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On the trail of detecting genetic (co)variation between resistance to parasite infections (*Diplectanum aequans* and *Lernanthropus kroyeri)* and growth in European seabass (*Dicentrarchus labrax*)

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ABSTRACT

The European seabass is one of the main commercial fish produced in Mediterranean marine aquaculture. Recently, its production has been negatively affected by losses due to frequent and recurring outbreaks of parasitic, bacterial and viral diseases. In recent years, the gill parasites *Diplectanum aequans* and *Lernanthropus kroyeri* are increasingly becoming more dominant and contributing more significantly to the observed losses. Genetic improvement for disease resistance represents an important strategy for controlling infectious diseases in farmed fish by increasing their robustness. In order to determine the possibility of including such trait in selective breeding programs, we need to comprehend whether additive genetic variation for resistance against *Diplectanum aequans* and *Lernanthropus kroyeri* exists. For this purpose, two open-sea parasite cohabitation trials (for two consecutive years) were performed in the commercial production sites of a private company (Nireus S.A), that had high infestation with *Diplectanum aequans* and *Lernanthropus kroyeri*. Juvenile European seabass (9425 offspring from 91 full-sib and half-sib families per year), originating from the company's breeding program were equally divided into two groups and transferred to two commercial farming sites located in the areas of Nafpactos and Sagiada, in western Greece, for the intended cohabitation studies. The parasite numbers on all the gill arches were counted and recorded at the end of the trials for the infestation levels. A third site (Palairos) without any parasite infestation was used as a control site. A multi-trait animal model was used to estimate the variance-covariance components and to evaluate the genetic parameters for Parasite Counts, recorded from all the gill arches of the fish, and their corresponding growth in sea cages. The estimated heritabilities for parasite count, using untransformed data, were 0.20 (*D. aequans*) and 0.28 (*L. kroyeri*) and for transformed data 0.29 and 0.26, respectively. The heritability estimates for body weight were 0.42−0.51 for *D. aequans* and 0.28−0.51 for *L. kroyeri* trials. Similarly, estimated heritabilities for the growth in sea cages were 0.43 and 0.29, respectively. Although parasite count has a low to medium unfavorable genetic correlation with body weight and growth (0.09−0.37), it seems that it is not significantly impairing selection for growth. Furthermore, the results of this study are very promising in terms of the existing potential for genetic improvement of parasite resistance, and provides a good basis for further genetic analysis using molecular markers.

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1. Introduction

The European seabass *Dicentrarchus labrax* (Linnaeus, 1758) is one of the most commercially important marine fish species cultured in sea cages in the Mediterranean area (Antonelli et al., 2016). The largest producers of European seabass are Greece, Turkey, Italy, Spain, Croatia and Egypt with a global aquaculture production of 191,003 tonnes in 2016 (FAO, 2016). In addition, the European aquaculture production of seabass has reached its highest levels ever in 2016. Greece (43,000 tonnes) and Spain (23,000 tonnes) represent 80 % of the EU total production (EUMOFA, 2019). However, the high density of fish in aquaculture farms especially in sea cages, can facilitate the spread of infectious diseases such as parasitic diseases amongst both farmed and wild fish populations in the Mediterranean sea (Antonelli et al., 2012; Whittington et al., 2002).

Parasitic diseases, among other diseases (e.g. VNN etc.), are one of the major problems facing marine aquaculture today, especially at sea cages, and could lead to morbidities, mortalities and economic losses (Eissa et al., 2020). However, parasitic diseases may not cause direct mortalities, but could increase production costs through treatment or reduce the quality of the product (Nowak, 2007; Abou Zaid et al., 2018) and may also have significant impact on growth and behavior of fish (Antonelli et al., 2016).

Two of the most common parasites for European seabass are *Lernanthropus kroyeri* (Van Beneden, 1851) and *Diplectanum aequans* (Wagener, 1857) (Dezfuli et al., 2007; Şirin and Tokşen, 2014; Tokşen, 2010). *Diplectanum aequans* is a monogean parasite of the genus *Diplectanum*, being the largest genus with approximately 80 species in the family *Diplectanidae* (Hayward, 1997). The family *Diplectanidae* comprises of 20 genera and more than 250 described species parasitizing the gills of a wide range of marine species such the European seabass (Whittington and Chisholm, 2008; Abou Zaid et al., 2018). The disease caused by *D. aequans* is often referred as diplectanosis, and has been recognized as one of the most significant ectoparasitic diseases of European seabass culture (Dezfuli et al., 2007; Athanasopoulou et al., 2009; Ogut and Uzun, 2012). The mode of attachment of *D. aequans* on the gills of fish inflicts significant injury to the gill filaments, which may lead to secondary infections from other pathogens such a bacteria and viruses (Yardimci and Pekmezci, 2012; Eissa et al., 2017).

Lernanthropus, the third largest genus of Copepoda with more than 100 species, is the most widespread genus of the family *Lernanthropidae* (Yardimci and Pekmezci, 2012; Sethi et al., 2018;). Some *Lernanthropus* species are strictly host specific, but many parasitize several species of fish of one or several genera (Tokşen et al., 2010; Chu et al., 2012; Sethi et al., 2018) such as *L. kroyeri* which has been frequently observed in European seabass farms (Antonelli et al., 2012; Yardimci and Pekmezci, 2012). Furthermore, mass mortalities due to *Lernanthropus kroyeri* have also been reported in European seabass as a result of heavy infections leading to large scale economic losses (Sobhana, 2009).

