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[Article]

Heritability of Cold Tolerance in Red Drum

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Abstract.—Heritability (h^2) of cold tolerance was estimated for red drum *Sciaenops ocellatus*, an economically important sciaenid fish in the southern USA. Nineteen families were generated via environmentally induced spawning of multiple sets of five broodfish (three dams × two sires) and were mixed in three common-garden replicate tanks for cold tolerance challenge. All offspring were assigned postmortem to parents based on genotypes at nuclear-encoded microsatellites. The cold tolerance trial was initiated when offspring were 230–251 d old (total length mean ± SD = 182 ± 26 mm). Temperature was decreased progressively from 25°C to 3°C over a 30-d period and was maintained at an average of 3.1°C until all fish expired. Mortality began when temperature reached 5°C. Cold tolerance of individual fish was quantified based on survival time and a cooling-degree-hours (CDH) index. The $h^2$of cold tolerance was estimated using an animal mixed model and a restricted maximum likelihood algorithm. The h^2 (mean ± SE) estimate was 0.32 ± 0.12 and indicated a significant genetic component to cold tolerance in red drum. Phenotypic correlations between cold tolerance and body weight or length were positive but low (0.18 and 0.23, respectively), suggesting that selective breeding for increased growth rate in red drum would not have a negative impact on cold tolerance in the selected strain.

The red drum *Sciaenops ocellatus* is an economically important sciaenid fish in the southern USA, especially in Texas and other states bordering the Gulf of Mexico. Dramatic declines in red drum populations in the northern and western Gulf of Mexico during the 1970s and early 1980s led to the implementation of harvest restrictions and the prohibition of commercial sale of wild red drum (Matlock 1990). Interest in the aquaculture of red drum intensified around this time (Gatlin 2000), both for stock enhancement and, following the moratorium on commercial harvest, to replace wild-caught red drum in the seafood market (Lutz 1999). Currently, aquaculture production of red drum involves the rearing of juveniles for stock enhancement by state agencies (McEachron et al. 1995) and the private commercial culture of food fish to a marketable size of 1.0–1.5 kg (Lutz 1999; D.M.G., personal observations).

A significant obstacle to development of red drum aquaculture is the species' relatively low tolerance to cold (Procarione 1986). High mortality during sudden or severe episodes of cold weather can result if fish are grown in outdoor facilities (Lutz 1999). Zootechnical practices for minimizing mortality risks from cold involve use of in-pond thermal refuges and indoor overwintering facilities (Lutz 1999) or advancing the rearing cycle via photoperiod manipulation of spawning time to restrict outdoor production to warmer seasons. Another potential approach to improve resistance of red drum to cold is inclusion of high levels of unsaturated lipids in the diet (Craig et al. 1995).

To date, no work on the genetics of cold tolerance in red drum has been carried out. Significant genetic effects on cold tolerance have been found for both intra- and interspecific crosses in a number of fish species, such as common carp *Cyprinus carpio* (Hulata 1995); Mozambique tilapia *Oreochromis mossambicus*, blue tilapia *O. aureus*, and their F_1 hybrids (Cnaani et al. 2000); and Nile tilapia *O. niloticus* (Charo-Karisa et al. 2005). Such findings highlight the potential for genetic improvement of cold tolerance in cultured fish. Assessing the potential for genetic improvement of cold tolerance in red drum requires knowledge of the heritability (h^2) of this trait.

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Development of a breeding program in red drum would potentially include improvement of other traits of interest in aquaculture production, such as growth rate. In such a case, knowledge of the correlation between cold tolerance and other characters (e.g., growth traits) that could be included in a selective breeding program is also essential (Falconer and McKay 1996; Lynch and Walsh 1998).

Estimating genetic parameters for traits such as cold tolerance in experimental aquaculture involves testing multiple families during cold challenges carried out under identical conditions. Molecular markers such as microsatellites are a useful tool to implement such tests, as pedigree of individual offspring can be inferred a posteriori from multilocus genotypes. Offspring from multiple families can thus be maintained under identical conditions (i.e., in the same tank) during a trial, thus avoiding the potential bias related to the rearing of families in separate tanks (Vandeputte et al. 2001).

