M., Waller K.I., Parsons J.E. & Thorgaard G.H. (2001) Association between DNA polymorphisms tightly linked to MHC class II genes and IHN virus resistance in backcrosses of rainbow and cutthroat trout. *Aquaculture* **194**, 283–289.

Palti Y., Parsons J.E. & Thorgaard G.H. (1999) Identification of candidate DNA markers associated with IHN virus resistance in backcrosses of rainbow (*Oncorhynchus mykiss*) and cutthroat trout (*O. clarki*).*Aquaculture* **173**, 81–94.

Panzarin V., Fusaro A., Monne I., Cappellozza E., Patarnello P., Bovo G. & Cattoli G. (2012) Molecular epidemiology and evolutionary dynamics of betanodavirus in southern Europe. *Infection, Genetics and Evolution : Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases* **12**, 63–70.

Panzarin V., Patarnello P., Mori K., Rampazzo E., Cappellozza E., Bovo G. & Cattoli G. (2010) Development and validation of a real-time TaqMan PCR assay for the detection of betanodavirus in clinical specimens. *Archives of Virology* **155**, 1193–203.

Peducasse S., Castric J., Thiery R., Jeffroy J., Le Ven A. & Laurencin F.B. (1999) Comparative study of viral encephalopathy and retinopathy in juvenile sea bass *Dicentrarchus labrax* infected in different ways. *Diseases of Aquatic Organisms* **36***,* 11-20.

Perez-Rufaza A., Marcos C., 2014. Ecology and distribution of *Dicentrarchus labrax* (Linnaeus 1758), in Biology of European sea bass, Sanchez Vasquez F.J.,Munoz-Cueto J.A. (eds.), CRC Press, Boca Raton, 3-33.

Pszczola, M., Strabel, T. & Calus, M.P.L. (2014) Size of required reference population updates to achieve constant genomic prediction accuracy across generations. Proceedings, 10th World Congress of Genetics Applied to Livestock Production

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J. & Sham, P.C. (2007) PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*, 81(3), 559–575.

Qian D., Shi Z., Zhang S., Cao Z., Liu W., Li L., Xie Y., Cambournac I., Bonami J.R. (2003) Extra small virus-like particles (XSV) and nodavirus associated with whitish muscle disease in the giant freshwater prawn, Macrobrachium rosenbergii. *Journal of Fish Diseases* **26**, 521–527.

Ransangan J. & Manin B.O. (2012) Genome analysis of Betanodavirus from cultured marine fish species in Malaysia. *Veterinary microbiology* **156**, 16–44.

Ransangan J., Manin B.O., Abdullah A., Roli Z. & Sharudin E.F. (2011) Betanodavirus infection in golden pompano, *Trachinotus blochii*, fingerlings cultured in deep-sea cage culture facility in Langkawi, Malaysia. *Aquaculture* **315**, 327–334.

Rastas, P., Calboli, F.C.F., Guo, B., Shikano, T. & Merilä, J. (2016) Construction of Ultradense Linkage Maps with Lep-MAP2: Stickleback F2 Recombinant Crosses as an Example. *Genome biology and evolution*, 8(1), 78–93.

Rastas, P., Paulin, L., Hanski, I., Lehtonen, R., Auvinen, P. & Brudno, M. (2013) Lep-MAP: Fast and accurate linkage map construction for large SNP datasets. *Bioinformatics*, 29(24), 3128–3134.

Resende, M.F.R., Munoz, P., Resende, M.D.V., Garrick, D.J., Fernando, R.L., Davis, J.M., Jokela, E.J., Martin, T.A., Peter, G.F. & Kirst, M. (2012) Accuracy of Genomic Selection Methods in a Standard Data Set of Loblolly Pine (Pinus taeda L.). *Genetics*, 190(4), 1503–1510.

Robledo, D., Palaiokostas, C., Bargelloni, L., Martínez, P. & Houston, R 2017. Applications of genotyping by sequencing in aquaculture breeding and genetics. *Reviews in Aquaculture*, (February).