The existence of additive genetic variance, in the form of heritability estimates, has been proved for different health related traits, such as disease resistance in aquaculture (Gjedrem, 2010). The designs that were used to confront and control fish diseases consist of improving nutrition, reducing the fish density, using vaccinations and commercial antimicrobials and antiparasitic substances; nevertheless, all are costly and do not have a permanent effect (Gjedrem, 2010; Houston, 2017). Another alternative to improve disease resistance is selective breeding and this was demonstrated in many farmed fish species (Gjedrem, 2015; Mahapatra et al., 2008; Ødegård et al., 2007). Several studies have reported significant estimates of heritability for disease resistance in aquaculture species, especially in salmonids. (Correa et al., 2017; Gjerde et al., 2011; Klemme et al., 2020; Robledo et al., 2018; Silverstein et al., 2008; Tsai et al., 2016; Yáñez et al., 2019).

In Atlantic salmon, the presence of significant genetic variation in resistance to sessile *Caligus rogercresseyi* (count on fins) has been documented, with heritability values ranging between 0.12 (Correa et al., 2017; José M. Yáñez et al., 2014b) and 0.32 (Lhorente et al., 2014), demonstrating the feasibility of genetic improvement of the trait. Similar heritability estimates (0.1 to 0.3) were reported for resistance to sea lice (*Lepeophtheirus salmonis*) (Gharbi et al., 2015; Houston et al., 2019; Kolstad et al., 2005; Òdegôrd et al., 2011; Tsai et al., 2016). Furthermore, several studies have shown the existence of genetic variation in a gill parasite (Amoebic gill disease - AGD), being one of the largest threats to salmon aquaculture, where the heritability estimates were ranging from 0.16 to 0.48 (Robledo et al., 2018; Taylor et al., 2009, 2007).

In European seabass, a moderate heritability of 0.26 was recently reported (Doan et al., 2017), suggesting that selective breeding is a potential tool for VNN (Viral Nervous Necrosis) prevention and control. VNN is a major threat for European seabass, where outbreaks of VNN can lead to a mortality of 80–100% at the larval stage (Breton et al., 1997; Doan et al., 2017; Munday et al., 2002). In fish survived VNN infection, the disease can become chronic leading to low growth rates (Faggion et al., 2021; Vendramin et al., 2014).

Effective selective breeding programs can offer cumulative and permanent improvement in host resistance (Bishop and Woolliams, 2010; Yáñez et al., 2014a). The aquaculture species and fish in particular, have the advantage of having large family sizes which allows for family-based selective breeding programs. Moreover, utilizing genetic selection for disease resistance can lead to over 10 % increase in survival per generation in some cases (Gjedrem, 2015). Therefore, in animal breeding aquaculture programs, apart from growth traits, a major challenge is to include disease resistance traits in order to produce improved stocks that would achieve some degree of lower disease or parasite prevalence (Gjedrem, 2010, 2012, 2015; Òdegôrd et al., 2011; Yáñez et al., 2014b; Houston, 2017). The objective of this study was to investigate the existence of additive genetic variance, using a polygenic pedigree-based model, in parasite resistance of the European seabass, and consequently to evaluate indirectly the feasibility to improve the parasitic resilience through selective breeding approaches. The main traits of commercial interest were resistance to parasites (as expressed by the number of parasites hosted in each fish) and growth in an environment being heavily infected by parasites (in natural cohabitation trials).

2. Materials and methods

2.1. Ethics statement

Both experimental trials were carried out in commercial rearing conditions. The minimum number of fish used was based on similar research studies (Thodesen et al., 2001). Appropriate anesthesia was applied before any fish handing (pit-tagging, fin-clipping, weighting and sampling) in order to minimize fish suffering. Parasite challenge was achieved through natural cohabitation at the designated sea cages farms. Final parasite counts were performed in dead fish.

2.2. Parasite infection trial

In total, 9245 European seabass (*D. labrax*) juveniles originating from the Nireus S.A. breeding program, comprising of totally (for both years) 182 full-sib and half-sib families (91 full-sib families per year of which 35 were maternal half-sib families), were individually pit-tagged and fin-clipped, divided into two groups (approximately 25 full-sibs from each family per group) and transferred to two sites selected for parasite cohabitation trials, in an environment heavily infested with the monogenean *D. aequans* (*D. aequans* site, in Nafpactos GR 07 FISH 003) and the copepod *L. kroyeri* (*L. kroyeri* site, in Sagiada GR 32 FISH 012). The trials were conducted twice in two consecutive years, and each challenge test had a duration of six months period. During the trial, weight recordings were taken every two months and parasites in each fish were counted at the end of each trial. Number of fish per site and

year are shown in Table 1.

