Evidence of euryhalinity of the Gulf corvina, *Cynoscion othonopterus*

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Short title: Euryhalinity of the Gulf corvina, *Cynoscion othonopterus*

Summary

The effects of environmental salinity on physiological responses, growth, and survival of the Gulf corvina, *C. othonopterus*, were evaluated in a 6-week completely randomized design experiment. Corvina (17.2 ± 2.3 g mean initial body weight) were subjected to salinities of 5, 15, 25, and 35‰ and fed a commercial feed with protein and lipid contents of 46 and 14%, respectively. Plasma osmolality increased significantly with salinity, ranging from 335.1 ± 5.3 mOsm/kg in fish maintained at 5‰, to 354.8 ± 6.8 mOsm/kg in fish kept in seawater, while a significant inverse relationship was observed between salinity and moisture content of whole fish, ranging from 73.8 ± 0.7 (measured at 5‰) to 76.9 ± 1.0% (measured at 35‰). In spite of this, growth indices (final weight, weight gain, specific growth rate, condition factor, survival) were not altered, suggesting that, like other members of the family Sciaenidae, the Gulf corvina is a strong osmoregulator. The isosmotic point for this species was estimated to correspond to a salinity of 9.8‰. The present study represents the first set of experimental data on salinity tolerance of *C. othonopterus* and confirms the euryhalinity of this species.

Keywords: Salinity, Osmolality, Euryhaline, *Cynoscion othonopterus*

The recent near-collapse of the shrimp farming industry in Northwest Mexico, to a large extent caused by shrimp diseases (Rosales-Leija *et al*. 2012), has catapulted the interest in the production of marine fish. The Gulf corvina, *Cynoscion othonopterus*, a member of the family Sciaenidae native to northwest Mexico that is highly appreciated for its top-quality meat and that supports a commercial fishery of over 3,000 MT (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación 2012), is currently being evaluated as a candidate for aquaculture in Sonora, Mexico. Reproduction in captivity and growout in sea-cages is currently being explored as an alternative to shrimp culture. In general, fish belonging to the family Sciaenidae, such as the spotted sea trout, *Cynoscion nebulosus* (Cuvier), and the shi drum, *Umbrina cirrosa* (Linnaeus, 1758), are considered euryhaline species, *i.e.,* capable of tolerating a wide range of environmental salinities (Miranda and Sonski 1985; Kucera *et al*. 2002; Doroudi *et al*. 2006; Mylonas *et al*. 2009). For aquacultural purposes, this feature may be advantageous for the diversification of finfish production in low salinity waters, which have a different ionic profile compared to natural seawater. Interestingly, for some marine fish species, growth and/or survival are compromised when reared below full-strength seawater salinity (Sampaio and Bianchini 2002), while the opposite has been observed for others (Imsland *et al*. 2008). As a member of the Sciaenidae family, the Gulf corvina is also thought to be a euryhaline species, for it displays a reproductive seasonal migration to the Colorado River Delta in the northern portion of the Gulf of California, where it encounters lower environmental salinities and spawns (Rowell *et al*. 2005; Encinas-Rivera 2008). However, no experimental studies of the growth response and tolerance of this species to a wide range of environmental salinities are available in the literature. In the present study, the effects of environmental salinity on physiological responses, growth, and survival of *C. othonopterus* were investigated.

Juvenile *C. othonopterus* of the same cohort, originating from wild spawners and reared in full-strength seawater, were obtained from the "Centro Reproductor de Especies Marinas del Estado de Sonora" at Kino Bay, Sonora, Mexico. Fish were transported to the Wet Laboratory of Aquaculture Nutrition of the Kino Bay Experiment Station (KBES), University of Sonora at Kino Bay, Sonora, Mexico. Fish were stocked into a $10m^3$ fiberglass raceway. Because in its natural habitat the Gulf corvina feeds mainly on sardine (*Cetengraulis mysticetus*) (Román-Rodríguez 2000), fish were fed a commercial fish feed for seawater carnivorous fish (Nutripec, Agribrands Purina, Jalisco, México), with dietary protein and lipid contents of 46

and 14%, respectively. After one week and with an overall mean weight (± standard deviation, SD) of 17.2 ± 2.3 g, fish were transferred into experimental tanks for initiation of the study.

