

REGULAR PAPER

Stronger effects of heterozygosity on survival in harsher environments

Simone Vincenzi^{1,2}  | Dušan Jesenšek³ | John C. Garza^{1,2} | Alain J. Crivelli⁴

¹Department of Ocean Sciences, University of California, Santa Cruz, California, USA

²Fisheries Ecology Division, Southwest Fisheries Science Center, National Marine Fisheries Service, Santa Cruz, California, USA

³Tolmin Angling Association, Most na Soci, Slovenia

⁴Station Biologique de la Tour du Valat, Arles, France

Correspondence

Simone Vincenzi, Department of Ocean Sciences, University of California, Santa Cruz, 110 Shaffer Road, Santa Cruz, CA 95060. Email: simon.vincenz@gmail.com

The hypothesis that the effects of heterozygosity vary with environmental conditions was tested using six populations of marble trout *Salmo marmoratus* from western Slovenia as a model system. The hypotheses tested were: stronger effects of heterozygosity on survival in populations characterized by low average survival; no effects of heterozygosity on probability of surviving flash floods owing to their largely non-selective effects across traits; stronger effects of heterozygosity on survival for fish born after floods than fish born before. A significant effect of heterozygosity on survival was found in populations characterized by low average survival. There were no effects of heterozygosity on probability of surviving flash floods, but in one population a positive correlation between heterozygosity and survival for fish born after the extreme events was found, probably because crowding in a small section of the stream caused more intense competition for resources.

KEYWORDS

fitness, inbreeding, individual quality, marble trout, *Salmo marmoratus*

1 | INTRODUCTION

Understanding within-population variation in components of fitness and how they are affected by genetic variation, is a central topic in ecology and evolutionary biology (Chapman *et al.*, 2009). The concept of individual quality has often been used to explain inter-individual heterogeneity in performance (Bergeron *et al.*, 2011; Moyes *et al.*, 2009; Wilson & Nussey, 2010). Heterogeneity in individual quality may result from complex interactions among genetic, maternal, environmental and demographic factors at different stages of life (Bergeron *et al.*, 2011). In particular, the strength of the relationship between individual quality and either fitness or particular components of fitness (e.g., survival, growth) may depend on the quality of the environment. A stronger correlation between individual quality and fitness may be expected in harsher (e.g., food-poor, stronger competition for resources) environments (Reed *et al.*, 2007), while in more favourable environments there may be enough resources available for all individuals to survive to sexual maturity and reproduce (Leung & Forbes, 1997). In addition, the effects of individual quality on survival might be greater during extreme events (e.g., cold spells, floods, fires) and in the post-extreme event environment, due to higher densities

and stronger competition for resources (Jenouvrier *et al.*, 2015; Keller *et al.*, 1994).

Heterozygosity is often considered a proxy for individual quality and a positive correlation between heterozygosity and fitness-related traits has often been found in fishes (David, 1998; Pujolar *et al.*, 2005). Multiple hypotheses on the proximate mechanisms of a positive heterozygosity–fitness correlation have been advanced (e.g., functional overdominance, avoidance of inbreeding depression) and, as expected, the scale of such effects is generally small (Chapman *et al.*, 2009). Although investigations have been encouraged (Chapman *et al.*, 2009), the hypothesis of whether the effects of heterozygosity on fitness components vary with environmental conditions has rarely been tested in natural populations of fish species.

Here, this hypothesis was tested using as a model system six marble trout *Salmo marmoratus* Cuvier 1829 populations living in western Slovenia (Vincenzi *et al.*, 2016). Average survival probabilities of *S. marmoratus* differ greatly among populations and three of them had been affected by flash floods causing massive mortality (Vincenzi *et al.*, 2016). Specifically, the hypotheses tested were: positive effects of heterozygosity on survival in populations with lower average survival rates or when in competition with other species (i.e., harsher, poorer environment); no effect of heterozygosity on surviving flash

floods owing to their largely non-selective effects across traits; greater effects of heterozygosity for fish born after the occurrence of flash floods than born before, due to higher population density and stronger competition for resources after the flood.