Trials for parasite resistance were executed initially in the period from September 2017 to January 2018 (named "2017 trial"), and then in the period from August 2018 to December 2018 in next year (named "2018 trial"). Every year, different full-sib and half-sib families were created, 25 offspring from each family were individually pit tagged at 15 g and transferred to the selected open sea farming areas (*L. kroyeri*-Sagiada and *D. aequans*-Nafpactos), where heavy parasitism of the two parasite infested species (*D. aequans* and *L. kroyeri*) was previously confirmed. In parallel, 1000 offspring of the same year class, products of the same year's crossings (i.e. full- and half-sibs of the fish participating in the trials) were stocked in a fish cage next to the experimental one, in order to monitor the infection level through frequent (every 15 days) sampling and counting. After transfer to the sea, individual fish weight recordings were performed to fish participating in the experiment in two-month intervals. Final weight recording was performed after six months in sea cages, and all the fish were sacrificed at the end of the trials and parasite enumerations were made within a time span of 4–5 days in case of *L. kroyeri* and over a period of 15–20 days in the case of *D. aequans* by daily sampling. The parasite resistance trait (PC) was defined according to the counts of parasite found on each individual fish. Parasites were counted by experienced personnel being fish health experts' members of Nireus Pathology Department. Health experts counted the number of parasites on each gill arch on both sides of the fish, using microscopes in the case of *D. aequans* and stereoscopes in the case of *L. kroyeri*. No dual parasite contamination was observed and each site had a natural infestation from one parasite only. For *D. aequans*, a total of 1608 and 1197 fish were examined in years 2017 and 2018, respectively. For *L. kroyeri*, the first year's trial (2017 trial) was not utilized as was deemed unsuitable for parasite counting and contribution to data analysis, due to unexpected and unjustifiable high mortalities that affected the experimental results of the *L. kroyeri* cohabitation. In the next year (2018 trial), recordings were made in 1576 out of the 2425 fish initially introduced into the farm. Therefore, results for *L. kroyeri* presented herein refer only to the second year (2018). The recorded traits in the trials were: weight at tagging (**WAT**), weight at four months after placement in the cages (**W4mSea**), weight at six months after placement in the cages (**W6mSea**), growth at sea cages (**Growth at sea** = W6mSea – weight before transfer to sea cages) and parasite count (**PC**).

Finally, in order to obtain an estimate of the effect of fish growth performance in the trial populations and the effect of parasite infection on the selection decisions of the breeding program, offspring of the same families described above (i.e. full- and half-sibs of the fish from the 2017 and 2018 trials) were also farmed in a third site (control site in Palairos) where none of the two parasites under study was present. Therefore, this third site was used as a reference population or control site. The growth performance of the populations in the two trial sites for both parasites was compared to that of the population in the control site.

2.3. Data analysis

In order to compare the growth performance of populations of the two trial sites between them and with a third site without dominant presence of the parasites of interest, the average z-scores of average family growth were estimated and the population pedigree information from Nireus breeding program was used. The SPSS Statistics 25 software (https://www.ibm.com/analytics/spss-statistics-software) was used for descriptive statistics and correlations between sites.

Parasite counts (PC) is a discrete-categorical variable with a large number of categories that approximates the normal distribution. However, it seems that in our data set the distribution was expressing some skewness (i.e. 3.18). As in most cases of genetic analysis of parasite counts, data transformation was utilized (Bishop et al., 1996) and several transformations, e.g. $\ln(PC + 1)$, $\ln(PC + 25)$, $\sqrt[3]{x}$, $\sqrt{x}$, x^2, $1/x^2$ and Box Cox for the PC values, were tested in order to remove the skewness and to better approximate the normal distribution, so as to have more unbiased results in the estimation of the genetic parameters. As in other parasite count genetic parameter studies (Vagenas et al., 2007), the best transformation for our data was the ln transformation [i.e. $\ln(PC + 1)$]. In all the genetic analyses both the original PC values and the transformed ones were used.

Estimation of variance and covariance components was carried out for all the recorded traits in the trials using restricted maximum likelihood methodology, through the VCE 6.0 software (Groeneveld, 2010). The genetic parameters were estimated using a multi-trait animal model:

$$y = \mu + Xb + Za + e$$

where μ is the mean for each trait, y is the vector of phenotypic records, b is the vector of fixed effects, a is the vector of random animal genetic effects, e is the vector of random residual errors and X, Z are the design matrices relating fixed and random effects, respectively, to observations. The year was used as fixed effect in the analysis of *D. aequans* but not in the *L. kroyeri* analysis, since for the latter parasite data from only one year were analyzed (2018 trial) to avoid biases due to the high mortality rate in the first-year trial period (2017 trial). Furthermore, days in sea cages were fitted as a covariate in parasite count in both trials, since all fish from both trials could not be harvested on the same day and those that have stayed more days in the sea cages might had more parasites due to longer exposure time.

3. Results

3.1. Descriptive statistics of D. aequans and L. kroyeri infection trial

Kroyeri infection trial

For years 2017 and 2018, 1608 and 1197 fish were examined

Table 1
Descriptive statistics for parasite cohabitation trials (standard deviations in parentheses).

	Initial No	Final No	WAT(g)	W4mSea(g)	W6mSea(g)	Growth at sea (g)	Parasite count	Survival (%)
	D. aequans							
2017	2228	1608	**17.5** (4.1)	**90.0** (24.0)	**126.0** (44.2)	**108.0** (42.2)	**26** (32.5)	**72**
2018	2297	1197	**12.7** (2.4)	**58.2** (12.1)	**214.6** (53.9)	**185.0** (52.5)	**47** (41.6)	**52**
	L. kroyeri							
2017*	2222	272	**17.0 (4.2)**	–	–	–	–	**13**
2018	2425	1576	**19.0** (4.4)	**53.1** (10.9)	**174.3** (38.6)	**155.2** (36.9)	**24.4** (11.7)	**64**

WAT = weight at tagging.
W4mSea = weight at four months at sea.
W6mSea = weight at six months at sea.
* The trial was aborted due to high mortality rate at the early stages of the trial.

respectively, for the presence of *D. aequans*. In the case of *L. kroyeri* the first-year trial data (2017 trial at *L. kroyeri*site) could not be used for evaluation due to the fact that this population suffered unexpected and unjustifiable high mortalities (87 %), so it was decided not to count parasites on fish from this trial. In second-year trial (2018), recordings were taken to 1576 out of 2425 fish initially introduced in sea cages.