The study was conducted in polyethylene, circular tanks (71 cm diameter, 0.4 m^2 bottom area), filled with 200 l of water. Each tank was provided with a submerged airstone for water aeration supplied by a 1.0-HP blower (Fuji, Model VFC40, Saddle Brook, New Jersey, USA). Salinities of 5, 15, 25, and 35‰ were tested in a completely randomized design experiment. Each experimental treatment was assigned to three replicate tanks. Fish were stocked into tanks at a rate of 6 individuals per tank (30 fish per m^3). For acclimation, salinity was lowered at a rate of 1‰/h by gradually adding freshwater to the experimental tanks using irrigation button drip emitters (Submatic Irrigation Systems, Model BE ½-10, Austin, Texas, USA). Once acclimation of all treatments was achieved, fish were maintained in the tanks for six weeks.

All fish were fed the commercial feed described earlier to moderate excess, dividing the daily ration into three equal portions, administered at 09:00, 14:00, and 19:00 h. Uneaten feed and feces were siphoned out of the tanks daily. In order to keep water clean throughout the experiment, a daily water exchange of 50% was applied using water previously adjusted for salinity and aerated for 24 h. Daily measurements of water temperature, dissolved oxygen, and salinity were taken with a multi-function oxygen meter (YSI, Model Y85, Yellow Springs, OH, USA). Weekly, pH was measured with a handheld pH meter (Oakton®, model Double Junction pHTestr 1, Vernon Hills, Illinois, USA).

At the end of the experiment, a freezing-point technology osmometer (Advanced Instruments, Inc., Model 3320, Norwood, Massachusetts, USA) was employed to evaluate the osmolality (reported as mOsm/kg) of experimental waters and plasma of all fish. Following the Mexican technical specifications for the production, care and use of experimental animals (Norma Oficial Mexicana 2001), fish were anesthetized with MS222 before caudal severance. Then, approximately 1 ml of blood was withdrawn from the caudal blood vessel of each fish with a 25-gauge needle and 1-cc syringe, and placed into a 1.7-ml micro centrifuge tube (Costar Corning Incorporated, 1.7 mL Corning, NY, USA) kept on ice. Fish were then sacrificed by severing of the spinal cord and frozen for further analysis. Blood samples were centrifuged at 850 x g for 15 min to separate cells from plasma. Total osmolality was then measured using 20 µL of plasma. Both plasma and experimental water samples were analyzed in duplicate. Plasma and culture water osmolality data were

both regressed against salinity. The intersection between the two regression lines estimated the isosmotic point.

Duplicate composite samples (of 8 g each) of three whole fish from each of three experimental tanks, were taken to determine moisture (Method 930.15) and ash (Method 942.05) content, following the procedures of the Association of Official Analytical Chemists (2005).

Length (mm) and weight (g) of fish were individually measured at the beginning and end of the study. Weight gain was calculated from the difference between final minus initial weight. Survival rate was calculated from the difference between final and initial numbers of fish per tank: (final number of fish x 100)/initial number of fish). The Fulton's condition factor (K) (Ricker 1975), a measurement of the robustness of fish, was calculated as $K = [(\text{weight}/\text{length}^3)] \times 100$. In addition, the specific growth rate (SGR) was calculated as SGR = [ln (final weight – initial weight)] [100] / time (days).

Using a significance level of $P \leq 0.05$, one-way analysis of variance (ANOVA) was employed to evaluate treatment differences in fish performance (growth indices, survival, K, FCR, and SGR), plasma osmolality, moisture, and ash content of whole fish, while Repeated Measures ANOVA was employed to analyze water quality data (dissolved oxygen, temperature, and pH). Differences among treatments were identified by Duncan's method. Percent survival rates were arcsine-transformed prior to statistical analysis; untransformed values are presented. Data analyses were performed using Statistical Analysis System software (SAS Institute, Inc. 1989-95).

The measurements (treatment means ± SD) of temperature at the salinity treatments 5, 15, 25, and 35‰ were 25.9 ± 2.0, 25.7 ± 1.9, 26.5 ± 2.0, and 26.7 ± 2.1°C, respectively. For dissolved oxygen, they were 7.1 ± 0.4, 7.0 ± 0.4, 6.7 ± 0.4, and 6.7 ± 0.4 mg/l, respectively, while for pH, they were 7.5 ± 0.1, 7.5 ± 0.1, 7.7 ± 0.1, and 7.7 ± 0.1, respectively. These parameters are within the range of values either observed in the natural habitat of this species, or employed in studies in which satisfactory growth and survival of other fishes belonging to the same family has been recorded (Neill 1990; Rowell et al. 2005; Martínez-Llorens *et al*. 2011; Minjarez-Osorio *et al.* 2012). Hence, it is considered that adequate overall water quality was maintained throughout this study.

