

THE IMPORTANCE OF ENSURING GENETIC VARIABILITY WHEN ESTABLISHING SELECTION PROGRAMMES IN AQUACULTURE

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INTRODUCTION

The great potential of aquaculture genetic improvement programmes is unquestionable given the high fecundity of fish species that allows intense selection and therefore fast genetic gains. Following the clear success of Atlantic salmon programmes, that have led to genetic progresses comparable to those in livestock species (Gjedrem and Rye, 2016), numerous selective breeding programmes have been started in the last decades for different fish species. In fact, 80% of the current aquaculture production in Europe originates from improved stocks (Janssen et al., 2016).

The success of any breeding programme critically depends on the way in which the base population of breeders is built, as the genetic variability initially available will determine the genetic progress achieved in the subsequent selection programme (Fernández et al., 2014). This is particularly important in aquaculture, as base populations can be created from very few individuals given that thousands of offspring from a single mating pair can be obtained. This would lead to small effective population sizes (N_e) and therefore to large rates of loss in genetic variability.

Estimates of N_e in commercial aquaculture populations are very scarce. The objective of the present study was to estimate N_e in commercial populations of three of the most important species in European aquaculture, including turbot, gilthead seabream and common carp. Estimates of N_e were obtained from genomic information and were used to evaluate the genetic status of the populations and to detect bottlenecks that occurred in the past.

MATERIAL AND METHODS

Fish used in this study come from different experiments carried out in turbot, gilthead seabream and common carp under the European project FISHBOOST (<http://www.fishboost.eu/>). Data analysed came from broodstock sampled from different commercial European breeding programmes for these species. Turbot data were obtained from the broodstock maintained at CETGA (Aquaculture Cluster of Galicia, Spain), a population that is representative of the main European breeding programmes that have the same Atlantic origin. Turbot programmes practice artificial fertilization and perform single-pair matings. Seabream data came from the breeding programme of the Andromeda Group (Greece) where mass spawning is performed. Carp data came from broodstock of the Amur Mirror Carp strain, recently created at the University of South Bohemia (Czech Republic) from crosses between two different strains.

RAD-sequencing was used to obtain genotypes for Single Nucleotide Polymorphisms (SNPs) for each population. Information on the number of samples and SNPs used for N_e estimation after quality control is detailed in Table 1.

Linkage disequilibrium (LD) between SNP pairs (r_{Δ}) was estimated using the Burrows' Composite Method (Weir, 1979) adjusted by sample size (S) and standardized by the allele frequencies. Current N_e was estimated from unlinked SNPs (recombination rate $c = 0.5$), using Waples' method (Waples, 2006; Do et al., 2014):

$$\hat{N}_e = \frac{1}{3(\hat{r}_\Delta^2 - 1/S)}$$

Estimates of N_e from LD in the last 15 generations were obtained using a modification of the initial formula of Hill (1981) that reinterprets the relationship between time and genetic distances (Caballero and Santiago, unpublished). The fundamental difference of this method resides in the idea that the demographic history of a population persists until the present, affecting in this way LD patterns. This implies that the genetic distance between a SNP pair does not reflect the N_e of a specific generation, but all genetic distances between all SNP pairs are taken into account to estimate N_e in each generation.

Table 1. Number of broodstock and SNPs (n_{SNP}) analysed, physical and genetic genome length (L , in Mb/cM), SNP density (d , in number of SNPs/Mb) and current N_e estimate for each population.

Population	No. broodstock		n_{SNP}	L	d	Current N_e
	Males	Females				
Turbot	23	23	18,125	524 / 1,343	35	28 (23, 35)
Seabream	57	60	21,754	~786 / 1,406	28	40 (30, 55)
Carp	40	20	12,311	1,830 / 3,944	7	22 (19, 27)

RESULTS AND DISCUSSION

Our results revealed that current N_e for all populations analysed (Table 1) was lower than the critical value of 50 individuals recommended to avoid inbreeding depression and retain fitness in the short-term (Frankham et al., 2002). These low N_e estimates could be due to the way in which base populations were established (low number of breeding individuals and/or strong relationships between them) or to a suboptimal inbreeding control. In the particular case of turbot, current N_e could be underestimated as the material used included fish from different breeding programmes.

Estimates of N_e across time (Figure 1) evidenced bottlenecks 4 – 11 generations ago. This point in time coincides with the number of generations for which selection programmes of turbot and seabream have been practised (Janssen et al., 2016). The recent creation of the carp strain analysed also coincides with the observed bottleneck.

