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Genetic effects on tolerance to acute cold stress in red drum, *Sciaenops ocellatus* L.

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Abstract

Genetic effects on cold-stress tolerance were assessed for red drum (*Sciaenops ocellatus*), an economically important sciaenid fish in the southern USA. Thirty-five families were generated via 'natural' spawning of multiple sets of five breeders (three dams × two sires) in individual brood tanks. Offspring from the 35 families were transferred abruptly from an acclimation temperature of ~24 °C to 5.7 °C and maintained subsequently at an average temperature of 6.5 °C. Offspring were assigned *a posteriori* to individual broodfish (dam and sire) based on genotypes at nuclear-encoded microsatellites. Heritability of the survival–time probability function was estimated using a proportional hazard approach and an animal-additive model. The estimated heritability was 0.20 (95% CI: 0.07–0.40), indicating a significant genetic component to acute cold-stress tolerance in red drum.

Keywords: cold-stress tolerance, heritability, *Sciaenops ocellatus*, microsatellites

Introduction

The red drum, *Sciaenops ocellatus* L., is an estuarine-dependent marine fish that frequents coastal waters of the western Atlantic Ocean and the Gulf of Mexico. The species is cultured both by state agencies producing juveniles for stock enhancement (McEachron, McCarty & Vega 1995) and by the private sector producing red drum destined to be marketed at a size of 1.0–1.5 kg (Lutz 1999). Production of red drum in culture, however, is limited by the species' low tolerance to cold temperature (Lutz 1999). Extensive mortality of 'wild' red drum in bays and estuaries along the coast of the Gulf of Mexico and of cultured red drum in outdoor facilities has been documented during severe episodes of cold weather (Gunter 1941; Gunter & Hildebrand 1951; Lutz 1999). Mortality typically occurs during very sudden and sharp temperature declines in shallow aquaculture ponds or inshore waters where water temperature can decline by up to 15 °C in a few hours (Moore 1976).

Zootechnical practices used to minimize mortality risks from cold involve the use of in-pond thermal refuges and indoor over-wintering facilities (Lutz 1999) or advancing the rearing cycle via photoperiod manipulation of spawning time in order to restrict outdoor production to warmer seasons. Another potential approach to improve the resistance of red drum to cold is inclusion of high levels of unsaturated lipids in the diet (Craig, Neill & Gatlin III 1995). Characterization of a genetic basis for tolerance to acute cold stress would be useful in the design of selective breeding programmes to improve resistance of red drum in culture to cold stress (Lynch & Walsh 1998). To date, information on variation in tolerance to acute cold stress in red drum is limited to the study by Procarione and King (1993), who compared temperatures lethal to 50% (LT50) in strains of red drum from Texas and South Carolina. No difference in LT50 between red drum from the two geographic origins was detected.

In this study, we used a cold-challenge approach to estimate the magnitude of genetic effects on tolerance to acute cold stress in red drum from coastal waters of Texas. Offspring from 35 families were mixed in the same tanks according to the 'common garden' principle, and tolerance to acute cold stress was assessed as the duration of survival following abrupt transfer to cold temperature. A proportional,

hazard mixed model where genetic effects were modelled as a random, additive-animal effect was used to estimate genetic variance and heritability.

Materials and methods

Production of red drum families and initial rearing

Families of red drum were generated during spawning of multiple sets of breeders (generally three females × two males) conditioned in the same brood tank for spawning at the Texas Parks and Wildlife Department (TPWD) Coastal Conservation Association/Central Power and Light (CCA/CPL) Marine Development Center (MDC) in Flour Bluff, Texas. Depending on the contribution of individual dams and sires present in a brood tank, each spawning event could give rise to up to six dam × sire combinations, with the mixture of offspring generated potentially including full-sib, half-sib and unrelated fish.

