Available online at www.sciencedirect.com
SCIENCE DIRECT®
Marine Environmental Research 62 (2006) S249–S252
MARINE ENVIRONMENTAL RESEARCH
www.elsevier.com/locate/marenvrev

Short communication

Reproductive endocrine dysfunction in Atlantic croaker exposed to hypoxia

Peter Thomas *, Md. Saydur Rahman, James A. Kummer, Susan Lawson

The University of Texas at Austin, Marine Science Institute, 750 Channel View Drive, Port Aransas, TX 78373, USA

Abstract

Although there is extensive evidence for impaired endocrine function in fishes exposed to environmental chemicals, information is currently lacking on reproductive endocrine effects of other environmental stressors such as hypoxia. The effects of ten weeks exposure to low dissolved oxygen (DO: 2.7 ppm and 1.7 ppm) on reproductive morphometric and endocrine responses in female Atlantic croaker (*Micropogonias undulatus*) were investigated in controlled laboratory studies, and compared to the effects observed in fish collected from hypoxic sites in Mobile Bay, Alabama. Exposure of croaker to moderate hypoxia during ovarian recrudescence, both in the laboratory and at the field sites, caused significant impairment of ovarian growth as well as decreased production of fully grown oocytes, resulting in dramatic reductions in the number of viable gametes (fecundity). Ovarian dysfunction was associated with significant decreases in endocrine indicators of the estrogen signaling pathway regulating production of vitellogenin, the yolk protein precursor sequestered by the growing oocytes. The results indicate that reproductive morphometric and endocrine biomarkers in croaker are sensitive to moderate hypoxia, and are potentially useful as early warning indicators of reproductive failure.

Keywords: Hypoxia; Atlantic croaker; Biomarkers; Reproductive function

* Corresponding author. Tel.: +1 361 749 6768; fax: +1 361 749 6777.
E-mail address: thomas@utmsi.utexas.edu (P. Thomas).

0141-1136/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marenvres.2006.04.031

There is mounting concern over the recent dramatic increase in the incidence of seasonal hypoxia in the northern Gulf of Mexico and other coastal regions of the world due to increased nutrient loading (Diaz, 2001). Many marine and estuarine species do not survive prolonged exposure to hypoxic conditions (dissolved oxygen, DO, <2 ppm), whereas other species such as Atlantic croaker (*Micropogonias undulatus*) appear to tolerate these conditions and remain in moderately hypoxic environments if food organisms are available (Bell and Eggleston, 2005; Eby and Crowder, 2002). However, even for these hypoxia-tolerant species, the long term effects of frequent and persistent exposure to sublethal low DO conditions are unknown. Gonadal recrudescence is one of the most sensitive stages of the reproductive cycle to stressors, and impairment of gametogenesis (e.g. a decrease in fecundity) can have serious consequences at higher levels of biological organization on the maintenance of population size. Therefore, the effects of chronic laboratory and field exposure to moderate hypoxia on reproductive endocrine function in female Atlantic croaker during the period of oocyte and ovarian growth (exogenous vitellogenesis) were investigated.

Adult one-year old female croaker, collected with a trawl, were transferred to nine 2000 L-recirculating sea water tanks (8 per tank) at the beginning of the ovarian cycle in September and maintained for ten weeks under ambient conditions of temperature (24 °C, decreasing to 23 °C) and photoperiod (12L:12D, decreasing to 11L:13D), and fed commercial pellets (5% body weight/day) to promote ovarian recrudescence (oocyte development is synchronous and is completed within a 10-week period). The DO levels were lowered in two-thirds of the tanks from 5.7 ppm (control: high DO; 80% saturation) to 2.7 ppm (moderate DO, 38% saturation) or 1.7 ppm (low DO, 24% saturation) over a period of a week by reducing tank aeration. DO concentrations were monitored continuously with oxygen meters and air flow adjusted when necessary to maintain DO levels within 0.02 ppm of the target concentrations. Water quality (ammonia, pH) was maintained and the fish continued feeding under these experimental conditions. At the end of the ten-week exposure period the ovaries had fully recrudesced in the control group (80% DO saturation), with a mean gonadosomatic index (GSI) of ~15, whereas ovarian growth was significantly decreased in a concentration dependent manner in the groups exposed to medium DO (mean GSI 6.9) and low DO (mean GSI 3.9, Table 1). Ovarian growth is primarily due to the sequestration of the yolk precursor protein, vitellogenin, by the growing oocytes under

Table 1
Effects of chronic exposure to hypoxia in the laboratory on reproductive and endocrine function in female Atlantic croaker

Parameter	*N*	Oxygen saturation		
		80%	38%	24%
GSI (%)[a]	8	15.17 ± 1.23	6.86 ± 1.63*	3.93 ± 1.13**
Estradiol-17β (ng/mL)	8	5.78 ± 0.77	3.45 ± 0.79*	0.821 ± 0.22**
Estrogen receptor mRNA[b]	8	54.61 ± 3.51	22.65 ± 5.67**	22.68 ± 4.95**
Vitellogenin (μg/mL)	8	1439 ± 127	1119 ± 154	487 ± 154**

All measurements are mean ± SEM. All data were analyzed using one-way analysis of variance (ANOVA) followed by Fisher's PLSD test.