Mean plasma osmolality, which varied from 335.1 ± 5.3 mOsm/kg in fish maintained at 5‰, to

354.8 ± 6.8 mOsm/kg in fish kept in seawater, lies within the range of values generally observed in marine fish (335-480 mOsm/kg) (Jobling 1995; Sampaio and Bianchini 2002; Resley *et al*. 2006), and it is notably similar to values found in other members of the Sciaenidae family like the red drum, *Sciaenops ocellatus* (350 mOsm/kg, measured in seawater) (Crocker *et al*. 1983), the shi drum, *U. cirrosa* (350-409 mOsm/kg, measured in 40‰ water) (Mylonas *et al*. 2009), and the dusky kob, *Argyrosomus japonicus* (362 mOsm/kg, measured in 35‰ water) (Bernatzeder *et al*. 2008). In the present study, the differences detected in the plasma osmolality values, significantly lower for fish kept at 5 and 15‰, with respect to fish at 25 and 35‰, along with the significantly higher moisture contents observed as salinity decreased, suggest some degree of physiological stress imposed by the low salinity. However, from the very small slope found for the linear relationship between salinity and plasma osmolality (0.69 mOsm/kg/‰, Figure 1) and the fact that none of the growth responses measured, survival, or the ash content of whole were statistically affected by the salinities imposed (Table 1), it seems evident that fish were able to adapt satisfactorily to low salinity. These results represent the first set of experimental data on the salinity tolerance of *C. othonopterus* and confirm the euryhaline nature of this species. Relative constancy of plasma osmolality, with little or no effects on growth in response to salinity, has also been observed in other euryhaline teleosts. For example, plasma osmolality of the rabbitfish (*Siganus rivulatus*) varied from 398 to 435 mOsm/kg after being exposed for 3 weeks to salinities ranging from 10 to 50‰, while growth of this highly euryhaline species was only slightly affected (Saoud *et al*. 2007). Similarly, Resley *et al*. (2006) reported that over the salinity range of 5 to 35‰, plasma osmolality of juvenile cobia varied from 318.8 to 335.5 mmol/kg, but growth performance of fish was not influenced. Furthermore, findings of the present study agree with the overall range of salinity tolerance, from 5 to 45‰, found for sciaenids such as *S. ocellatus*, *U. cirrosa*, *A. regius*, *A. japonicus*, and *A. inodorus* (Wurts and Stickney 1993; Fielder and Bardsley 1999; Tomasso and Kempton 2000; Doroudi *et al*. 2006; Ferreira *et al*. 2008; Partridge *et al*. 2008; Mylonas *et al*. 2009; Partridge & Lymbery 2009; Márquez *et al*. 2010). Other teleost fish also considered as strong osmorregulators include the widely studied salmonids (Varsamos *et al*. 2005), as well as some flatfish (Sampaio and Bianchini 2002; Imsland *et al*. 2008), some groupers (Tsui *et al*. 2012; Cheng *et al*. 2013), and some cyprinids (Kolbadinezhad *et al*. 2012), among others. Conversely, stenohaline species, unable to adapt to large variations in salinity, display wider changes in plasma osmolality

often accompanied by acute or lethal effects when subjected to salinity challenge. For instance, baseline plasma osmolality of the sunshine bass (hybrid of white bass *Morone chrysops* ♀ × striped bass *M. saxatilis* ♂) and the palmetto bass (striped bass ♀ × white bass ♂) (360 and 351 mmol/kg, respectivelty), increased to 415 and 530 mmol/kg, respectively, after a 24-h stepwise elevation in salinity from 1 to 52‰. Both species were unable to survive at high salinity (Myers and Kohler 2000). Similar responses have been observed in other fresh and marine fish exposed to high or low salinity, respectively (Bystriansky *et al*. 2007; Suchy 2007). The findings of the present study, conducted for 6 weeks within the salinity range of 5-35‰, support further investigation of the osmorregulatory capacity of the Gulf corvina. For example, long-term exposure to a further extended low salinity range should be examined. Taking into account that the survival rates observed at salinities below full-strength seawater (83.0-88.7%) were numerically, but not statistically, lower than that observed in seawater (100.0%), this approach would help elucidate the effects of long-term exposure to low salinity on this and other response variables. Different sizes of fish, including larvae, juveniles, subadults, and adults could also be included, taking into account that salinity has been shown to vary with size/age for certain species (Rajabi and Khodabandeh 2013).