2 | MATERIALS AND METHODS

2.1 | Study area and species description

Detailed descriptions of the biology and ecology of *S. marmoratus* can be found in Vincenzi *et al.* (2016). The hypotheses on effects of heterozygosity on survival varying with environmental conditions were tested on the *S. marmoratus* populations of lower Ildrija (Ildri), upper Ildrija (Uldri), Lipovesck (Lipo), Zadlascica (Zadla), Trebuscica (Trebu) and Zakojska (Zak). In Ildri, *S. marmoratus* live in sympatry with rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) (Vincenzi *et al.*, 2011); in all other streams, *S. marmoratus* live in allopatry. Three populations have been affected by flash floods, Lipo (flash floods in 2007 and 2009), Zadla (2007) and Zak (2007), leading to > 55% decreases in survival with respect to sampling occasions with no floods (Vincenzi *et al.*, 2016). In Zak, the only fish surviving the 2007 flood were living in a small stretch in the uppermost part of the stream that was less affected by flows and debris (Vincenzi *et al.*, 2017). Estimated average survival probabilities (\pm SE) in sampling occasions with no floods were: Ildri (0.38 ± 0.01), Uldri (0.51 ± 0.02), Lipo (0.45 ± 0.06), Zadla (0.33 ± 0.05), Trebu (0.5 ± 0.02), Zak (0.49 ± 0.01) (Vincenzi *et al.*, 2016). Inbreeding coefficient estimated using genomic data was similar across populations and between 0.60 and 0.75 (current authors, unpublished data).

2.2 | Sampling

Populations were sampled either annually in June (Zak) or September (Zadla, Trebu) or bi-annually in both months (Ildri, Uldri, Lipo). Tagging started in different years for different populations: 1996 Zak; 2004 Ildri and Uldri; 2006 Zadla, Lipo and Trebu. Sampling protocols are described in detail in Vincenzi *et al.* (2016), but briefly, fish were captured by electrofishing and fork length (L_F , mm) and live mass (M_T , g) recorded. If a captured fish had $L_F > 110$ mm and had not been previously tagged or had lost a previously applied tag, it received a Carlin tag (Carlin, 1955) and age was determined by reading scales. The adipose fin was also removed from all fish captured for the first time, including those not tagged due to small size. Fish are aged as 0+ year in the first calendar year of life, 1+ year in the second year and so on. Sub-yearlings are smaller than 110 mm in June and September, so fish were tagged when at least aged 1+ years. We included data up to 2014 for each population.

2.3 | SNP discovery and genotyping

DNA was extracted from dried fin clips using the Dneasy 96 filter-based nucleic acid extraction system on a BioRobot 3000 (Qiagen, Inc., <http://www.qiagen.com>), following the manufacturer's protocols. Extracted DNA was diluted 2:1 with distilled water and used for PCR amplification of population-specific single nucleotide polymorphisms

(SNP). SNPs were assayed with 96.96 genotyping IFC chips on an EndPoint Reader 1 (Fluidigm, Inc.; <http://www.fluidigm.com>), using the manufacturer's recommended protocols. Genotypes were called using SNP genotyping analysis software (Fluidigm). Two people called all genotypes independently and discrepancies in the scores were resolved either by consensus, by re-genotyping, or by deletion of that genotype from that data set. A proportion of individuals were sampled and genotyped more than once (e.g., in case of tag loss or when the fish was sampled when < 110 mm and then later at tagging), as determined by observed identical genotypes and compatible age and length data and one of the samples was excluded from the analyses. Matching genotypes of individuals with different tags is a method of genetic tagging that allows reconstructing the life histories of individuals after tag loss.

We used 118 SNPs for Lipo [mean \pm SD minor allele frequency (MAF) of the SNPs: 0.23 ± 0.15], 94 for Zak (0.28 ± 0.13), 95 for Ildri (0.27 ± 0.14), 95 for Uldri (0.26 ± 0.14), 95 for Trebu (0.28 ± 0.14), 95 for Zadla (0.30 ± 0.11). The location of SNPs in the genome is unknown. Individual expected heterozygosity was calculated as the observed proportion of an individual's loci that were heterozygous (i.e., multi-locus heterozygosity). It has been found that newly proposed measures of variation are no more powerful at detecting relationships than multi-locus heterozygosity (Chapman *et al.*, 2009). The final dataset included 3,416 *S. marmoratus* total, from Ildri, $n = 781$; Uldri, 502; Lipo, 384; Zadla, 220; Trebu, 278 and Zak, 1,241.