Broodfish were caught offshore of the south Texas coast. Sexual maturation and spontaneous spawning, starting at the beginning of April 2004, was induced environmentally as described by McCarty (1987). Offspring from 35 spawning events involving 20 brood tanks were used in the experiment. Details relative to larval rearing may be found in Saillant, Ma, Wang, Gatlin III and Gold (2007). On 27 July, fish were transported to the Aquacultural Research and Teaching Facility (ARTF) at Texas A&M University in College Station, where they were maintained in three 10 m^3 tanks until 16 September and in three 400 L tanks thereafter. Tanks were connected to recirculating systems that provided nitrifying biofiltration. The photoperiod was maintained at a 12-h light/12-h dark cycle; water temperature was maintained at 25 ± 4 °C by controlling ambient air temperature. Water quality was monitored weekly and maintained within the optimal ranges for red drum juveniles (Neill 1987) via addition, as needed, of a mixture of well water and concentrated synthetic sea water adjusted to a salinity of 11 g L^{-1}. Fish were fed a commercial diet (EXTRU 400; 400 g kg^{-1} crude protein, 100 g kg^{-1} lipid; Rangen, Angelton, TX, USA) to apparent satiation daily.

Cold tolerance challenge

On 10 February 2005, 428 red drum were weighed and measured for total length. Fish had been tagged previously (16 September 2004) with Passive-Integrated-Transponder (PIT) tags (Biomark, Boise, ID, USA). Water temperature over an acclimation period of 4 days before the beginning of the trial averaged 23.9 °C (range = 22.3–25.6 °C).

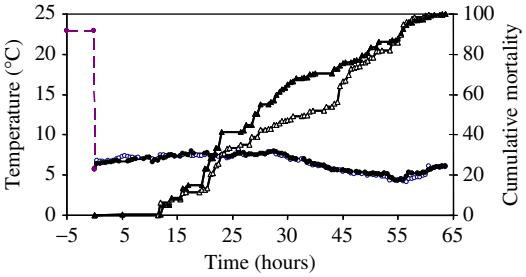

Figure 1 Temperature (circles) and cumulative mortality (triangles) during the cold tolerance challenge in replicate tanks (white and black symbols respectively).

On 24 February, fish were transferred directly from acclimation tanks to two 400 L tanks (212 and 216 fish per tank), where water temperature had been brought to 5.7 °C beforehand. Water temperature was subsequently maintained at an average value of 6.5 °C (range = 4–8 °C, Fig. 1) until all fish expired. The two experimental tanks were connected to the recirculating system described above; water temperature was controlled via D1-33 chiller units (Frigid Units, Toledo, OH, USA). Water turnover in each tank was $\sim 20\%\,h^{-1}$, with supplemental aeration provided via airstones. The temperature in each tank was monitored every hour during the trial and dead fish were removed at each temperature check point. Death was defined as the point when fish had lost balance, ceased body, fin and opercular movements and were unresponsive to touch. Death parameters (date, time and water temperature) were recorded for each expired individual.

Genotyping and pedigree analysis

A tissue sample (caudal fin clip) was taken from experimental fish on 10 February and from all but six possible dams and sires (57 dams and 40 sires sampled). Fin clips were stored at room temperature in 95% ethanol. DNA was extracted from tissue samples using an alkaline-lysis protocol (Saillant, Fostier, Haffray, Menu, Thimonier, Laureau & Chatain 2002). Possible dams and sires (broodfish) and experimental fish were genotyped using a panel of five microsatellites (Multiplex Panel 4) combined for multiplex PCR amplification and electrophoresis as

described in Renshaw, Saillant, Bradfield and Gold (2006). Experimental progeny that were not matched to a single dam and sire based on Multiplex Panel 4 (128 individuals total) and all possible parents were genotyped using Multiplex Panels 2 and 3 (Renshaw *et al.* 2006) as needed to achieve unambiguous parental assignment. Characteristics of the microsatellite markers included in multiplex panels are available in Saillant, Cizdziel, O'Malley, Turner, Pruett and Gold (2004); genotypes of all possible parents and offspring are available upon request from the authors. Assignment of offspring to individual dams and sires based on microsatellite genotypes was implemented using the program PROBMAX v. 1.2 (Danzmann 1997) available at http://www.uoguelph.ca/~rdanzman/software/probmax/.