Osmolality of culture water also increased directly with salinity. Significant ($P < 0.05$) positive linear relationships were found between salinity and both culture water (r = 0.99) and plasma osmolality (r = 0.78). The isosmotic point for *C. othonopterus*, *i.e.*, the point of intersection between these lines, was estimated to be 9.8‰ (Figure 1), which is comparable to the that of the flounder *Paralichthys orbignyanus* (10.9‰) (Sampaio and Bianchini 2002), another euryhaline marine fish.

The osmoregulatory capacity of the Gulf corvina observed in the present study is consistent with changes in salinity that this species successfully faces along its annual reproductive migration (Rowell et al. 2005; Encinas-Rivera 2008). In fact, it appears that *C. othonopterus* not only withstands lower salinities down to 26‰ in the Colorado River Delta, but requires these estuarine conditions for successful spawning and nursing (Rowell *et al.* 2005). As a consequence of the construction of upstream dams that stopped the flow of the Colorado River into the Gulf of California, capture fisheries of the Gulf corvina completely disappeared in the 1960s (Román-Rodriguez 1998). After controlled pulses of Colorado River water were released into the Gulf of California in the early 1990s, commercial fishery of this species re-emerged (Román-Rodriguez 2000;

190 Rowell *et al*. 2005; Encinas-Rivera 2008). The mechanisms of osmotic regulation of euryhaline marine fish,
191 which include high drinking rates of sea water, active uptake of ions along the digestive tract, coupled with
192 osmotic intake of water, have been comprehensively studied and reviewed by various authors (Evans, 1999;
193 Wilson and Laurent 2002; Hirose *et al*. 2003; Versamos *et al*. 2005). It would be of interest to examine these
194 physiological aspects in future studies of Gulf corvina.

195 With respect to the magnitude of growth of the Gulf corvina observed in the present study, the mean
196 SGR values, which varied from 0.9 to 1.3%/d, are at the lower end of the range of SGR values (fluctuating
197 from 0.7 to approximately 4%/d) documented for a variety of species and sizes of sciaenids, such as *A.*
198 *japonicus*, *S. ocellatus*, *Pseudosciena crocea*, *Nibea michthioides*, and *Totoaba macdonaldi* (Jirsa *et al*. 1997;
199 McGoogan and Gatlin 1999; Duan *et al*. 2001; Turano *et al*. 2002; Wang *et al*. 2006; Pirozzi *et al*. 2010;
200 Minjarez-Osorio *et al*. 2012). However, it is worth pointing out that the present study was conducted in indoor
201 tanks with limited space, and that important aspects such as the nutritional requirements for optimum growth
202 of this species are still unknown. It is expected that, once these requirements are fulfilled, greater growth rates
203 can be obtained for this species, especially when reared in adequate infrastructure for commercial culture,
204 *e.g*., floating or submersible cages. The estimated mean values of the condition factor for the Gulf corvina
205 varied from 0.9 to 1.0 (Table 1), and were very similar to estimates of other members of the Sciaenidae family
206 like *Micropogonias furnieri* (ranging from approximately 1.0 to 1.2) (Manickchand-Heileman and Kenny
207 1990), and *T. macdonaldi* (reported mean value of 1.1) (Minjarez Osorio *et al*. 2012).

208 In conclusion, plasma osmolality and whole body moisture content of the Gulf corvina, *C.*
209 *othonopterus*, were statistically influenced after being reared for 6 weeks within the salinity range of 5 to
210 35‰. However, none of the growth responses, as evaluated by final weight, weight gain, specific growth rate,
211 condition factor, or survival of fish were affected, indicating that the Gulf corvina is a strong osmorregulator.
212 The present study represents the first set of experimental data on salinity tolerance of *C. othonopterus* and
213 confirms the euryhalinity of this species.
214
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Table 1. Plasma and culture water osmolality, moisture and ash content of whole fish, and growth response of *C. othonopterus* reared at different salinities (means ± SD).