For *D. aequans* the average number of parasites at family level was 19, having a minimum of three and maximum of 45 parasites for 2017 trial, and 26 for the 2018 trial, with a minimum of 6 and a maximum of 59 parasites. Though, the average number of parasites per fish was 26 and 47 for trial years 2017 and 2018, respectively. The average number of parasites per fish for *L. kroyeri* was 24 with a maximum of 84 parasites, although fish without any parasites were also recorded. The minimum and maximum values for average number of parasites per family were 12 and 44, respectively. Table 1 shows the descriptive statistics for parasite counts, growth traits and mortality with their standard deviations. In addition, distribution of family parasite count per year for all trials can be found in the bar plots of Supplemental Fig. 1.

3.2. Genetic parameters estimation - D. aequans trial

Heritability of parasite count for *D. aequans* was medium ($h^2 = 0.2$) and the parasite count does not seem to affect bodyweight (phenotypic correlations -0.03 to 0.05), but the genetic potential of bodyweight and growth seems to be slightly related with high parasites count (genetic correlations 0.2 to 0.28 with bodyweight at different ages and 0.37 with growth at sea) (Table 2).

Regarding transformed data, the heritability of parasite count was 0.29 (Table 3), which is higher compared to that in the untransformed data (0.20). The phenotypic correlations with body weight were ranging between 0.1−0.13 (Table 3) and were higher than the respective ones in the untransformed data (-0.03−0.05, Table 2), while the genetic correlations were ranging between 0.17−0.29 (Table 3) being lower than those in the untransformed data (0.2−0.37, Table 2).

Moreover, the heritability estimates (h^2) of bodyweight and growth traits for *D. aequans* (weight at tagging: 0.51, weight after four months in sea cages: 0.43, weight after six months in sea cages: 0.42 and growth at sea cages: 0.43, Table 2) are within the range observed in the breeding program and other studies. ThePhenotypic and genetic correlations between bodyweight at different ages were high (0.72−0.82 and 0.88−0.95, respectively) (Table 2). However, both phenotypic and genetic correlations were lower when bodyweight measurements were associated with the growth at cages (growth at sea: 0.37−0.72 and 0.6−0.85, respectively).

Table 2
Heritability estimates of traits for *Diplectanum aequans* (on diagonal with bold font), genetic correlation (above diagonal) and phenotypic correlation (below diagonal) between the traits.

	WAT	W4mSea	W6mSea	Parasites count	Growth at sea
WAT	**0.51 (0.04)**	0.95 (0.01)	0.88 (0.01)	0.20 (0.06)	0.60 (0.03)
W4mSea	0.80 (0.05)	**0.43 (0.01)**	0.94 (0.01)	0.26 (0.05)	0.66 (0.03)
W6mSea	0.72 (0.04)	0.82 (0.00)	**0.42 (0.01)**	0.28 (0.05)	0.85 (0.02)
Parasites count	0.05 (0.05)	0.01 (0.01)	−0.03 (0.01)	**0.20 (0.01)**	0.37 (0.06)
Growth at sea	0.37 (0.04)	0.54 (0.01)	0.72 (0.01)	0.00 (0.01)	**0.43 (0.01)**

Table 3
Heritability (on diagonal with bold font), phenotypic (below diagonal) and genetic correlations (above diagonal) for log transformation parasite count PC (ln+1) (*D. aequans*), bodyweight at different ages and absolute growth in sea cages. Standard error of the estimates is given in parentheses.

	WAT	W4mSea	W6mSea	Parasites count	Growth at sea
WAT	**0.51 (0.00)**	0.95 (0.01)	0.88 (0.02)	0.17 (0.06)	0.60 (0.05)
W4mSea	0.87 (0.01)	**0.43 (0.02)**	0.94 (0.01)	0.23 (0.06)	0.70 (0.04)
W6mSea	0.79 (0.01)	0.87 (0.01)	**0.42 (0.02)**	0.23 (0.05)	0.85 (0.02)
Parasites count	0.11 (0.02)	0.11 (0.02)	0.10 (0.02)	**0.29 (0.07)**	0.29 (0.05)
Growth at sea	0.48 (0.03)	0.59 (0.02)	0.77 (0.01)	0.13 (0.03)	**0.44 (0.00)**

3.3. Genetic parameters estimation - L. kroyeri trial Kroyeri trial

For the copepod *L. kroyeri,* heritability of parasite count was estimated at 0.28 and 0.26 for untransformed and transformed data, respectively, which might be considered as a quite high estimate for a disease resistance trait. For both data, heritability estimates of bodyweight and growth at sea cages were very similar (Tables 4 and 5). The

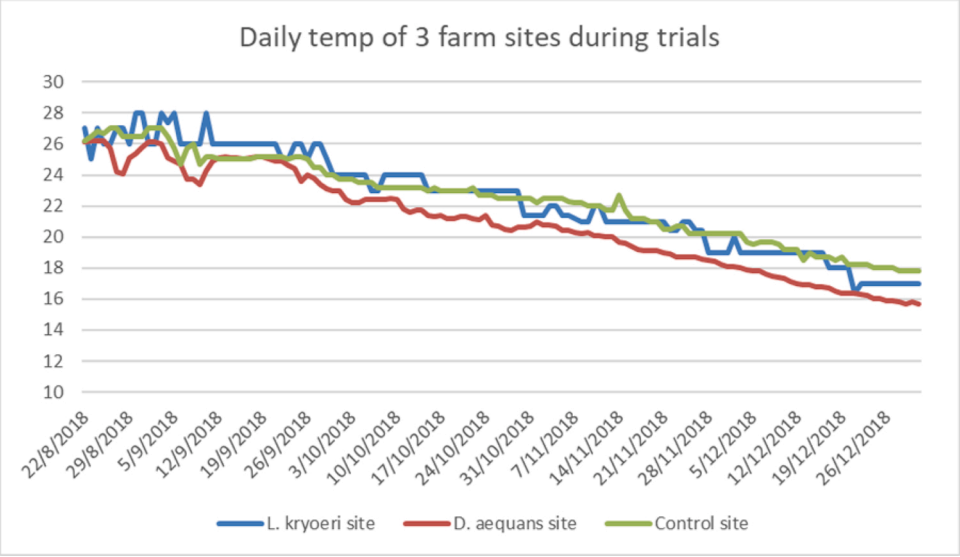

Fig. 1. Temperature profiles in three different sites for the trial period.