Rodriguez M.F., LaPatra S., Williams S., Famula T. & May B. (2004) Genetic markers associated with resistance to infectious hematopoietic necrosis in rainbow and steelhead trout (*Oncorhynchus mykiss*) backcrosses. *Aquaculture* **241**, 93–115.

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(1–4), 139–147.

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(5), 494–505.

Saillant, E., Fostier, A., Menu, B., Haffray, P. & Chatain, B. (2001) Sexual growth dimorphism in sea bass Dicentrarchus labrax. *Aquaculture*, **202**, 371-387.

Saillant, E., Dupont-Nivet, M., Sabourault, 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(1), 105–112.

Sakamoto, T., Danzmann, R.G., Gharbi, K., Howard, P., Ozaki, A., Khoo, S.K., Woram, R.A., Okamoto, N., Ferguson, M.M., Holm, L., Guyomard, R. & Hoyheim, B. (2000) A Microsatellite Linkage Map of Rainbow Trout (Oncorhynchus mykiss) Characterized by Large Sex-Specific Differences in Recombination Rates. Genetics, 155, 1331-1345.

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(3), 229–239.

Sargolzaei, M., Chesnais, J.P. & Schenkel, F.S. (2014) A new approach for efficient genotype imputation using information from relatives. *BMC Genomics*, **15**:478.

Scapigliati G., Buonocore F., Randelli E., Casani D., Meloni S., Zarletti G., Tiberi M., Pietretti D., Boschi I., Manchado M., Martin-Antonio B., Jimenez-Cantizano R., Bovoc G., Borghesan F., Lorenzen N., Einer-Jensen K., Adamse S., Thompsone K., Alonso C., Bejar J., Cano I., Borrego J.J. & Alvarez M.C. (2010) Cellular and molecular immune responses of the sea bass (*Dicentrarchus labrax*) experimentally infected with betanodavirus. *Fish & Shellfish Immunology* **28**, 303-311.

Senapin S., Jaengsanong C., Phiwsaiya K., Prasertsri S., Laisutisan K., Chuchird N. & Flegel T.W. (2012) Infections of MrNV (*Macrobrachium rosenbergii* nodavirus) in cultivated whiteleg shrimp *Penaeus vannamei* in Asia. *Aquaculture* **338-341**, 41–46.

Shao, C., Niu, Y., Rastas, P., Liu, Y., Xie, Z., Li, H., Wang, L., Jiang, Y., Tai, S., Tian, Y., Sakamoto, T. & Chen, S. (2015) Genome-wide SNP identification for the construction of a

high-resolution genetic map of Japanese flounder (Paralichthys olivaceus): Applications to QTL mapping of Vibrio anguillarum disease resistance and comparative genomic analysis. *DNA Research*, 22(2), 161–170.

Shetty M., Maiti B., Santhosh K.S., Venugopal M.N. & Karunasagar I. (2012) Betanodavirus of marine and freshwater fish: distribution, genomic organization, diagnosis and control measures. *Indian Journal of Virology: An Official Organ of Indian Virological Society* **23**, 114–123.

Skliris G.P., Krondiris J.V., Sideris D.C., Shinn A.P., Starkey W.G. & Richards R.H. (2001). Phylogenetic and antigenic characterization of new fish nodavirus isolates from Europe and Asia. *Virus Research* **75**, 59–67.

Skliris G.P. & Richards R.H. (1999) Induction of nodavirus disease in seabass, *Dicentrarchus labrax*, using different infection models. *Virus Research* **63**, 85–93.

Sommerset I., Skern R., Biering E., Bleie H., Fiksdal I.U., Grove S. & Nerland A.H. (2005) Protection against Atlantic halibut nodavirus in turbot is induced by recombinant capsid protein vaccination but not following DNA vaccination. *Fish & Shellfish Immunology* **18**, 13–29.

Sommerset I. & Nerland A.H. (2004) Complete sequence of RNA1 and subgenomic RNA3 of Atlantic halibut nodavirus (AHNV). *Diseases of Aquatic Organisms* **58**, 117–25.