Data analysis

Tolerance of fish to acute cold stress was characterized as duration (time) of survival following transfer to cold temperature. The distribution of survival-time data was bimodal (Fig. 1) and could not be normalized via transformation, thus precluding the use of a simple linear model to analyse genetic effects. Estimation of genetic effects therefore used the semi-parametric Cox survival model (Cox 1972). In this model, estimation of the effects of fixed and random factors on survival probability is based on the proportional hazard principle and does not require assumptions regarding the shape of the overall distribution of survival times.

The distribution of survival-time data was used to estimate the hazard function of an individual fish according to the following proportional hazard mixed model (Ducrocq & Sölkner 1998):

$$\lambda(t, x_{\mathrm{m}}, z_{\mathrm{m}}) = \lambda_{0,s}(t)\exp(x'_{\mathrm{m}}\beta + z'_{\mathrm{m}}u) \qquad (1)$$

where $\lambda(t)$ is the hazard function of an individual fish (m) and represents the probability of death at time t, given that the fish was alive immediately before t; $\lambda_{0,s}(t)$ is the baseline hazard function at time t; β is the vector of fixed covariates affecting the hazard; u is the vector of random animal additive values; and x'_{m} and z'_{m} are design matrices for β and u respectively.

A Cox model (Cox 1972) was used to evaluate the effects of fixed and random factors on the hazard variable ($\lambda(t)$). In the Cox model, the effects of different levels of factors on $\lambda(t)$ are assumed to be multiplicative (proportional) on a log scale at any time t as indicated in Eq. (1), while parameters of the baseline hazard function remain arbitrary (i.e. not estimated) as described in Cox (1972). Computations were implemented in the Survival Kit (Ducrocq & Sölkner 1998) available at http://www.nas.boku.ac.at/1897.html. The effect of replicate tank was evaluated as a fixed covariate; the significance of this fixed effect on the estimated hazard function ($\lambda(t)$) was tested via a likelihood-ratio test that compared the likelihood of the full model (including both fixed and random effects) with the likelihood of a model excluding the effect of replicate tank.

Genetic effects were modeled as a random, animal-additive effect, the levels of which were assumed to follow a multi-normal distribution with covariances between levels being induced by genetic relationships. The distribution parameters of the variance of additive genetic effects were estimated using the Bayesian approach described in Ducrocq and Casella (1996). Heritability of tolerance to acute cold stress was estimated after Yazdi, Visscher, Ducrocq and Thompson (2002) as the quantity

$$h^2 = \frac{\sigma_a^2}{1 + \sigma_a^2}$$

where σ_a^2 is the mode of the approximated marginal posterior distribution of the additive genetic variance. The 5% and 95% percentiles of the approximated posterior distribution of σ_a^2 were used to derive 95% confidence limits for h^2.

Genetic parameters for body mass and total length were also estimated. Variance and covariance components of each trait and their standard errors were estimated using the restricted-maximum-likelihood (REML) method as implemented in VCE-5® (Neumaier & Groeneveld 1998) and using the animal model

$$y = \mathrm{X}b + \mathrm{Z}a + e \qquad (2)$$

where y is the vector of observations (mass or length), b is the vector of fixed effects of common replicate tank, a is the random vector of additive breeding values, X and Z are the design matrices for b and a, respectively, and e is the vector of errors. Heritability estimates were derived as the ratio of the estimate of additive genetic variance to the total phenotypic variance (Lynch & Walsh 1998).

Phenotypic correlations between tolerance to acute cold stress (survival time) and growth traits (mass or total length) were estimated using Spearman's rank correlation (r_{p}); the significance of r_{p} was tested using critical values of the coefficient distribution (Zar 1984). Genetic correlations were estimated as the correlation of estimated breeding values (EBVs) of each animal for each trait (Lynch & Walsh 1998).