Table 4
Heritability estimates of traits for *L. kroyeri* (on diagonal with bold font), genetic correlation (above diagonal) and phenotypic correlation (below diagonal) between the traits.

	WAT	W4mSea	W6mSea	Parasites count	Growth at sea
WAT	**0.51**	0.80	0.67	0.40	0.47
	(0.05)	(0.04)	(0.06)	(0.11)	(0.09)
W4mSea	0.49	**0.28**	0.93	0.28	0.78
	(0.03)	**(0.04)**	(0.02)	(0.11)	(0.05)
W6mSea	0.41	0.77	**0.34**	0.22	0.94
	(0.03)	(0.01)	**(0.05)**	(0.11)	(0.01)
Parasites	0.32	0.19	0.14	**0.28**	0.09
count	(0.03)	(0.03)	(0.03)	**(0.03)**	(0.11)
Growth at sea	0.25	0.63	0.84	0.05	**0.29**
	(0.04)	(0.02)	(0.01)	(0.03)	**(0.04)**

Table 5
Heritability (on diagonal with bold font), phenotypic (below diagonal) and genetic correlations (above diagonal) for log transformation parasite count PC (ln+1) (L. *kroyeri*), bodyweight at different ages and absolute growth in sea cages. Standard error of the estimates is given in parentheses.

	WAT	W4mSea	W6mSea	Parasites count	Growth at sea
WAT	**0.51**	0.80	0.67	0.42	0.47
	(0.05)	(0.05)	(0.07)	(0.06)	(0.09)
W4mSea	0.60	**0.28**	0.93	0.30	0.78
	(0.03)	**(0.04)**	(0.02)	(0.10)	(0.05)
W6mSea	0.51	0.82	**0.34**	0.24	0.94
	(0.04)	(0.03)	**(0.05)**	(0.11)	(0.01)
Parasites	0.32	0.20	0.16	**0.26**	0.12
count	(0.03)	(0.03)	(0.03)	**(0.03)**	(0.11)
Growth at sea	0.33	0.67	0.87	0.06	**0.29**
	(0.04)	(0.02)	(0.03)	(0.03)	**(0.40)**

phenotypic correlations between parasite count and bodyweight at different ages were also similar for untransformed and transformed data (0.14 to 0.32 and 0.16 to 0.32, respectively). Finally, the genetic correlation estimates showed that the parasite count affects low to moderately the genetic potential of bodyweight (at different ages) and growth at sea cages in both transformed and untransformed data (0.09 to 0.4 and 0.12 to 0.42, respectively) (Tables 4 and 5).

3.4. Growth comparison between sites

In *D. aequans* site the average weight of fish population for the experimental period of 2018 was 185 g and in *L. kroyeri* site it was 155 g, although the average temperature differs by 1.5 °C (higher in *L. kroyeri* site compared to *D. aequans* site). As mentioned previously, offspring of the same families were placed in cages in a third reference/control site (Palairos) where the two parasites of interest were not present, so as to study the possible effect of parasites on fish growth. The average growth of full-sibs farmed in the control site was 260 g. The possible impact of parasitism in fish growth is evident due to the different values of thermal growth coefficients (TGC) existing in the three different areas (Fig. 1). Growth performance in the three different sites is presented in Table 6.

Ranking of families from best to worst was based on the average z-score of offspring growth. Comparing the ranking of families in the three different sites can provide an indication about the severity of the effect of parasitism in the selection decision process and, more specifically, in the characterization of an individual as "preferable" or "non-preferable" selection candidate. This is because every year many half-sib families are produced based on a breeding design (originating from the same female or male), and that provides the ability to check the ranking of each breeder based on the performance of its offspring. The growth at sea and z-scores of offspring for each female breeder are provided in Fig. 2. It can be observed that in some families (e.g. third family) the mean z-score

Table 6
Measurements of average weights (standard errors in parentheses) in different time-periods at the three sites.

	Control (Ref) Av. weight (g)	*D. aequans* (*Da*) Av. weight (g)	*L. kroyeri* (*Lk*) Av. weight (g)
June	45 (0.19)		
July	67 (0.26)	57 (0.27)	53 (0.25)
August	112 (0.47)		
September	160 (0.81)	130 (0.62)	116 (0.59)
October	221 (1.24)		
November	266 (1.85)	214 (1.52)	174 (1.01)
December	307 (2.04)	242 (1.69)	208 (1.19)
Day degrees	4415	4176	4410
TGC	16.69	13.49	9.75

TGC stands for Thermal Growth Coefficient (Iwama and Tautz, 1981).

values for growth at sea were the same in the control site and *D. aequans* site and a slightly higher within-family variation was expressed in the control site. However, the same family in the *L. kryoeri* site had lower z-score and expressed much higher within-family variation than the control site. It can be seen that the growth potential both between- and within-family was affected, and was affected more in the *L. Kryoeri* trial rather than in the *D. aequans* trial.