Sonesson A.K. (2007) Within-family marker-assisted selection. *Genetics Selection Evolution* **39**, 301–317.

Sonesson, A.K. & Meuwissen, T.H.E., 2009. Testing strategies for genomic selection in aquaculture breeding programs. *Genetics, selection, evolution : GSE*, 41, p.37.

Starkey W.G., Ireland J.H., Muir K.F., Shinn A.P., Richards R.H. & Ferguson H.W. (2000) Short communication Isolation of nodavirus from Scottish farmed halibut, *Hippoglossus hippoglossus* (L). *Journal of Fish Diseases* **23**, 419–422.

Storset A., Strand C., Wetten M., Kjøglum S. & Ramstad A. (2007) Response to selection for resistance against infectious pancreatic necrosis in Atlantic salmon (*Salmo salar* L.). *Aquaculture* **272**, 62–68.

Su Y.C., Chiu H.W., Hung J.C. & Hong J.R. (2014) Beta-nodavirus B2 protein induces hydrogen peroxide production, leading to Drp1-recruited mitochondrial fragmentation and cell death via mitochondrial targeting. *Apoptosis* **19**, 1457–1470.

Sugaya T., Mori K., Nishioka T., Masuma S., Oka M., Mushiake K. & Nakai T. (2009) Genetic heterogeneity of betanodaviruses in juvenile production trials of Pacific bluefin tuna, *Thunnus orientalis* (Temminck & Schlegel). *Journal of Fish Diseases* **32**, 815–823.

Tan C., Huang B., Chang S.F., Ngoh G.H., Munday B., Chen S.C. & Kwang J. (2001) Determination of the complete nucleotide sequences of RNA1 and RNA2 from greasy grouper (*Epinephelus tauvina*) nervous necrosis virus, Singapore strain. *Journal of General Virology* **82**, 647–653.

Tanaka S., Mori K., Arimoto M., Iwamoto T. & Nakai T. (2001) Protective immunity of sevenband grouper, *Epinephelus septemfasciatus* Thunberg, against experimental viral nervous necrosis. *Journal of Fish Diseases* **24**, 15–22.

Tanaka S., Aoki H. & Nakai T. (1998) Pathogenicity of the nodavirus detected from diseased sevenband grouper *Epinephelus septemfasciatus*. *Fish Pathology* **33**, 31-36.

Tang K.F.J., Carlos R.P., Redman R.M. & Lightner D.V. (2007) Development of in situ hybridization and RT-PCR assay for the detection of a nodavirus (PvNV) that causes muscle necrosis in *Penaeus vannamei*. *Disease of Aquatic Organisms **75***, 183–190.

The united voice of the European aquaculture production industry – Annual report 2016.

The EU fish market, 2016 Edition.

The European Commission 2016.

Thiéry R., Cozien J., Cabon J., Lamour F., Baud M. & Schneemann A. (2006) Induction of a protective immune response against viral nervous necrosis in the European sea bass, *Dicentrarchus labrax*, by using betanodavirus virus-like particles. *Journal of Virology* **80**, 10201–10207.

Thiéry R., Cozien J., de Boisséson C., Kerbart-Boscher S. & Névarez 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.

Thiéry R., Raymond J.C. & Castric J. (1999) Natural outbreak of viral encephalopathy and retinopathy in juvenile sea bass, *Dicentrarchus labrax*: study by nested reverse transcriptase–polymerase chain reaction. *Virus Research* **63**, 11–17.

Thiéry R., Arnauld C. & Delsert C. (1999) Two isolates of sea bass, *Dicentrarchus labrax* L., nervous necrosis virus with distinct genomes. *Journal of Fish Diseases* **22**, 201–207.

Thiéry R., Peducasse S., Castric J., Leven A., Jeffroy J. & Laurenci F.B. (1997) Experimental transmission of viral encephalopathy and retinopathy to juvenile sea bass (*Dicentrarchus Labrax*). *Fish Pathology* **17***,* 118-122.