In this study, we estimated h^2 of cold tolerance in red drum. Offspring from multiple families were mixed in the same tanks in a common-garden design for cold tolerance trials. Microsatellites developed specifically for red drum (Saillant et al. 2004) were used in a posteriori parentage assignment; h^2 was estimated using animal mixed models. The phenotypic correlation between cold tolerance and growth traits (i.e., weight, length, and condition) was also estimated.

Methods

Mating design and rearing.—Broodfish used to generate experimental offspring were wild red drum caught in Texas coastal waters and held at the Texas Parks and Wildlife Department (TPWD) Coastal Conservation Association–Central Power and Light Company (CCA–CPL) Marine Development Center (MDC) in Flour Bluff. Individual broodfish were allocated to brood tanks, each generally containing three dams and two sires, and were conditioned for spawning by manipulation of temperature and photoperiod. Offspring from 10 spontaneous spawning events involving a total of 7 brood tanks were used in the experiment. Spawning events occurred between 8 and 29 May 2003.

Offspring from each spawning event were grown in 1–2-acre prefertilized ponds (Colura 1987) until they reached an average size of about 30 mm (33–46 d postfertilization). At that time, 200 fish were sampled at random from each pond and were further grown in cages at the MDC until late July, when they were transferred to the Aquacultural Research and Teaching Facility (ARTF) in College Station, Texas. Fish were then maintained in 110-L aquaria or 400-L replicate tanks connected to a recirculating system (Goff and Gatlin 2004).

On 12 January 2004 (228–249 d postfertilization), 210 fish selected from offspring groups of the various spawning events were individually tagged with passive integrated transponder tags (Biomark, Boise, Idaho) and their weights and total lengths (TLs) were recorded. Mean (±SD) weight was 71.0 ± 33.5 g, and mean TL was 182.2 ± 25.8 mm. Fish were allocated at random to three replicate tanks described below (70 fish/tank).

Cold tolerance challenge.—The three replicate tanks were connected to a recirculating system equipped with mechanical (sand) filtration and nitrifying biofiltration. Throughout the experiment, photoperiod was 12 h light : 12 h dark; water turnover was approximately 20% per hour, and supplemental aeration was provided by a regenerative blower and air stones. Water quality was monitored weekly and was maintained within the optimal range for red drum juveniles (Neill 1987; Neill et al. 2004) by adding, on an as-needed basis, a mixture of well water and concentrated synthetic seawater adjusted to a salinity of 11‰. Fish were fed a commercial diet (40% crude protein, 10% lipid; Rangen, Inc., Angelton, Texas; EXTRU 400) to apparent satiation daily.

Water temperature in the three tanks during the experiment was controlled using three chiller units (Frigid Units, Toledo, Ohio; D1-33). Fish were acclimated to a temperature of 25°C. The cold tolerance challenge was initiated on 14 January 2004 (230–251 d postfertilization). Water temperature in each tank was reduced by approximately 1°C per day until it reached 12°C. Thereafter, the temperature was reduced at the rate of about 1°C every 2 d until it reached approximately 3°C. Temperature could not be decreased further and was therefore maintained at this minimum achievable temperature (average = 3.1°C, range = 2.3–4.8°C; Figure 1) until all fish had expired. Temperature was recorded once daily until 6 February and twice daily thereafter. Mortality (death) was defined as the point at which a fish had lost balance; ceased body, fin, and opercular movement; and was unresponsive to touch.

Expired fish were removed with a scoop net at each temperature check point, and death parameters (date, time, and water temperature) and fish TL and weight were recorded for each individual. Fin clips (~2–3 mm^2) from each fish were taken at death and stored in 95% ethanol for subsequent genotyping and pedigree analysis. Fin clips from all possible parents (i.e., dams and sires in each brood tank) had been removed previously and were similarly stored in 95% ethanol.