2.4 | Survival analysis

The goal was to estimate the effects of heterozygosity on variation in survival for each population while accounting for year of birth and season and occurrence of flash floods, where applicable. The hypotheses are: stronger effects of heterozygosity on survival in populations with lower survival probabilities (Zadla) or with *S. marmoratus* living in sympatry with *O. mykiss* (Ildri); no effect of heterozygosity on surviving flash floods; a stronger effect of heterozygosity for fish born after, rather than before, the occurrence of flash floods in Zak and Lipo, due to higher juvenile production post-flood (Vincenzi *et al.*, 2016, 2017) and consequent higher competition for resources (e.g., food, shelter). For the latter, it was also predicted that a stronger effect in Zak than in Lipo due to higher density–stronger competition of fish born after the flood in Zak (Vincenzi *et al.*, 2017). For Zadla, the sample size was too small for testing the last of the three hypotheses.

Two relevant probabilities can be estimated from a capture history matrix: $\hat{\phi}$, the probability of apparent survival and p , the probability that an individual is captured given that it is alive (Thomson *et al.*, 2009). The Cormack–Jolly–Seber (CJS) model was used as a starting point for the analyses (Thomson *et al.*, 2009). Previous work has found no or only minor effects of population density, water temperature, body size, or sex on survival in *S. marmoratus* (Vincenzi *et al.*, 2016).

For each population, the best recapture model as reported in Vincenzi *et al.* (2016) was used. A seasonal effect (season) was modelled as a simplification of full-time variation, dividing each year in two periods: June to September (summer) and September to June (winter). Since the length of the two intervals (summer and winter) was

different, the probability of survival was estimated on a common annual scale, including season as a potential predictor of probability of capture and survival in all populations that have been sampled twice a year. Flood (0 for no flood occurring during the sampling interval and 1 for a flood) was included as a potential predictor of probability of survival for Lipo, Zak, Zadla.

Fish with fewer than 50 SNPs (~ half of total SNPs across populations) genotyped were excluded from the analysis (between 2 and 15% of data across populations). Heterozygosity was not standardized within populations since values on their natural scale are more easily interpretable and the ranges of individual heterozygosities were very similar among population. Previous studies on *S. marmoratus* have shown that a large fraction of the variability in survival is explained by year of birth, season and the occurrence of floods (Vincenzi *et al.*, 2016). Therefore, models tested included a year-of-birth component (year-class, pre-flood or post-flood, constant), a time component (season, flood, or constant) and a heterozygosity component (linear, cubic splines, or constant). Year class is the year of birth of a fish and pre-flood–post-flood is a categorical variable identifying fish born before or after the flash floods in Lipo and Zak. Since just a few fish were born in Lipo between the flash floods of 2007 and 2009 (Vincenzi *et al.*, 2017), fish born in 2008 and later in Lipo were post-flood. We did not include sampling occasion in our model of survival, in order to avoid it masking the role of other determinants of survival of expected much smaller effect size (such as heterozygosity).

For each model, we tested additive and multiplicative interactions among predictors (see R code for full list of models). Models

TABLE 1 Population-specific models of apparent *Salmo marmoratus* survival ϕ within $\Delta AIC < 2$ from the best model

Model	npar	ΔAIC	Weighting
Zak			
ϕ (heter * pre.post + flood)p(age)	7	0.00	0.51
ϕ (heter + pre.post + flood)p(age)	6	0.95	0.32
trebu			
ϕ (heter) p(time)	10	0.00	0.54
ϕ (bs(heter) p(time))	12	1.23	0.29
Zadla			
ϕ (heter + flood)p(const)	4	0.00	0.80
Lldri			
ϕ (bs(heter)) p(bs(age))	8	0.00	0.43
ϕ (bs(heter)+season) p(bs(age))	9	1.15	0.24
Uldri			
ϕ (const) p(bs(age))	5	0.00	0.30
ϕ (bs(heter)) p(bs(age))	8	1.16	0.17
ϕ (heter)p(bs(age))	6	1.42	0.15
ϕ (season)p(bs(age))	6	1.44	0.15
Lipo			
ϕ (pre.post + flood)p(const)	26	0.00	0.98

Full list of models is at https://github.com/simonevincenzi/Heter/blob/master/Survival_models.pdf

*, Interaction between predictors. Time, interval between two consecutive sampling occasions (only used for recapture models); season, categorical variable for summer (June to September) and winter (September to June); heter, expected individual heterozygosity; pre.post, categorical variable for fish born before (pre) or after (post) flash floods; bs, b-splines; npar, number of parameters of the survival model.