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.

Toffolo V., Negrisolo E., Maltese C., Bovo G., Belvedere P., Colombo L. & Valle L.D. (2007) Phylogeny of betanodaviruses and molecular evolution of their RNA polymerase and coat proteins. *Molecular phylogenetics and evolution* **43**, 298–308.

Tsai, H.-Y., Hamilton, A., Tinch, A.E., Guy, D.R., Bron, J.E., Taggart, J.B., Gharbi, K., Stear, M., Matika, O., Pong-Wong, R., Bishop, S.C. & Houston, R.D. (2016) Genomic prediction of host resistance to sea lice in farmed Atlantic salmon populations. *Genetics Selection Evolution*, 48(1), 47.

Tsai, H.Y., Robledo, D., Lowe, N.R., Bekaert, M., Taggart, J.B., Bron, J.E. & Houston, R.D. (2016) Construction and Annotation of a High Density SNP Linkage Map of the Atlantic Salmon (Salmo salar) Genome. *G3 (Bethesda, Md.)*, 6(7), 2173–2179.

Tsai, H.Y., Hamilton, A., Tinch, A.E., Guy, D.R., Gharbi, K., Stear, M.J., Matika, O., Bishop, S.C. & Houston, R.D. (2015) Genome wide association and genomic prediction for growth traits in juvenile farmed Atlantic salmon using a high density SNP array. *BMC genomics*, 1–9.

Tu J., Chen W., Fu X., Lin Q., Chang O., Zhao L., Lan J., Li N. & Lin L. (2016) Susceptibility of Chinese Perch Brain (CPB) Cell and Mandarin Fish to Red-Spotted Grouper Nervous Necrosis Virus (RGNNV) Infection. *Int J Mol Sci* **17**.

Ucko M., Colorni A. & Diamant A. (2004) Nodavirus infections in Israeli mariculture. *Journal of Fish Diseases* **27**, 459–469.

Vallejo, R.L., Leeds, T.D., Fragomeni, B.O., Gao, G., Hernandez, A.G., Misztal, I., Welch, .J., Wiens, G.D. & Palti, Y. (2016) Evaluation of genome-enabled selection for bacterial cold water disease resistance using progeny performance data in rainbow trout: Insights on genotyping methods and genomic prediction models. *Frontiers in Genetics*, 7(MAY), 1–13.

Vallejo, R.L., Leeds, T.D., Gao, G., Parsons, J.E., Martin, K.E., Evenhuis, J.P., Fragomeni, B.O., Wiens, G.D. & Palti, Y. (2017) Genomic selection models double the accuracy of predicted breeding values for bacterial cold water disease resistance compared to a traditional pedigree-based model in rainbow trout aquaculture. *Genetics Selection Evolution*, 49(1), 17.

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., 2009b. 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., Baroiller, J.F., Haffray, P., Quillet, E., 2009b. Amélioration génétique des poissons: quelles réalisations et quels défis pour demain ? Cah. Agric. 18, 262–269.

Vandeputte, M., Haffray, P., 2014. Parentage assignment with genomic markers: a major advance for understanding and exploiting genetic variation of quantitative traits in 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.

VanRaden, P.M. (2008) Efficient methods to compute genomic predictions. *Journal of dairy science*, 91(11), 4414–4423.

Van Regenmortel M.H.V., Fauquet C.M., Bishop D.H.L., Cartens E.B., Estes M.K., Lemon S.M., Maniloff J., Mayo M.A., McGeoch D.J., Pringle C.R. & Wickner R.B. (2000) *Virus Taxonomy: Classification and Nomenclature of Viruses. Seventh Report of the International Committee on Taxonomy of Viruses.* Academic Press, San Diego.

Vendramin N., Toffan A., Mancin M., Cappellozza E., Panzarin V., Bovo G., Cattoli G., Capua I. & Terregino C (2014) Comparative pathogenicity study of ten different betanodavirus strains in experimentally infected European sea bass, *Dicentrarchus labrax* (L.). *Journal of fish diseases* **37**, 371–383.