Estimated breeding values for growth traits were Best Linear Unbiased Predictors (BLUPs) as computed using PEST 4.2.3 (Groeneveld & Kovac 1990) and were based on the model described in Eq. (2). Estimated breeding values for tolerance to acute cold stress were solutions computed in the survival kit (Ducrocq & Sölkner 1998) based on the model described in Eq. (1). Spearman's rank correlation coefficient was used to estimate and test the significance of genetic correlations.

Results

Six fish (1.4% of the offspring sampled) could not be assigned unambiguously to dam or sire based on multilocus genotypes. Another five fish had incomplete records and one fish died prematurely. Records from these 12 individuals were not included in the statistical analysis. Complete records were available for 416 fish.

Temperature following transfer averaged 6.5 °C (range = 4.1–8.0 °C, Fig. 1). Most fish lost balance and showed limited movement immediately after transfer, and remained in this state until death. The first mortality was observed 12 h after transfer; thereafter, death occurred continuously till all fish had expired after 63.5 h (Fig. 1). The average time to death was 34.3 ± 14.6 h, while 50% of the fish were dead after 32 h; the average fish mass and total length recorded before the transfer were 185.3 ± 63.1 g and 25.5 ± 2.7 cm.

The total number of dam × sire combinations represented in the sample was 35, with the number of contributing pairs from individual brood tanks varying between one and six (average 2.9). Family sizes were unequal, with number of offspring per family ranging between 1 (10 families) and 77 (one family).

Likelihood-ratio tests indicated a significant effect ($P < 0.05$) of replicate tank on the hazard function ($\lambda(t)$) of an individual. This fixed effect was thus included in the final model used to estimate genetic variance. The estimate of genetic variance (95% CI) was 0.24 (0.08–0.66) and corresponded to a heritability (h^2) estimate of 0.20 (0.07–0.40). Heritability estimates for body mass and length (± SE) were 0.23 ± 0.08 and 0.26 ± 0.09 respectively.

Estimates of phenotypic correlations between cold tolerance and mass and length were 0.39 and 0.38 respectively. Estimates of genetic correlations between EBVs for tolerance to acute cold stress and EBVs for body mass and total length were 0.34 and 0.40 respectively. All estimated correlations (phenotypic and genetic) differed significantly from zero.

Discussion

The objective of this study was to assess the magnitude of genetic effects on tolerance to acute cold stress in red drum. The estimate of heritability (h^2) was 0.20 and differed significantly from zero, indicating the occurrence of significant genetic variation for tolerance to acute cold stress in red drum. The heritability value also suggests that selective breeding for increased tolerance to cold stress in aquaculture would be effective (Lynch & Walsh 1998). The animal model used here, however, assumed that all genetic effects were additive. Our estimate would be biased if non-additive (genetic) effects such as dominance, epistasis and/or maternal effects occurred. There are few assessments to date of non-additive genetic effects on cold tolerance in fish species. Charo-Karisa, Rezk, Bovenhuis and Komen (2005) found that a high proportion of the phenotypic variance of cold tolerance in Nile tilapia, *Oreochromis niloticus* L., was explained by a common environment/full-sib effect, suggesting occurrence of significant, non-additive genetic effects. The occurrence of non-additive genetic effects on cold tolerance was also inferred by Cnaani, Hallerman, Rona, Wellera, Indelman, Kashi, Gall and Hulata (2003) during analysis of a quantitative trait locus (QTL) affecting cold tolerance in F_2 progeny of hybrids between *Oreochromis aureus* (Steindachner) and *Oreochromis mossambicus* (Peters). The occurrence of non-additive genetic effects on acute cold-stress tolerance in red drum would bias the estimates of heritability upwardly (Gjerde 1986). Because many breeders present in brood tanks did not contribute to offspring, or did not mate with multiple partners, most mating groups generated in our experiment represented incomplete factorial-mating designs, precluding assessment of non-additive genetic effects. Further assessment of the magnitude of non-additive genetic effects utilizing appropriate experimental design is warranted.