In general, the magnitude of our estimates for recent N_e agrees with that found in commercial fish populations. For gilthead seabream, estimates ranged from 14 to 18 between photoperiod-controlled broodstock groups (Brown et al., 2005). The main reason for these low estimates was the practice of mass spawning as this mating system led to a high variance in family size and to an unequal number of breeding males and females (fewer males than females contributed to each spawning). For coho salmon, estimates lower than 50 ($N_e = 20, 46$) have been also obtained for a population selected for four generations for increasing harvest body weight (Gallardo et al., 2004). For a breeding nucleus of the same species, estimates of 34 – 39 fish were obtained (Yáñez et al., 2014). Estimates lower than 50 were also obtained by Su et al. (1996) and Pante et al. (2001) for rainbow trout. An exception has been the GIFT (Genetically Improved Farmed Tilapia) selection programme where the creation of the base population was carefully planned and N_e after seven generations of selection for growth rate was 88 (Ponzoni et al., 2010).

Our estimates of ancestral N_e suggest that important bottlenecks occurred when selective breeding programmes started probably due to a reduced number of breeders constituting the base populations. This agrees with a recent study by Prado et al. (2018) who indicate that current N_e for turbot wild populations of Atlantic origin is very high ($N_e > 1,000$).

In summary, our results highlight the need of broadening the genetic composition of base populations from which selection programmes start. The results also suggest that methods

designed to increase N_e within all farm populations analysed here need to be implemented in order to manage genetic variability and ensure the sustainability of the breeding programmes. Failure to do that could lead in the future to the necessity of replacing some broodstock with wild stock with the consequent loss of the genetic progress achieved to date. Increasing the number of parents selected and equalizing their contributions is needed. Thus, practicing artificial fertilization and single-pair matings should be considered in those schemes where mass spawning is currently employed. For schemes already performing single-pair matings, implementing Optimal Contribution Selection (Meuwissen, 1997; Grundy et al., 1998), that maximizes genetic gain while restricting the rate of inbreeding (and therefore controlling N_e), and factorial mating designs would be recommendable practices. Where these interventions are not enough for increasing N_e above critical values, another option could be to interchange genetic material coming from different genetically improved stocks.

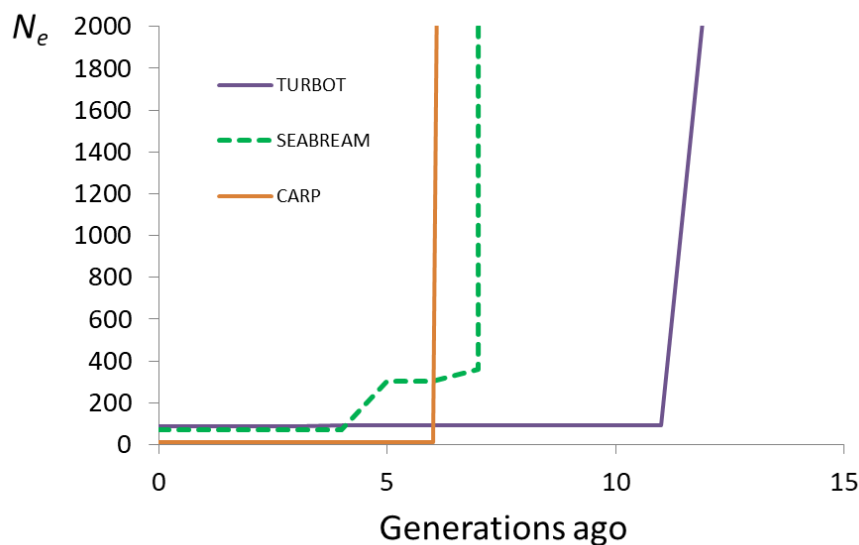


Figure 1. Estimates of effective population size (N_e) across the last 15 generations for the different commercial populations analysed.

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ABSTRACT: The success of any breeding programme critically depends on the way in which the base population of breeders is built. This is particularly important in aquaculture, as given the high fecundity of fish species, base populations can be created from very few individuals. This would lead to small effective population sizes (N_e) and large rates of loss in genetic variability. In this study, we used RAD-sequencing data to estimate N_e from linkage disequilibrium in commercial populations of three of the most important species in European aquaculture (turbot, seabream and carp). Our results revealed that current N_e estimates were lower than the critical value of 50 individuals recommended to ensure short-term sustainability of the breeding programmes. These estimates agree with those found in other commercial fish populations. In addition, we detected important bottlenecks at the time when selection programmes started, highlighting the need of broadening the genetic composition of base populations from which aquaculture selection programmes start.

Keywords: aquaculture, base population, effective population size, linkage disequilibrium