Vendramin N., Patarnello P., Toffan A., Panzarin V., Cappellozza E., Tedesco P. & Cattoli G. (2013) Viral Encephalopathy and Retinopathy in groupers (*Epinephelus* spp.) in southern Italy: a threat for wild endangered species. *BMC Veterinary Research* **9**:20.

Vendramin N., Padrós F., Pretto T., Cappellozza E., Panzarin V., Bovo G. & Terregino C. (2012). Viral encephalopathy and retinopathy outbreak in restocking facilities of the endangered freshwater species, *Salaria fluviatilis* (Asso). *Journal of Fish Diseases* **35**, 867–71.

Verrier E.R., Dorson M., Mauger S., Torhy C., Ciobotaru C., Hervet C. & Quillet E. (2013) Resistance to a rhabdovirus (VHSV) in rainbow trout: identification of a major QTL related to innate mechanisms. *PloS One* **8**:55302.

Villanueva, B., Fernández, J., García-Cortés, L.A., Varona, L., Daetwyler, H.D. & Toro, M.A. (2011) Accuracy of genome-wide evaluation for disease resistance in aquaculture breeding programs. *Journal of animal science*, 89(11), 3433–3442.

Vimal S., Farook M. A., Madan N., Abdul Majeed S., Nambi K.S.N., Taju G., Sundarraj N., Venu S., Subburaj R., Thirunavukkarasu A.R. & Sahul Hameed A.S. (2016) Development, distribution and expression of a DNA vaccine against nodavirus in Asian Seabass, Lates calcarifier (Bloch, 1790). *Aquaculture Research* **47**, 1209–1220.

Voorrips, R.E. (2002) MapChart: Software for the Graphical Presentation of Linkage Maps and QTLs. *Journal of Heredity*, 93(1), 77–78.

Wang, H., Misztal, I., Aguilar, I., Legarra, A., and Muir, W. M. (2012). Genome- wide association mapping including phenotypes from relatives without genotypes. Genet. Res. 94, 73–83. doi: 10.1017/S0016672312000274

Wang, L., Liu, P., Huang, S., Ye, B., Chua, E., Wan, Z.Y. & Yue, G.H. (2017) Genome-Wide Association Study Identifies Loci Associated with Resistance to Viral Nervous Necrosis Disease in Asian Seabass. *Marine Biotechnology*, 1–11.

Wang, L., Bai, B., Huang, S., Liu, P., Wan, Z.Y., Ye, B., Wu, J. & Yue, G.H. (2017) QTL Mapping for Resistance to Iridovirus in Asian Seabass Using Genotyping-by-Sequencing. *Marine Biotechnology*. DOI 10.1007/s10126-017-9770-8

Wang L., Huang S.Q., Xia J.H., Liu P., Wan Z.Y. & Yue G.H. (2015) Genome-wide discovery of gene related SNPs in Barramundi *Lates calcarifer*. *Spinger, Conservation Genetics Resources* **7**, 605-608.

Wang, C.M., Bai, Z.Y., He, X.P., Lin, G., Xia, J.H., Sun, F., Lo, L.C., Feng, F.Z., Ze, Y. & Yue, G.H. (2011) A high-resolution linkage map for comparative genome analysis and QTL fine mapping in Asian seabass, Lates calcarifer. *BMC genomics*, 12(1), 174.

Wargo, A.R., Kell, A.M., Scott, R.J., Thorgaard, G.H., Kurath, G., 2012. Analysis of host genetic diversity and viral entry as sources of between-host variation in viral load. Virus Res. 165, 71–80. doi:10.1016/j.virusres.2012.01.010

Watanabe K., Nishizawa T. & Yoshimiru M. (2000) Selection of brood stock candidates of barfin flounder using an ELISA system with recombinant protein of barfin flounder nervous necrosis virus. *Diseases of Aquatic Organisms* **41**, 219-223.

