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SHORT PAPERS AND NOTES

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COMPARISON OF CULTURED AND WILD SHEEPSHEAD MINNOW (*CYPRINODON VARIEGATUS*) HEALTH CONDITION METRICS USED IN TOXICITY EFFECTS ASSESSMENT—

Toxicity tests are typically conducted on laboratory-cultured populations and measure contaminant effects on individual health metrics to assess effects on wild populations. Culture conditions in which test species are reared are designed for optimal growth and health of test organisms to isolate effects of toxicant exposure; however, these laboratory populations may not be representative of their wild counterparts. Four standard health condition metrics [hepatosomatic index (HIS) gonadosomatic index (GSI), fecundity, and condition factor] were compared between cultured and wild-caught sheepshead minnow (*Cyprinodon variegatus*) to determine if laboratory cultured fish were representative of wild populations. Wild fish were more robust (e.g., higher condition factor) than cultured fish but yielded fewer eggs per female per unit body weight and had lower HSI and GSI. These results demonstrate greater individual fitness of the laboratory-cultured populations for this species. The interpretation of toxicity test results should consider higher reproductive potential and individual fitness of cultured fish when applied in comparisons to wild populations.

Toxicity tests that measure fish sensitivity to contaminants are generally conducted using laboratory-cultured populations. Data collected from these tests are then used to assess impacts to fish in wild populations. Cultured fish are maintained in highly controlled conditions designed to optimize health, reproduction, and survival independent of toxicity treatments (Cripe et al., 2008), whereas wild populations are exposed to a multitude of stressors and selection pressures such as predators, competition, varying food quality and quantity, and environmental variation. The differences in stressors experienced by wild and cultured fish and the potential influence of ambient conditions on overall individual health may cause toxicant effects measured on laboratory-cultured fish to underestimate effects to wild populations.

Previous comparisons of cultured and wild fish have reported both physical and biochemical differences; however, studies have focused on fish cultured under hatchery conditions. Gross morphological differences, such as general body shape, size, and coloration have been noted between cultured and wild fish for herring and turbot (Balbontin et al., 1973; Blaxter, 1975; Ellis et al., 1997). Differences in fecundity and maturation rate between cultured and wild fish are also well recognized (Scott, 1962; Bagenal, 1969; Wooton, 1973; Blaxter, 1975). Cultured fish may have higher lipid content than wild fish due to high feeding rates of artificial diets, which may contribute to differences in the lipid composition of eggs from cultured females. However, comparisons using fish reared in hatcheries (e.g., salmonids, herring), which typically have higher crowding pressures than their wild counterparts, may not be applicable to fish cultured under conditions established for toxicity testing.

Metrics regularly used to assess general fish health condition include fecundity, hepatosomatic index (HSI), gonadosomatic index (GSI), and condition factor (Williams, 2000; Lloret et al., 2002). Fecundity, or reproductive potential, is directly related to environmental conditions, habitat, and nutritional status (Bromage et al., 1992). GSI is indicative of reproductive cycling and is sensitive to environmental conditions such as temperature (Kamanga et al., 2002). HSI has been frequently correlated to toxicity exposure (e.g., Everaarts et al., 1993; Pinkney et al., 2001) and is used to assess energetic reserves as a measure of health condition, nutritional history, and growth rates (Guderley et al., 1996; Chellappa et al., 2006). All of these metrics are influenced by fish microhabitat, climate, and nutrition, as well as being useful indicators of exposure to chemical stressors.

We compared these health metrics between cultured and wild sheepshead minnow (*Cyprinodon variegatus*; Cyprinodontidae) to determine if effects measured during toxicity tests under laboratory conditions may underestimate effects on wild populations. The sheepshead minnow is a standard test species commonly used to evaluate chemical toxicity and as a surrogate in ecological risk assessments (Hansen and Parrish, 1977). Sheepshead minnows are found from Massachusetts to the Yucatan Peninsula as well as in the West Indies and South America. Adults and juveniles inhabit shallow waters of salt marshes, inlets, and bays and are capable of living in a wide variety of environmental conditions (Hardy, 1978). The sheepshead minnow is a euryhaline, [0–35 parts per thousand (ppt)]

fish that tolerates a wide range of temperatures (0.6–45°C) as well as waters with low dissolved oxygen (Hardy, 1978; Bennett and Beitinger, 1997). Cultured fish used in toxicity tests are maintained with a regulated feeding schedule, high availability of food, limited number of individuals in tanks, and consistent salinity and water temperature; these fish are not subject to interspecific interactions. This study tested the hypothesis that health condition metrics in cultured fish would be indicative of greater individual fitness than those collected from wild populations.