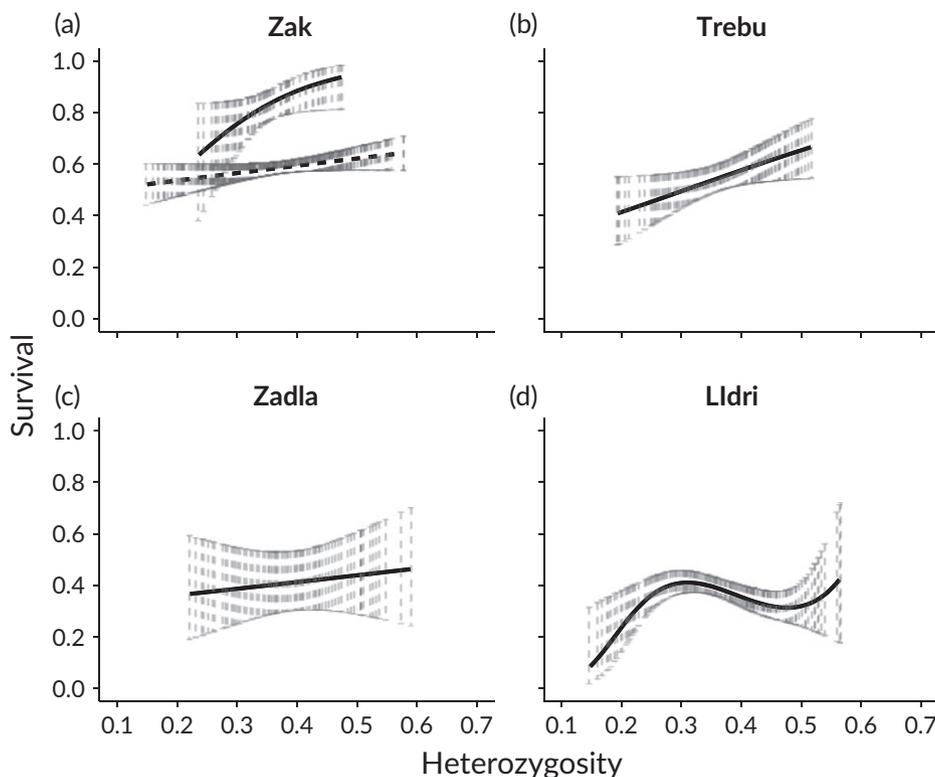


FIGURE 1 Relationship between heterozygosity and apparent survival ϕ in the four *Salmo marmoratus* populations for which models including heterozygosity were strongly supported: (a) Zakojska (Zak; Pre, fish spawned before the 2007 flash flood; Post, fish spawned after the 2007 flash flood); (b) Trebuscica (Trebu); (c) Zadlascica (Zadla); (d) Ildrija (Lldri). For both Zak and Zadla, survival probabilities are for sampling occasions with no extreme event. ■, 95% CI. (—) Post and (-----) Pre

were considered to provide the same fit to data when ΔAIC between them was < 2 and, in the case of multiple models with $\Delta AIC < 2$, the model with the fewest parameters was selected as the most supported (Burnham & Anderson, 2002). We did not use null-hypothesis significance testing for model; variable selection and heterozygosity was assumed to explain variation in survival (relative to models not including heterozygosity) when it was included in the most supported model. We carried out the analysis of survival using the marked package (Laake *et al.*, 2013) in R (<http://www.r-project.org>). Data and code are available at <https://github.com/simonevincenzi/Heter>

3 | RESULTS

Models including heterozygosity as a predictor of survival were strongly supported in Lldri, Zadla, Trebu and Zak (Table 1). In Zak, the effects of heterozygosity varied with year of birth (*i.e.*, before or after the flash flood of 2007; Table 1). For these populations, the relationship between heterozygosity and survival was positive (Figure 1), although in Lldri a cubic spline mostly predicted very low survival probabilities for fish with low heterozygosity (Figure 1(d)). No effect of heterozygosity on fish survival was observed in Lipo, Zadla or Zak for the sampling occasion in which flash floods occurred (Figure 2), but a positive effect of heterozygosity on fish survival was observed in Zak after the flash flood of 2007 (Table 1 and Figure 1(a)).