In addition, Pearson's correlation coefficients for growth performance between offspring from families in the control and parasite sites were estimated. A medium to high positive correlation was observed between full-sib families, with a highest value of 0.73 between *D. aequans* and control sites. In the case of maternal half-sib families, a medium to high positive correlation was also observed, having a highest value of 0.78 between *D. aequans* and control sites. Ranking correlations among maternal half-sib families were, in average, higher compared to those recorded for full-sib families. Correlations of growth and z-scores are presented in Table 7.

4. Discussion

Parasite infections are considered to be one of the major health risks of Mediterranean fish farming in the years to come. Our results showed a mortality rate between 28 % (*D. aequans*) and 87 % (*L. kryoeri*) (Table 1), which is significantly higher than the mortality rate of the same years in the control site (11–15 %). The high mortality (87 %) in the 2017 cohabitation trial with *L. kryoeri* led us not to utilize this year's data in our analysis. Moreover, there are various reports from the aquaculture industry for increasing losses from parasitic diseases and increasing costs from the use of anti-parasitic compounds in the Mediterranean aquaculture (L. Papaharisis, Avramar S.A., personal communication). Parasites show a great variability regarding their life cycle, host specificity strategies and their impact on host and, accordingly, require different defense strategies. The opportunities to minimize the parasite impacts through non–invasive strategies, such as selective breeding, should be examined thoroughly. To our knowledge, this is the first effort to identify the existence of additive genetic variation in parasite resistance traits, using a polygenic model, in order to promote its utilization in selective breeding programs of a Mediterranean aquaculture fish species.

The heritability estimates obtained before and after transformation for parasite count in *D. aequans* were 0.2 and 0.29 and for *L.kroyeri* 0.28 and 0.26, respectively. In another ectoparasite of intensively farmed aquaculture species (i.e. Atlantic Salmon), higher heritability estimates were reported by Lhorente et al. (2012) for parasite (*Caligus rogercresseyi*) load (0.34 for total number of sessile lice per fish) and by Gjerde et al. (2011) for parasite count (0.35) and parasite density (0.3) of another lice parasite (*Lepeophtheirus salmonis*). Sessile louse are copepod (as *L.kroyeri*) ectoparasites that parasitize in epidermal tissue (but can be also found on gills especially the *Lepeophtheirus salmonis*) of various species of salmonids creating serious problems both on the production

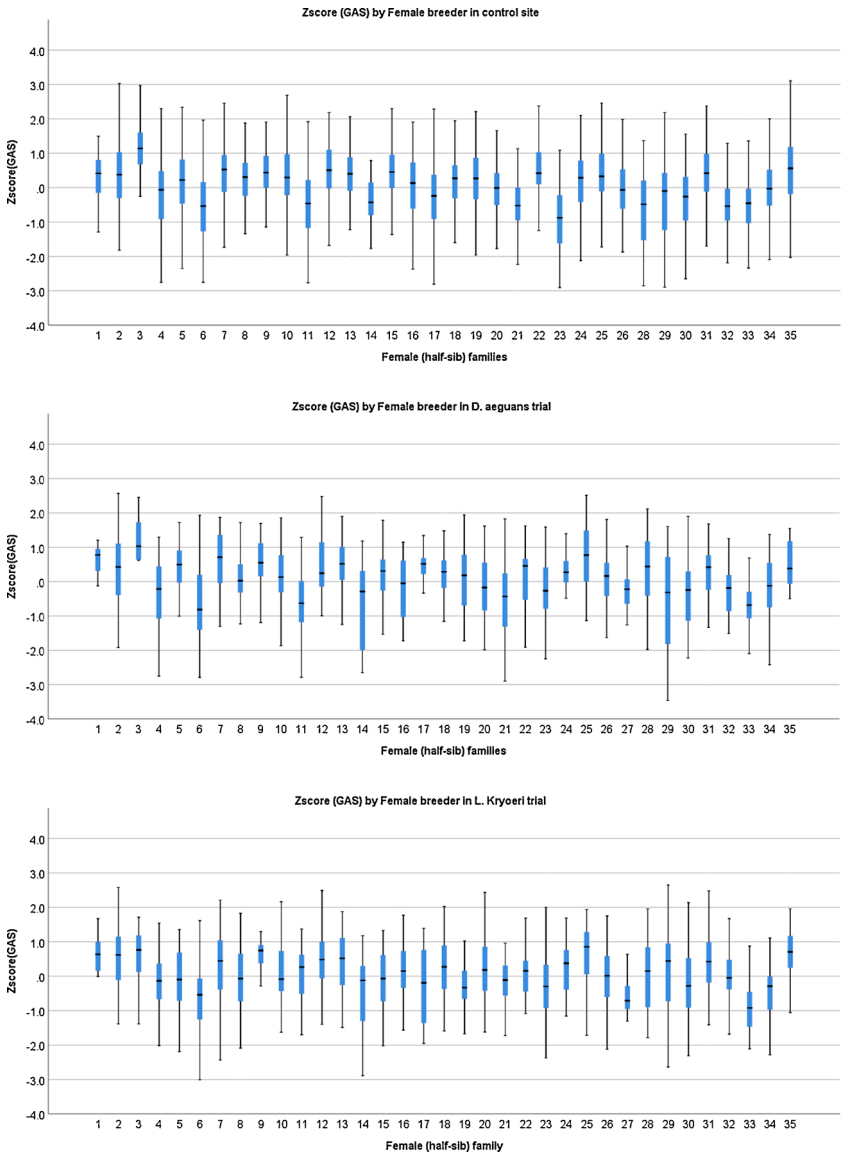

Fig. 2. Box plots of growth at sea z-scores per female in three different sites.