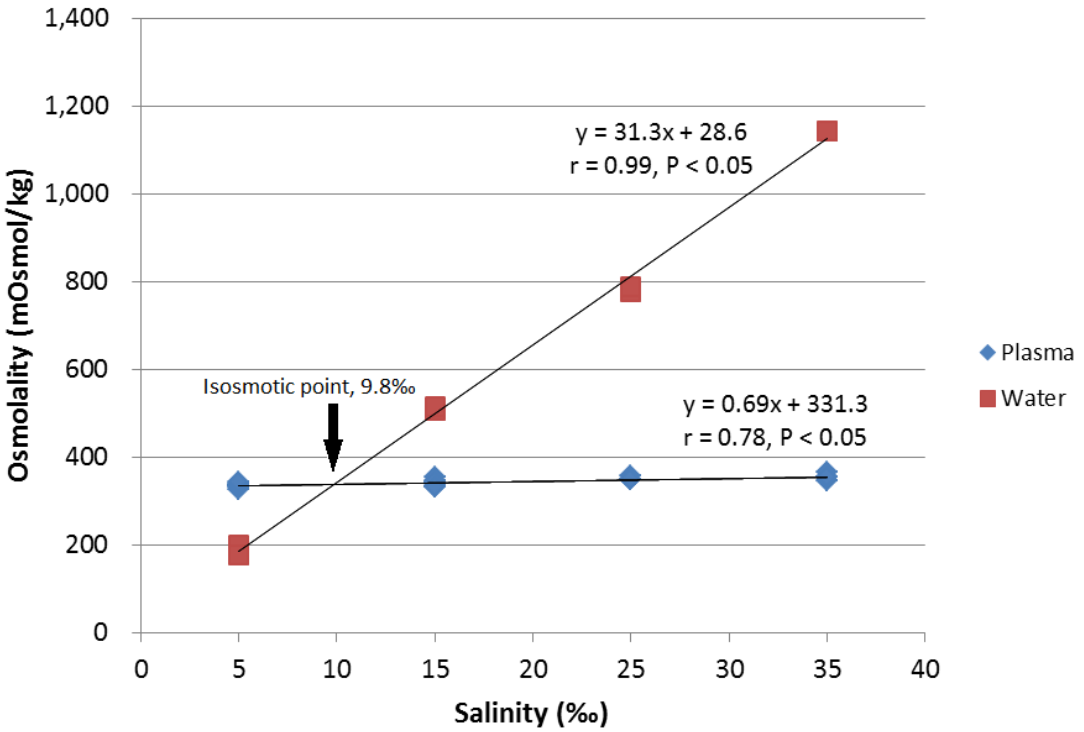
Salinity (‰)	Plasma osmolality[1] (mOsmol kg^{-1})	Culture water osmolality[2] (mOsmol kg^{-1})	Initial weight (g)	K	Final weight (g)	Weight gain (g)	SGR (% d^{-1})	Survival (%)	Moisture content[3] (%)	Ash content[3] (% of dry weight)
5	335.1 ± 5.3[b]	188.5 ± 14.8	17.9 ± 2.1	1.0 ± 0.1	29.4 ± 3.5	10.3 ± 4.1	1.0 ± 0.4	83.0 ± 0.0	76.9[a] ± 1.0	12.6 ± 1.4
15	340.4 ± 8.6[b]	510.0 ± 5.7	16.8 ± 1.2	1.0 ± 0.1	28.8 ± 1.1	11.1 ± 1.2	1.1 ± 0.1	88.7 ± 9.8	75.5[b] ± 0.5	12.8 ± 1.2
25	351.3 ± 4.0[a]	782.0 ± 7.1	17.3 ± 2.2	0.9 ± 0.1	26.7 ± 3.9	8.5 ± 2.3	0.9 ± 0.1	86.7 ± 9.8	75.0[b] ± 0.9	13.3 ± 1.3
35	354.8 ± 6.8[a]	1,143.5 ± 0.7	16.9 ± 3.6	0.9 ± 0.1	28.0 ± 4.3	11.3 ± 2.2	1.3 ± 0.4	100.0 ± 0.0	73.8[c] ± 0.7	13.6 ± 0.8
ANOVA *Pr* > F	0.0002	-	0.8794	0.0505	0.7978	0.5964	0.4711	0.0855	< 0.0001	0.4930

Means with different superscripts in the same column are significantly different ($P < 0.05$). Abbreviations: K = condition factor; SGR = specific growth rate.

[1]Means of blood samples taken from all individuals from each of 3 replicate tanks. Each sample was analyzed in duplicate.

[2]Means of culture water samples analyzed in duplicate.

[3]Means of duplicate samples of three pooled whole fish from each of three experimental tanks.



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365 Figure legends

366 **Fig. 1.** Regression lines between salinity and culture water or plasma osmolality of *C. othonopterus*
367 reared at different salinities. The point of intersection between the lines represents the estimated isosmotic
368 point (9.8‰).