4 | DISCUSSION

The results of this study confirmed the hypothesis of stronger effects of heterozygosity on survival in poorer environments, with the exception of the population of Trebuscica, for which the most-supported model predicted a positive effect of heterozygosity on survival, although average survival was the second highest among the 6 *S. marmoratus* populations.

The consequences of extreme events on trait selection are still poorly understood; in some cases, all phenotypes are affected equally by an extreme event, while in others, some phenotypes may respond better than others to the unusually harsh conditions, which act as an intense selection episode. For instance, song sparrows *Melospiza melodia* that survived a severe population bottleneck caused by a harsh winter were a non-random subset of the pre-crash population with respect to inbreeding, indicating that natural selection favoured outbred individuals (Keller *et al.*, 1994). However, flash floods and debris flows such as those that are occurring with increasing frequency in western Slovenia are unlikely to directly select for particular phenotypes through differential survival, due to their catastrophic, unpredictable effects (Vincenzi *et al.*, 2017). Consistent with this, it was not found that more heterozygous fish had higher chances of surviving flash floods.

In Zakojska, a strong, positive relationship between heterozygosity and survival was found for fish spawned after the 2007 flash flood. In contrast, no effects of heterozygosity on survival was found for fish spawned after the flash floods in Lipovscek. High average survival for fish born after the flash flood may indicate particularly favourable

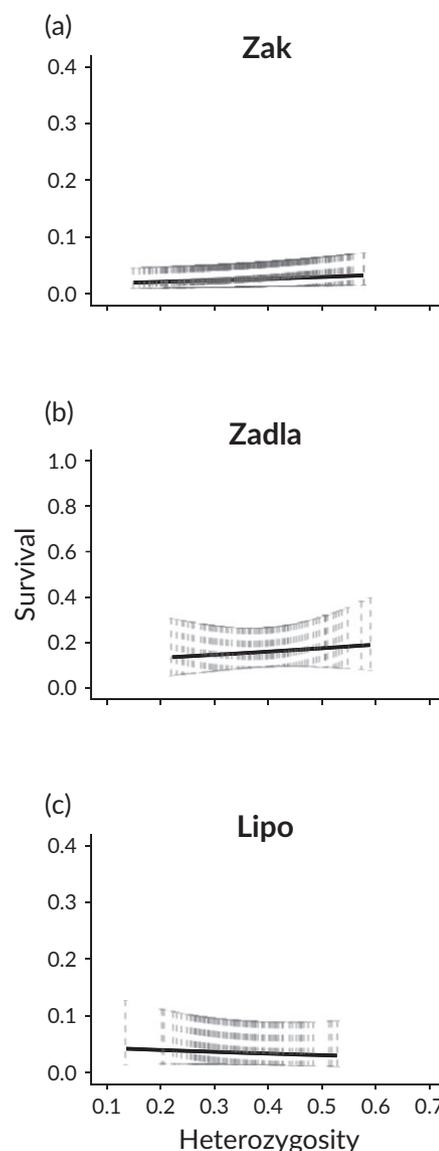


FIGURE 2 Relationship between heterozygosity and probability of surviving (○) a flash flood in the populations of (a) Zakojska (Zak), (b) Zadlascica (Zadla), and (c) Lipovscek (Lipo). See online code for model ranking and parameter estimates

environmental conditions; however, high survival may also be due to a combination of lower cannibalism and fewer older fish competing for space, while intra-cohort competition for food and space may still be intense. The different relationships found in the two populations are consistent with the hypothesis that a stronger correlation between individual quality and fitness is expected in harsher environments. In Zakojska, crowding in a small section of the stream after the 2007 flash flood caused more intense competition and may have contributed to stronger selection for heterozygosity than prior to the flood.

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ORCID

Simone Vincenzi  <https://orcid.org/0000-0002-8436-8608>

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