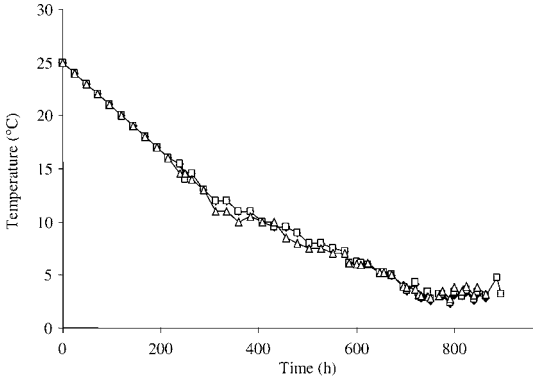

FIGURE 1.—Temperature during an assay used to determine cold tolerance in juvenile red drum held in tanks during 2004. Temperature was decreased gradually from 25°C to 3.1°C (average minimum) over 30 d. Symbols denote individual replicate tanks (70 fish/tank).

Genotyping and pedigree analysis.—The DNA was extracted from tissue samples by using an alkaline lysis protocol (Saillant et al. 2002). Possible parents (broodfish) and offspring were genotyped at four microsatellites (*Soc19*, *Soc85*, *Soc402*, and *Soc428*). The microsatellite loci, polymerase chain reaction (PCR) methods, electrophoresis of PCR products, and scoring of genotypes are described by Saillant et al. (2004). Genotypes of all possible parents and offspring are available from the Texas A&M Department of Wildlife and Fisheries Science (wfsc.tamu.edu/doc).

Assignment of offspring to parents was based on matching of offspring microsatellite genotypes to expectations (derived according to Mendelian principles) in one of the six possible full-sibling families resulting from the cross of one of the three dams and one of the two sires present in each brood tank. Parental assignment was implemented using Probmax version 1.2 (Danzmann 1997). All offspring sampled were unambiguously assigned to dam and sire.

Data analysis.—Individual cold tolerance was quantified using a cooling-degree-hours (CDH) statistic (after Cnaani et al. 2000), calculated as

$$\mathrm{CDH} = \sum_{i=1}^{k} [t_i \times (\theta_0 - \theta_i)],$$

where t_i is the number of hours between temperature measurements i and $i + 1$, θ_0 is the acclimation temperature (25°C), θ_i is the temperature measured at check-point i, and k is the check-point when mortality of the fish was recorded. Fulton's condition coefficient (K; Ricker 1975) was calculated as $10^5 \times (W/L^3)$, where W and L are individual weight and TL at death, respectively.

TABLE 1.—Summary statistics of individual cold tolerance in 202 red drum held in tanks and subjected to a gradual decrease in temperature from 25°C to 3.1°C (average minimum) over 30 d: cooling-degree-hours (CDH), weight, TL, and condition factor (K) recorded at death.

Variable	Mean	SD
CDH	9,733.9	838.1
Weight (g)	88.1	43.1
TL (mm)	186	27
K	1.28	0.11

Variance and covariance components and their standard errors were estimated using the restricted maximum likelihood (REML) method as implemented in VCE-5 (Neumaier and Groeneveld 1998) and using the animal model $\{y = X_b + Z_a + e\}$, where y is the vector of observations (CDH), 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 random errors.

Initial analysis revealed that nonadditive (genetic) effects on cold tolerance were either negligible or could not be distinguished from additive effects. The model employed, therefore, was based on the assumption that all genetic effects are additive. Heritability estimates were derived as the ratio of the estimate of additive variance to the total phenotypic variance. Phenotypic correlations between CDH and each of the growth traits (W, L, or K) were estimated by using Pearson's linear correlation coefficient; significance of correlation coefficients was tested using t-tests (Sokal and Rohlf 1981).

Results

Eight fish were lost during the acclimation period or during the early steps of the decrease in temperature. Records ultimately were available for 202 fish. The average (±SD) CDH per fish over the experimental trial was 9,733.9 ± 838.1 (Table 1). Mean (±SD) weight of offspring at death was 88.1 ± 43.1 g and mean TL was 186 ± 27 mm (Table 1). Feeding activity was much reduced when the temperature fell below 14°C, although some food pellet intake was still observed. No food intake was observed when temperature fell below 7°C. Mortality (death) first occurred when the temperature reached 5°C (655 h into the experiment, or 7,387 CDH; Figures 1, 2). Mortality increased dramatically between 8,500 and 9,500 CDH; complete mortality occurred at 12,550 CDH (Figure 2). Most of the mortality occurred during the final phase of the experiment, when temperature averaged 3.1°C. All fish were unambiguously assigned to their parents based on multilocus genotypes. The total number of families represented in the sample was 19, and the