Watanabe K., Suzuki S., Nishizawa T., Suzuki K., Yoshimizu M. & Ezura Y. (1998) Control strategy for viral nervous necrosis of Barfin flounder. *Fish Pathology* **33**, 445-446.

Weng, Z.-Q., A. Wolc, R.L. Fernando, J.C.M. Dekkers, J. Arango, P. Settar, J.E. Fulton, N.P. O'Sullivan, and D.J. Garrick (2014) Prediction accuracy of pedi- gree and genomic estimated breeding values over generations in layer chickens. In: Proc 10th World Congress of Genetics Applied to Livestock Production (WCGALP). http://bit.ly/1kHYGRj

Wetten M., Aasmundstad T., Kjøglum S. & Storset A. (2007) Genetic analysis of resistance to infectious pancreatic necrosis in Atlantic salmon (*Salmo salar* L.). *Aquaculture* **272**, 111–117.

Wolc, A., Kranis, A., Arango, J., Settar, P., Fulton, J.E., O'Sullivan, N.P., Avendano, A., Watson, K.A., Hickey, J.M., de los Campos, G., Fernando, R.L., Garrick, D.J. & Dekkers, J.C.M. (2016) Implementation of genomic selection in the poultry industry. Animal Frontiers, 6, 23-31.

Wolc, A., Kranis, A., Arango, J., Settar, P., Fulton, J.E. & O'Sullivan, N. (2014) Applications of Genomic Selection in Poultry. *Proceedings, 10th World Congress of Genetics Applied to Livestock Production*.

Wu Y.-C., Tsai P.-Y., Chan J.-C. & Chi S.-C. (2016) Endogenous grouper and barramundi Mx proteins facilitated the clearance of betanodavirus RNA-dependent RNA polymerase. *Developmental and Comparative Immunology* **59**, 110–120.

Yamashita H., Mori K., Kuroda A. & Nakai T. (2009) Neutralizing antibody levels for

protection against betanodavirus infection in sevenband grouper, *Epinephelus septemfasciatus* (Thunberg), immunized with an inactivated virus vaccine. *Journal of Fish Diseases* **32**, 767–75.

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

Yáñez J.M., Naswa S., López M.E., Bassini L., Cabrejos M.E., Gilbey J., Bernatchez L., Norris A., Soto C., Eisenhart J., Simpson B., Neira R., Lhorente J.P., Schnable P., Newman S., Mileham A. & Deeb N. (2014b) Development of a 200K SNP array for Atlantic salmon: exploiting across continents genetic variation. *Proceedings, 10th World Congress of Genetics Applied to Livestock Production,* August 17th-22th, 2014, Vancouver, BC, Canada.

Yazdi, M.H., Sonesson, A.K., Woolliams, J.A. & Meuwissen, T.H.E. (2010) Combined detection and introgression of QTL in outbred populations. *Genetics Selection Evolution*, **42**:16

Yoshikoshi K. & Inoue K. (1990) Viral nervous necrosis in hatchery-reared larvae and juveniles of Japanese parrotfish, *Oplegnathus fasciatus* (Temminck & Schlegei). *Journal of Fish Diseases* **13**, 69-77.

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. *Proceedings, 10th World Congress of Genetics Applied to Livestock Production,* August 17th-22th, Vancouver, BC, Canada.

Yuasa K., Koesharyani I. & Mahardika K. (2007) Effect of high water temperature on betanodavirus infection of fingerling Humpback grouper (*Cromileptes altivelis*). *Fish Pathology* **42**, 219–221.

Yuasa K., Koesharyani I., Roza D., Mori K., Katata M. & Nakai T. (2002) Short communication immune response of humpback grouper, *Cromileptes altivelis* (Valenciennes), injected with the recombinant coat protein of betanodavirus. *Journal of Fish Diseases* **25**, 53-56

Yue, G.H. (2014) Recent advances of genome mapping and marker-assisted selection in aquaculture. *Fish and Fisheries*, 15(3), 376–396.