Methods.—Wild adult fish were collected from unpolluted marsh pools in Santa Rosa Sound near Gulf Breeze, FL, during peak spawning season (15 June 2009, salinity = 23 ppt, temperature = 29°C) using seine nets. Fish were anesthetized in the field using tricaine methanesulfonate and preserved by injecting approximately 0.1 mL of formalin into the body cavity. The fish were then stored in 10% formalin in sealed containers until analysis. A total of 140 females and 53 males were collected. A random subset of the females (n = 33) were separated for GSI, HSI, and condition factor comparisons, while the remaining females, representing a range of small (< 4.25 cm; n = 33), medium (> 4.25 to < 4.75 cm; n = 46), and large (>4.75 cm; n = 28) fish were designated for fecundity comparison. Based on von Bertalanffy growth curves for this species developed from fish ranging from 2 to 653 d posthatch (dph; Raimondo, 2012) and corroborated with growth curves estimated for field populations (Rowe and Dunsen, 1995), the predicted age of medium-sized fish is between 150 and 200 dph.

Cultured fish were reared in laboratory conditions according to those recommended in Cripe et al. (2008), which includes feeding fish frozen brine shrimp for 2 wk prior to sampling to artificially induce optimal fecundity. Females for fecundity analysis were spawned August 2009; adults for GSI, HSI, and condition factor were spawned in September 2010. Both sets of adults were spawned from laboratory-raised parental fish. Cultures received 20 ppt filtered seawater at 26 ± 2°C. Spawning was conducted by randomly placing three females and two males in a spawning chamber (20.5 × 26.5 × 22.5 cm, with a 6-mm polypropylene screen on the bottom) with an egg collection screen underneath. Eggs were collected and placed in incubation cups with nylon-screened bottoms to ensure water flow at a density of 30 eggs per cup. Eggs were maintained in incubation cups for approximately 5 d, at which point they hatched and were moved to larval trays. Larval fish were kept at a density of 50 larval fish per larval tray (27.2 × 28 × 10.5–cm glass tray; 6-cm water depth) and fed 24-hr hatched *Artemia* twice daily (for 14 d). Juvenile fish were kept at a density of 25 juveniles per tank (95 × 34 × 26 cm) and fed Tetramin® to satiation twice daily. Juveniles were cultured at this density and feeding schedule through adulthood. Throughout rearing, cultures were maintained at a constant 14-hr light:10-hr dark photoperiod. This photoperiod is equivalent to that which occurred in the month and location of the field collection, minimizing potential differences in reproduction between the two populations that could be attributed to day length. After 185 dph, 78 females used in the fecundity analysis were anesthetized and preserved with formalin using the methods described above for the wild fish. After 194 dph, 85 females and 45 males used in other analyses were anesthetized. Fish were handled and preserved according to an Animal Care and Use Plan approved by the U.S. Environmental Protection Agency Gulf Ecology Division.

All fish were measured for wet weight and standard length (mm), and then dissected. Livers and ovaries were weighed. Fish and organ weights were taken to the nearest 0.01 g. GSI was calculated as (ovary weight/total weight) × 100. HSI was calculated as (liver weight/total weight) × 100. For fecundity, ovaries were removed and eggs greater than or equal to 10 μm in width were separated and counted using a dissecting microscope. Condition factor was calculated using fish from the HSI analysis as 100,000 W/L^3, where W is wet weight (g) and L is standard length (cm).

All data sets were checked for normality using the Shapiro-Wilk test prior to conducting statistical analyses and were transformed where necessary. HSI and GSI were square-root transformed and compared using analysis of variance. To conform to a normal distribution, zeroes in the fecundity data were excluded and the remaining data were square-root transformed. The frequency of females without eggs was compared between wild and cultured fish using a 2 × 2 contingency table. Two analyses of covariance (ANCOVA) were used to compare the square-root transformed number of eggs per female among groups, one using length and another using weight as the covariate. ANCOVAs were first run with a full model that included the group × covariate interaction effect. Where the interaction effect was nonsignificant, the ANCOVA was rerun without the interaction in the model. A Mann-Whitney test was used to

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TABLE 1. Mean and standard error (SE) for hepatosomatic index (HSI), gonadosomatic index (GSI), fecundity, their covariates, and median and interquartile range (IQR) for condition factor. HSI and GSI were analyzed using analysis of variance (test static = F value), fecundity was analyzed using analysis of covariance with female weight as the covariate (test static = F value), and condition factor was analyzed using a Mann-Whitney test (test statistic = Z value).

	Cultured fish			Wild fish			Test statistic (df = 1)	*P*-value
	n	Mean	SE/IQR	n	Mean	SE/IQR		
HSI								
Male HSI	45	2.07	0.09	53	1.47	0.06	32.3	< 0.001
Female HSI	85	3.2	0.09	33	2.92	0.24	3.03	0.084
GSI	81	5.97	0.37	31	3.52	0.48	11.94	< 0.001
Fecundity								
Eggs/female	57	69.57[a]	8.39	78	66.441	5.28	0.2	0.651
Female weight (g)	57	3.17	0.09	78	3.5	0.12	11.37	< 0.001
Condition factor								
Males	45	3257	3092–3482	53	3593	3283–3781	4.26	< 0.0001
Females	85	3050	2831–3264	33	3500	3318–3899	5.42	< 0.0001

[a] Least squares adjusted means.

compare condition factor between cultured and wild fish for each sex.