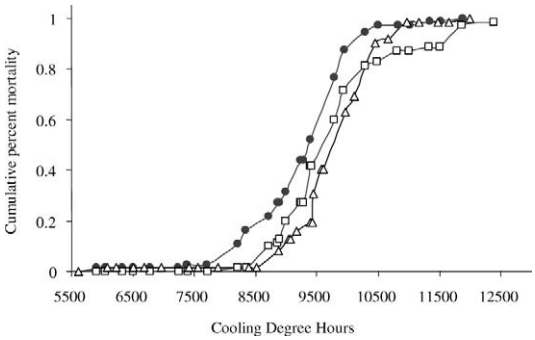

FIGURE 2.—Cumulative mortality (%) of juvenile red drum held in tanks during a cold tolerance challenge in 2004. Temperature was decreased gradually from 25°C to 3.1°C (average minimum) over 30 d. Survival time is expressed in cooling-degree-hours (see text for details). Symbols denote individual replicate tanks (70 fish/tank).

number of contributing pairs from individual brood tanks varied between one and five. Detailed contributions of individual families to the experimental groups are given in Table A.1. Family sizes were unequal; the number of offspring per family ranged between 1 (four families) and 36 (one family).

The h^2 estimate (mean ± SE) for cold tolerance was 0.32 ± 0.12 and differed by more than two standard errors from zero. Phenotypic correlations (r_p) between CDH and weight ($r_p = 0.23$) or TL ($r_p = 0.18$) were low but did differ significantly ($P < 0.05$) from zero, whereas that between CDH and K was not significant ($r_p = 0.09$, $P > 0.05$).

Discussion

In this study, progeny from 19 families of red drum were exposed to a progressive temperature decrease from 25°C to about 3°C and then were maintained at an average temperature of 3.1°C until all fish expired. Death was first observed when the temperature had reached 5°C, whereas 50% mortality occurred after the minimum achievable temperature (average = 3.1°C) had been maintained for approximately 1 d. Temperature-induced mortality for red drum in our experiments occurred at lower temperatures than those reported by Procarione (1986) and Craig et al. (1995) during similar cold tolerance challenge experiments where water quality conditions and diet composition, respectively, were tested. The differences in cold tolerance in these experiments could reflect, in part, the larger size of the red drum used in our experiment (average weight = 71.0 g, our study; 0.4 g, Procarione 1986; 3–15 g, Craig et al. 1995). Because most of the mortality in our study occurred when temperature had reached a low threshold of about 3.1°C, cold tolerance was quantified using a CDH parameter that essentially reflected the duration of survival under the treatment.

The cold tolerance h^2 (±SE) estimate based on the animal mixed model was 0.32 ± 0.12. To our knowledge, this result constitutes the first report of cold tolerance h^2 in red drum and demonstrates the occurrence of significant genetic effects on cold tolerance in red drum juveniles. Published reports of cold tolerance h^2 in fish include studies of Nile tilapia, blue tilapia, and their F_1 hybrids (Behrends et al. 1996; Charo-Karisa et al. 2005); estimates of h^2 in those studies were −0.05–0.31 (Nile tilapia), 0.33 (blue tilapia), and 0.31 (F_1 hybrids). Considering the phenotypic standard deviation observed in our experiment (837.8 CDH), an h^2 value of 0.32 would lead to a progress of about 270 CDH in one generation of truncative selection that retains the best 15% phenotypes. The generated progress would correspond to an average survival time about 14 h longer than the base (unselected) population in the condition of our challenge.

The experimental design in our study was based on the common-garden approach, thereby preventing a potential h^2 estimation bias that would occur if families were reared and tested in separate tanks. Fish were transferred into the common-garden tanks only 2 d before the beginning of the experiment. Rearing environment (i.e., rearing tank or aquarium) before entry into the common-garden tanks might therefore have affected fish cold tolerance during the assay. This effect could not be assessed from the present data set and would result primarily from eventual among-group differences in acclimation temperature (Beitinger et al. 2000). This source of bias, however, probably was minimal in our experiment given that temperature conditions in the various pre-experiment tanks and aquaria were identical. The pre-experiment rearing environment could also have affected fish size, and in turn, cold tolerance, as discussed below. We believe that the magnitude of this bias was minor, as the overall proportion of phenotypic variance in cold tolerance explained by fish size was only about 5% (versus 32% explained by genetic effects).