Phenotypic and genetic correlations between tolerance to acute cold stress and body mass and length were in the range 0.34–0.40 and differed significantly from zero. Positive phenotypic correlations between cold tolerance and fish size were found by Lankford and Targett (2001) in the sciaenid *Micropogonias undulatus* (L.) and by Charo-Karisa *et al.* (2005) and Atwood, Tomasso, Webb and Gatlin III (2003) in *O. niloticus*. No such correlations, however, were

found in brook trout, *Salvelinus fontinalis* (Mitchill), by Fry, Hart and Walker (1946) or in hybrids between *O. aureus* and *O. mossambicus* (Cnaani, Gall & Hulata 2000, Cnaani *et al.* 2003). Red drum used in our experiments were essentially of the same age, meaning that differences in fish size reflect differences in growth rate. A practical implication of a low correlation between growth rate and tolerance to acute cold stress in red drum aquaculture would be that selection for a large body size may result only in a slow increase in tolerance to acute cold stress. Simultaneous improvement in tolerance to acute cold stress and growth rate in a red drum breeding programme may therefore require genetic evaluation of breeders for both traits.

Finally, temperature decrease during our experiment was extremely abrupt in order to maximize expression of differences among families in tolerance to sudden temperature declines. Further evaluation of heritability of tolerance to the stress induced by more progressive temperature decrease, and correlation with cold tolerance as measured in our challenge, may be needed in order to design effective selective breeding strategies to improve cold tolerance in cultured red drum.

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Appendix A

Table A1 Summary statistics for 35 families (dam × sire combinations) of red drum subjected to cold-stress challenge

Dam	Sire	*n*	Average survival time ± SD	Average breeding value* ± SD
418	442	3	22.00 ± 14.00	0.28 ± 0.07
419	443	1	44.50	0.03
419	444	13	32.69 ± 12.71	0.17 ± 0.10
420	443	1	23.50	0.04
420	444	8	38.25 ± 12.56	0.07 ± 0.11
421	443	4	39.50 ± 16.37	0.10 ± 0.10
421	444	8	29.94 ± 9.73	0.28 ± 0.05
422	445	1	30.00	0.08
423	446	1	39.00	0.04
424	447	2	29.00 ± 0.00	0.22 ± 0.00
424	448	6	30.75 ± 11.38	0.23 ± 0.06
425	448	6	42.33 ± 14.54	−0.02 ± 0.08
426	449	26	28.48 ± 13.04	0.47 ± 0.10
426	450	14	37.11 ± 14.65	−0.23 ± 0.14
427	449	3	23.33 ± 0.58	0.15 ± 0.01
427	450	15	49.37 ± 13.10	−0.65 ± 0.11
428	450	42	40.93 ± 14.51	−0.44 ± 0.11
429	451	7	32.57 ± 13.71	0.23 ± 0.08
429	459	77	28.07 ± 10.60	0.60 ± 0.10
430	451	11	36.82 ± 14.28	0.07 ± 0.10
430	459	59	28.74 ± 14.38	0.47 ± 0.12
431	452	14	44.39 ± 13.09	−0.37 ± 0.09
432	452	7	40.36 ± 11.82	−0.17 ± 0.08
433	453	1	44.50	−0.08
434	453	4	46.00 ± 9.69	−0.18 ± 0.08
435	454	27	31.78 ± 15.38	0.15 ± 0.12
436	454	1	22.50	0.06
436	455	1	61.00	−0.72
437	454	2	40.75 ± 7.42	−0.04 ± 0.04
437	455	12	41.42 ± 16.17	−0.47 ± 0.16
438	456	1	43.50	0.04
439	456	9	35.22 ± 15.30	−0.11 ± 0.11
439	457	27	42.72 ± 15.10	−0.42 ± 0.11
440	458	1	44.50	0.00
441	458	1	14.00	0.21

*Breeding values were estimated in the survival kit (see the text).
n, number of individuals; SD, standard deviation.