Results and Discussion.—Results of HSI, GSI, fecundity, and condition factor analyses are presented in Table 1. HSI of cultured fish was higher than that of wild fish for both males and females, although only significantly different for males. Smaller livers may indicate poor environment and food quality and HSI is a predictor of energy reserves (Chapella et al., 2006), thus HSI was expected to be lower in wild fish due to greater stress of foraging and food availability. Although enlarged livers could indicate exposure to toxicants (Liu et al., 2011), the laboratory populations were cultured in a contaminant-free environment and field fish were collected from a pristine estuary within Santa Rosa Sound. These results indicate that either wild fish are exposed to greater food-related or other stress, or cultured fish are reared on food with higher fat content. Regardless, these results indicate potentially lower energy reserves of wild sheepshead minnow.

The median condition factor of wild fish was higher than that of cultured fish for both sexes, indicating a greater robustness in wild fish. Condition factor uses a weight:length ratio that may be an expression of nourishment, robustness, or general health (Williams, 2000). Since activity and migration of cultured fish is severely limited, increased condition factor of wild fish may be due to greater robustness resulting from higher activity. Male wild fish used in this analysis weighed slightly less than cultured males (wild = 3.20 g, cultured = 3.69 g) and there was no difference in weight of females between the two groups (wild = 3.05 g, cultured = 3.02 g). Wild fish of both sex were shorter (females = 4.36 cm, males = 4.36 cm) on average than cultured fish (females = 4.6 cm, males = 4.78 cm). Differences in condition factor are, therefore, attributed to the differences in length. These results are contrary to comparisons of hatchery-reared and wild fish, which found cultured fish weighed more than wild fish at a given length (Blaxter, 1975; Ellis et al., 1997). Comparison of wild fish with hatchery-reared fish, which has been the sole source of previous cultured–wild comparisons, do not appear to be applicable to fish cultured in the laboratory for toxicity testing.

Reproductive potential was measured as fecundity of females that contained eggs and the frequency of females without eggs. The ANCOVA found a significant interaction effect between length and group, so ANCOVA results are only presented for the model that included weight as a covariate. In this model, the average number of eggs per female was not significantly different between the two groups; however, cultured fish were smaller in this analysis, producing more eggs per female for a given body size (Table 1). There were no significant differences in the frequency of fish that had no eggs between cultured (0.269) and wild (0.271) populations (X^2 = 3.841; P = 0.978). These results indicate that fish cultured in the laboratory according to most recently published test methods (e.g., Cripe et al., 2008) have higher reproductive potential than their wild counterparts. Average GSI was also significantly higher in cultured than in wild fish, supporting the results of the fecundity analysis.

Although the age of wild fish were not precisely determined, this analysis is based on a randomly collected subsample that represents the adult cohort in wild populations. The objective of this study was not to determine if, at a given age, a cultured individual is different from a wild individual. Rather, the significance of these results is the demonstration that for this species, cultured populations have health conditions that are different from wild populations. Toxicity tests are conducted on one age group at a time and the extrapolation of these impacts to a population of mixed cohorts is a source of uncertainty in test result interpretation.

Conclusion.—Extrapolation of toxicity test results from cultured fish to wild populations may underestimate ecological effects. Population models developed from laboratory toxicity tests are a common use of laboratory test data to project potential population effects (e.g., Miller et al., 2007; Raimondo et al., 2009); however, higher reproductive potential of laboratory sheepshead minnow may overestimate growth of wild populations and underestimate potential toxicity effects. Health condition metrics compared between the two populations indicated that wild fish were more robust but had lower reproductive potential per body size and lower lipid reserves than cultured fish. These results support the original hypothesis that wild fish potentially invest more energy in survival in higher-stress environments and that demographic data collected during toxicity or other tests conducted under optimal conditions in the laboratory may be overestimates for natural populations. There are a multitude of factors that vary between natural environments and culture conditions that may influence the metrics measured here (e.g., seasonally varying temperatures; Raimondo, 2012). While this study does not attempt to associate causal mechanisms for these differences, it provides evidence that the constant, optimal conditions of laboratory cultures produce fish whose health condition varies considerably from that of wild fish. This study is the first to demonstrate these differences in fish used in toxicity testing, but is limited to only one species and one field population. Future research that performs similar comparisons for other commonly tested species needs to be conducted to determine the consistency of these conclusions in a more diverse array of species.

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