Zhan, X., L.Lourenc, D.A., Miszta, I., Aguila, I. & Legarr, A. (2016) Weighted Single-step Genomic BLUP: an Iterative Approach for Accurate Calculation of GEBV and GWAS. Front. Genet. 7:151. doi: 10.3389/fgene.2016.00151

Zheng X, Levine D, Shen J, Gogarten SM, Laurie C, Weir BS: A high-performance computing toolset for relatedness and principal component analysis of SNP data. Bioinformatics. 2012, 28 (24): 3326-3328. 10.1093/bioinformatics/bts606.

Zorriehzahra M.E.J., Ghasemi M., Ghiasi M., Karsidani S.H., Bovo G., Nazari A., Adel M., Arizza V. & Dhama K. (2016) Isolation and confirmation of viral nervous necrosis (VNN) disease in golden grey mullet (Liza aurata) and leaping mullet (Liza saliens) in the Iranian waters of the Caspian Sea. *Veterinary Microbiology* **190**, 27–37

Résumé

Le bar est une espèce économique majeure de l'aquaculture méditerranéenne. La nécrose nerveuse virale (VNN), une maladie qui affecte au moins 70 espèces aquatiques, est devenue la menace la plus grave pour l'aquaculture de cette espèce. Bien que de nombreuses études aient été réalisées afin de contrôler cette maladie, aucune procédure simple et efficace n'est disponible. Dans cette thèse, nous évaluons la variabilité génétique de la résistance à cette pathologie et le potentiel d'amélioration génétique pour lutter contre cette menace.

Après une introduction générale (premier chapitre) et une revue de la littérature sur la nodavirose en aquaculture (second chapitre), nous explorons dans le troisième chapitre la variabilité génétique de résistance de populations sauvages de bar, Atlantique Nord (NAT), Méditerranée ouest (WEM), Nord-Est Méditerranée (NEM) et Méditerranée Sud-Est (SEM). Pour ce faire, 2011 descendants d'un croisement factoriel complet, où 9 mères WEM ont été croisées avec 60 pères NAT, WEM, NEM et SEM (15 mâles par population), ont été élevés en "common garden". Après 202 jours, 1472 poissons ont été infectés par injection intrapéritonéale nodavirus à 15.8g de poids moyen. Le reste des poissons a été conservé pour collecter les paramètres de performance. Après la récupération du pedigree, nous révélons une forte variabilité de résistance en fonction de l'origine des pères (de 53 à 90%), les descendants de pères Est-Méditerranéens étant les plus résistants (83 à 90% de survie), les descendants WEM étant intermédiaires (62% de survie) et les descendants de père NAT étant les plus sensibles (53% seulement de la survie). Une héritabilité modérée mais significative pour la résistance (0,26 ± 0,11) a été estimée et des corrélations négatives entre la résistance et les traits de production ont été montrées. Dans le quatrième chapitre une recherche de loci à effets fort (QTL) sur la résistance a été effectuée avec une carte de liaison moyenne-densité. Pour cela, 1717 individus appartenant à 397 familles de plein-frères et leurs parents ont été génotypés pour 2722 marqueurs SNP imprimés sur une puce SNPs. À partir de 1274 loci significatifs, une carte de liaison contenant 24 groupes de liaison, ainsi que des cartes sexe-spécifiques et origine-spécifiques ont été construites. Ces résultats révèlent une hétérochiasmie, avec un taux de recombinaison 1,14 fois plus fort chez les femelles par rapport aux mâles. La recherche de QTL a été effectuée à partir de différentes méthodes, mais bien qu'aucun QTL pour le «temps de survie» ou la survie, n'ait été identifié, nous discutons de l'effet du plan expérimental utilisé. Dans le quatrième chapitre, une étude association génomique a été effectuée en deux étapes: non pondérée (GWAS) puis pondérée (wGWAS) à partir de modèles mixtes linéaires utilisant les mêmes SNP que pour la cartographie de QTL, l'objectif étant de détecter des SNPs liés à la résistance au VNN. Un SNP significatif expliquant 3.11% de la résistance appartenant à LG9 a pu être détecté. Le potentiel de prédiction de la génomique pour la résistance au VNN en utilisant différents modèles génomiques a enfin été évalué, mais aucune différence significative n'a été montrée entre les valeurs génétiques estimées à partir des données génomiques ou à partir du pedigree. En conclusion, cette étude montre forte variation génétique de la résistance au VNN des populations sauvages de bar avec des corrélations génétiques négatives avec les traits de production. Ces derniers résultats sont précieux pour aider à définir des stratégies d'amélioration génétique de la résistance au VNN du bar. Enfin, de premières hypothèses sur l'emplacement de QTL putatifs plaident pour une future cartographie fine pour localiser ces QTLs, une valeur ajoutée dans un schéma de sélection assistée par marqueurs pour améliorer la résistance au VNN du bar.