Table 7
Pearson correlations between growth (growth at sea) z-scores of European seabass families and maternal full- and half-sibs (females). Above the diagonal: families' correlations regarding growth during infestation, below the diagonal: females' correlation regarding growth during infestation.

	Control (Ref)	*L. kroyeri*	*D.aequans* (*Da*)
Control(Ref)		.53**	.73**
***L. kroyeri*(*Lk*)**	.68**		.61**
***D.aequans* (*Da*)**	.78**	.74**	

*significantly different from zero ($p < 0.05$).
**significantly different from zero ($p < 0.01$).

cycle of aquaculture farms (Brooker et al., 2018; Tully and Nolan, 2002).

The same heritability as in *L.kroyeri* untransformed parasite load was estimated for a fresh water tropical fish, the tambaqui fish (*Colossomama cropomum*), concerning the *Ichthyophthirius multifiliis* parasite, though, with very high standard error (0.28 ± 0.18) (Lira et al., 2020). *Ichthyophthirius multifiliis* is an endoparasite that attaches underneath the skin and the gill's epithelium of the fish and can lead to very large mortality rates (Matthews, 2005).

Amoebic gill disease (AGD) is considered to be a disease which significantly affects marine aquaculture, especially salmonids pecies, where *Neoparamoeba perurans* parasite attaches to gill lamellae eliciting focal necrosis, oedema, inflammation and hyperplasia of the gill epithelium (English et al., 2019; Slinger et al., 2021). Heritability estimates for this parasite were 0.25 for mean gill score and 0.36 for parasite load in Atlantic salmon (Robledo et al., 2018). Similar heritability estimates were found for AGD in a Tasmanian Atlantic salmon population, being 0.16 for gross gill score and 0.35 for digital image gill score (Taylor et al., 2007), whereas higher heritability estimates were reported in Taylor et al. (2009), ranging from 0.23 to 0.48 for mean gill score and depending on the number of rounds of re-infection. The highest heritability estimate (0.48) of the previous study corresponded to the third challenge trial after two rounds of infection.

In marine sites with profound infestation by the monogenean *D. aequans*, a very large variation in the number of parasites per fish (parasite count) is recorded, similar with that of another gill parasite the *Neoparamoeba perurans* in Atlantic Salmon. Unfortunately, we cannot conclude about the impact of monogeneans on fish survival as the collection of dead fish and verification of cause of death was not possible, although a profound impact on fish growth is recorded (Tables 6 and 7). Nevertheless, heritability of parasite resistance (for both parasites and both untransformed and transformed data) is within the

same range (Tables 2 to 4), if not higher in some cases, with heritabilities recorded for other disease resistance trials (Kolstad et al., 2005; Gjerde et al., 2011; Òdegôrd et al., 2011; Kube et al., 2012; Lhorente et al., 2012; Yáñez et al., 2014a; Doan et al., 2017; Ariede et al., 2020; Lira et al., 2020), providing enough space for improvement under a structured selective breeding program.

The copepod parasite *L. kroyeri* presents a significant risk for the Mediterranean aquaculture due to its impact on fish growth at farm level (Table 6) and the reduced acceptability of heavily parasitized fish by the market. In addition, available treatment options for seabass copepod parasites are limited and there could also be arising issues from possible development of resistance to treatment from these parasites, if such treatments are used on a regular basis (as in the case of salmonid fish species). Hence, alternative parasite management measures are invariably valuable and desired. The results herein provide a very promising opportunity to increase resistance to *L. kroyeri* through selective breeding. A high heritability estimate was recorded in the current trial (0.28) with not very unfavorable genetic correlations with growth traits (0.09 to 0.28, 0.4 was observed only with weight at tagging) (Tables 4 and 5). However, these heritability estimates were lower than those of other copepod parasites [i.e. sea louse (*Caligus rogercresseyi* and *Lepeophtheirus salmonis*)] in Atlantic Salmon (Lhorente et al., 2012; Gjerde et al., 2011), therefore, the results of this study have to be verified further by the use of a bigger data set.

Since the fish that have participated in the two trial sites (*D. aequans* and *L. kroyeri)* were full sibs, it was possible to estimate the genetic and phenotypic correlations for resistance to both parasitic diseases in a bivariate analysis. The genetic correlation was found to be 0.33 while the phenotypic one was lower (0.14). Based on the results, it seems that the parasite count of the two studied parasite species is not highly correlated. Therefore, selection for resistance to one parasite species does not necessarily mean that it will largely improve resistance to the other parasite species.

Data transformation of parasite count did not have any large impact on the genetic parameters estimated in our study. On the contrary, logarithmic transformation [ln(PC + 1)] of parasite count can inflate substantially, as in the case of *D. aequans*, the heritability estimate (but not the covariances with the other traits) and since Log transformations could sometimes lead to impossible predictions, it was decided, as O'Hara and Kotze (2010) suggested, that a model based on counts is more sensible, as it is easier to interpret and avoids the problems of deciding which transformation to use. Various methods (random regression models, MCMC utilizing Poisson or negative binomial models etc.) have been suggested as more appropriate for the genetic analysis of counts and repeated measurements of counts (Mair et al., 2015). However, in farm animal studies, no significant differences have been observed in the estimated genetic parameters, e.g. regarding the parasite faecal count in sheep, and the estimated breeding values between models, utilizing raw and transformed data, were highly correlated.