On the other hand, the h^2 estimate in our study may be biased by nonadditive genetic effects (e.g., dominance, epistasis, or maternal effects, or a combination thereof), given that the animal model assumed that all genetic effects were additive. Generally, nonadditive genetic effects are difficult to estimate because of the complex mating designs required (Varona and Misztal 1999). However, in a study of cold tolerance in Nile tilapia, Charo-Karisa et al. (2005) were able to estimate the magnitude of additive genetic effects and a common environment–full-sibling effect that included

nonadditive genetic effects. They found the proportion of the phenotypic variance explained by additive genetic effects (8%) to be much smaller than that due to the common environment–full-sibling effect (33%), suggesting the occurrence of significant nonadditive genetic effects on cold tolerance. Occurrence of significant nonadditive genetic effects on cold tolerance also was demonstrated in analysis of a quantitative trait locus affecting cold tolerance in the F_2 progeny of blue tilapia × Mozambique tilapia hybrids (Cnaani et al. 2003). Because the presence of significant nonadditive effects generates upwardly biased estimates of h^2 (Gjerde 1986), cold tolerance h^2 (in the narrow sense) in juvenile red drum may be less than we estimated. Further study employing a more robust experimental design is clearly warranted.

Phenotypic correlation between CDH and body weight ($r_p = 0.18$) or TL ($r_p = 0.23$), although significantly greater than zero, was relatively low. Positive correlations between cold tolerance and fish size in Nile tilapia were found by Charo-Karisa et al. (2005) and Atwood et al. (2003). However, neither Behrends et al. (1990) nor Cnaani et al. (2000, 2003) found evidence of a significant correlation between cold tolerance and fish size in Nile tilapia or blue tilapia × Mozambique tilapia hybrids. Formal evaluation of the magnitude of correlated response in cold tolerance to selective breeding for increased body weight in red drum would require estimation of the genetic correlation between the two traits. Genetic correlation could not be estimated reliably based on the present data set, but the low estimate of phenotypic correlation suggests that the genetic correlation also may be low (Lynch and Walsh 1998). In such a situation, selection for large body size might not result in a rapid increase of cold tolerance in the selected strains; simultaneous improvement of cold tolerance and growth rate in a breeding program would require genetic evaluation of breeders for both traits. Given that both growth rate and cold tolerance probably would be included in a red drum selective breeding program, further characterization of additive genetic variance of cold tolerance and genetic correlation with growth rate is warranted.

Finally, temperature decrease during our experiment was slow to allow efficient discrimination of differences in cold tolerance among families. Although the temperature decrease in coastal waters of the Gulf of Mexico during fall and early winter occurs more slowly than that in our challenge, red drum mortalities in the wild and in aquaculture facilities often have been attributed to more rapid declines in temperature during winter cold waves (Moore 1976; Lutz 1999). Further h^2 evaluation of tolerance to the stress induced by abrupt temperature decrease and correlation with cold tolerance as measured in our challenge may be needed to design effective selective breeding strategies for improving cold tolerance in cultured red drum.

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Appendix follows

Appendix: Numbers of Red Drum Offspring from Each Family Used in Cold Tolerance Trials

TABLE A.1.—Number of red drum offspring from each of 19 dam × sire combinations identified among 202 fish used in a cold tolerance challenge.

		Number of offspring			
Dam code	Sire code	Tank 1[a]	Tank 2[a]	Tank 3[a]	Total
1001	2001	3	0	6	9
1002	2001	6	6	7	19
1005	2002	1	1	1	3
1005	2003	6	4	3	13
1006	2002	11	15	10	36
1006	2003	1	1	0	2
1008	2004	4	0	0	4
1011	2006	2	1	0	3
1011	2007	7	6	3	16
1012	2007	2	6	5	13
1013	2006	1	1	3	5
1013	2007	5	4	4	13
1014	2008	12	10	10	32
1017	2010	1	3	0	4
1018	2010	7	11	9	27
1019	2010	0	1	0	1
1020	2013	1	0	0	1
1023	2014	1	0	0	1
1023	2015	1	0	0	1

[a] Replicate tank identification.