Summary

European seabass is one of the most economic species in aquaculture in Mediterranean areas. Viral nervous necrosis (VNN), a disease affecting at least 70 aquatic species, has become the most serious threat to seabass cultured industry. While numerous studies have been performed in order to control this disease, no simple and effective procedures are available. In this thesis, we question genetic variability and the potential of selective breeding as an opportunity to address thwart this threat.

After a general introduction (first chapter) and a deep literature review of nodavirus in aquaculture (second chapter), we explore in the third chapter the genetic variability of resistance of different wild populations of European seabass, namely Northern Atlantic (NAT), Western Mediterranean (WEM), Northern-East Mediterranean (NEM) and Southern-East Mediterranean (SEM). To address this question, 2011 fish derived from a full-factorial mating scheme, where 9 WEM dams were crossed with 60 sires originated from NAT, WEM, NEM and SEM (15 sires per population), were reared in "common garden". At 202 days, 1472 were challenged by nodavirus intraperitoneal injection at a mean body weight of 15.8 g. The rest of fish were kept in a single tank in order to collect performance traits. Strikingly, after pedigree recovery, we reveal a very strong and significant differentiation in VNN resistance among sires' origin (ranging from 53 to 90%), offspring from East Mediterranean sires being the most resistant (83-90% of survival), offspring from WEM sires being intermediate (62% of survival) and offspring from NAT sires being the most sensitive (53% of survival only). A moderate liability heritability for VNN resistance (0.26±0.11) was estimated and negative correlations between resistance and production traits were shown. In the fourth chapter, a search of Quantitative Trait Loci (QTL) linked to the resistance was performed using a medium linkage map as examined. Therefore, 1717 individuals belonging 397 full-sib families and their parents were genotyped for 2722 SNP markers spotted on a SNPChip. From 1274 significant loci, a 24 linkage groups medium-density linkage map was constructed, as well as sex-specific and Origin-specific linkage maps. From these results, we show a 1.14-fold sex-biased heterochiasmy in favor to female recombination rate. Finally, genome scans for QTLs were performed in different methods, and while no QTLs were identified for both "time to death" or survival, we discuss the effect of the experimental design used. In the fifth chapter, a two-step unweighted then weighted Genome-Wide Association Study (GWAS & wGWAS) was carried out based on linear mixed models using the same SNPs as for QTL mapping. The aim was to determine whether we can detect significant individual SNPs linked to resistance against VNN. After SNPs weight calculation, the wGWAS detected one significant SNP explaining 3.11% of the resistance belonging to LG9. Finally, the potential for genomics prediction for VNN resistance using the different genomic models was performed and extensively presented. However, no significant differences were observed between genomic-based estimated breeding values and pedigree-based estimated breeding values. In conclusion, this study depicts a large genetic variation for VNN resistance in wild seabass populations but with negative genetic correlations with production traits. These latter results are valuable to help to define strategies for genetic improvement of resistance against VNN of European seabass. Moreover, the first assumptions on the location of potential QTLs claim for a fine QTL mapping and an expectable add-value of the use of genomic information in potential marker-assisted selection to VNN resistance in European seabass.