Nevertheless, Parasite Count in non-Mediterranean species (i.e. salmon) seems to be in general a more heritable trait (0.28 to 0.48) (Taylor et al., 2009; Gjerde et al., 2011; Kube et al., 2012; Lhorente et al., 2012; Yáñez et al., 2014a; Tsai et al., 2016; Bois et al., 2019). Furthermore, the heritability estimates of bodyweight in different ages were 0.51, 0.43 & 0.42 for *D.Aequans* and 0.51, 0.28 & 0.34 for *L. kroyeri* (WAT, W4mSea and W6mSea, respectively) (Tables 2 and 4), whereas the heritability value for bodyweight (4–7 days after infestation with *Caligus rogercresseyi*) in Atlantic Salmon was 0.58 (Lhorente et al., 2012). The bodyweight heritability, as that of the parasite count, seems to be lower in our experiment compared to the respective one in salmon.

Moreover, in terms of production traits (e.g. bodyweight, growth) the current study indicates zero to low phenotypic correlation between weight at different ages (including growth at sea) and parasite count in both trial sites. However, the genetic correlations of growth at sea and parasite count were estimated to be low (0.09) to medium (0.37) in *L. kroyeri* and *D. aequans,* respectively, indicating that in the case of *D. aequans* selection for parasite resistance might possibly have some negative impact in selective breeding for growth. Although phenotypic correlations between bodyweight at different age and growth, after placement in the sea cages, is very low (near zero in many cases), the genetic correlations are medium to low. Such result may be an indication that larger fish or fish that grow faster may have more parasites attached to their gill just due to their bigger size. However, when the bodyweight, at the parasite counting, was used as a covariate, no significant differences were observed in the genetic parameters estimates (i.e. heritabilities, phenotypic and genetic correlations). Any small differences observed were at the third decimal point (data not shown). Similarly, when the bodyweight, before placement to the cages, was fitted as a covariate in the parasite count, the heritability and phenotypic correlation estimates remained of the same magnitude, however, the genetic correlations were minimized (-0.08 – 0.13 for bodyweight and -0.03−0.2 for growth at sea cages). Such result may verify the theory that larger fish have more parasites attached to their gills due to their size. However, the use of bodyweight, at a different stage than the one taken when the parasite are counted, as a covariate in a genetic evaluation model should be done with caution since there is no certainty if these genetic correlations are biased from the fact that these measurements are taken at a very different age and with more than six months difference in time. Our study was a natural cohabitation trial, therefore if the size of the fish has an effect on the number of parasites attached to their gills it is always possible that faster growers, even those that might have low starting weight (i.e. before the placement to the sea cages), will have more parasites to their gills due to their increased size in comparison to slow growers, independently of their starting weight. Furthermore, the low phenotypic correlations of parasite count in the *D. aequans* trial with bodyweight support such a scenario. Nevertheless, a selection verification experiment could shed some light concerning the most suitable model for future genetic evaluation of parasite resistance in European seabass.

Growth comparison between not infected and infected sites (by different dominant parasites) gives useful insights for parasites impacts and strategies to be followed in selective breeding programs. In Table 6, the Pearson correlation between the growth z-scores of the families in different sites give as an indication of the average magnitude of such impact and in Fig. 2 the z-scores for each family and their within family variation individually can be visualized and observe which families were more affected. Nevertheless, comparing the ranking of families in the three different sites (Table 7) can provide an indication about the severity of the effect of parasitism in the selection decision process and, more specifically, in the characterization of an individual as preferable (selected candidate) or non-preferable (non-selected candidate). High correlation between family evaluation among different sites for both female breeders (i.e. maternal half sib families) and full-sib families, are in position to provide a certainty that the effectiveness of selection process will not be significantly disrupted by the abundance of the two specific parasites and the selected offspring will continue to grow faster, compared to their unselected counterparts, even under these special environmental conditions. Furthermore, due to the low to medium correlations observed (Tables 2–5), the inclusion of parasite resistance as a selection objective will not exclude fish with great growth potential and will not delay improvement in growth rates of selected fish. Taking into account this scenario, when Estimated Breeding Values (EBVs) for parasite count (*L. kroyeri*) and growth traits were estimated concurrently, it was evident that simultaneous selection for low parasite count and high growth in a selection index would only very slightly impair selection on growth (data not shown).

Nevertheless, the results of this study look quite promising in terms of the existing potential for genetic improvement for disease resistance traits when it is compared to farm animals, and gives a good basis for further genetic analysis using molecular markers (i.e. Single Nucleotide Polymorphisms-SNPs), in order to identify genomic regions affecting resistance to infection from parasites.

5. Conclusion

Infections from both parasites under study exhibit substantial genetic variation regarding the number of parasites on the fish ($h^2 = 0.2-0.28$, Tables 2 and 4). However, infection from *L. kroyeri* could lead to more substantial production loss and, in addition, is expressing slightly higher genetic variation ($h^2 = 0.28$). Unfortunately, parasite count is a very laborious measurement that can only be taken after death and consequently any selection method applied have to concentrated on sib or progeny testing, something that would affect negatively, respectively, the accuracy and the generation interval of the selection and therefore delay the response to selection. Furthermore, due to the above technical and commercial reasons, such as the lack of treatment methods and the reduced acceptability by the markets, it was decided to further explore the genetic resistance to infection from this specific parasite using state-of-the-art genomic tools (research project in progress), such as the recently available 25 K SNP-array for the European seabass (Med_FISH array). Genomic Selection (GS) approaches are nowadays in position to simultaneously incorporate dense SNP marker genotypes with phenotypic data from related animals to predict animal specific Genomic Estimated Breeding Value (GEBV), which circumvents the need to measure directly the disease phenotype in potentially valuable breeders, with a higher precision than that obtained using sib-testing with pedigree information.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aqrep.2021.100767.

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