[a] GSI = (gonad weight/body weight) × 100.

[b] Arbitrary units.

* $p < 0.05$, compared to 80%.

** $p < 0.0002$, compared to 80%.

the influence of gonadotropin. Hepatic vitellogenin production in turn is regulated by estradiol-17β through its regulation and activation of the estrogen receptor, ERα (Khan and Thomas, 1998). Therefore, we investigated whether the diminished ovarian growth observed after exposure to hypoxia was associated with disruption of the estrogen signaling pathway regulating vitellogenin production. Circulating levels of estradiol-17β measured by radioimmunoassay were significantly lower in the moderate and low DO groups compared to controls and this correlated with decreased hepatic expression of ERα mRNA as measured by RT-PCR (Hawkins et al., 2000), a key estrogen-regulated gene controlling vitellogenin production (Table 1). Moreover, these hypoxia-induced declines in estrogen signaling were associated with decreased circulating levels of vitellogenin measured by radioimmunoassay (Copeland and Thomas, 1989; Table 1). These laboratory results indicate that there is a clear relationship between the decrease in plasma estrogen levels in response to hypoxia and the subsequent signaling cascade leading to impaired oocyte and ovarian growth. This impaired ovarian function is associated with a dramatic decrease in the production of viable eggs (fecundity, results not shown), because only the fully grown oocytes are capable of producing viable post-yolk sac stage larvae. Thus, endocrine measures of vitellogenesis are potentially useful as early warning indicators of reduced fecundity, with possible long term population consequences.

The same suite of reproductive and endocrine responses were measured in female croaker collected from low DO and reference sites in Mobile Bay, Alabama, in October 2004, at an earlier stage of ovarian development to further evaluate their potential as indicators of reproductive output. A similar pattern of reproductive dysfunction was observed in croaker collected at hypoxic field sites to that observed in the laboratory study. DO was measured one meter from the bottom where the croaker congregate. DO was monitored continuously over several weeks and single measurements were also taken at the time of collection using oxygen meters (YSI 600 XLM multi-Parameter Water Quality Monitor). Gonadal growth (GSI) was significantly reduced in croaker collected at the two hypoxic sites (A&B; DO below 2.5 ppm from July-September and variable in October) in comparison to those from the normoxic sites (C&D; DO usually ~5 ppm from July to October, Table 2). The decrease in GSI at the hypoxic sites was associated with a significant decline

Table 2
Effects of chronic exposure to hypoxia at field sites in Mobile Bay, Alabama on reproductive and endocrine function in female Atlantic croaker

Parameter	*N*	Mobile Bay, sampling sites			
		A (hypoxia)	B (hypoxia)	C (normoxia)	D (normoxia)
GSI (%)[a]	34–41	2.83 ± 0.4*	2.9 ± 0.46*	4.41 ± 0.56	4.34 ± 0.61
Fully grown oocytes (%)	8	5.63 ± 4.83*	7.55 ± 4.15*	31.85 ± 7.79	31.12 ± 10.59
Estradiol-17β (ng/mL)	6–7	1.52 ± 0.16	1.47 ± 0.37	1.82 ± 0.34	1.28 ± 0.34
Estrogen receptor mRNA[b]	5–6	69.84 ± 5.1[c]	78.73 ± 4.69	78.52 ± 4.82	88.4 ± 3.2
Vitellogenin (μg/mL)	18–21	227.5 ± 43.03**	179.6 ± 43.27**	643.2 ± 75.37	468.5 ± 90.66

All measurements are mean ± SEM. All data were analyzed using one-way analysis of variance (ANOVA) followed by Fisher's PLSD test.

[a] GSI = (gonad weight/body weight) × 100.

[b] Arbitrary units.

[c] $p < 0.05$, compared to D.

* $p < 0.05$, compared to C.

** $p < 0.0001$, compared to C.

in the production of fully grown oocytes capable of producing viable offspring (determined by counting the number of large vitellogenic oocytes in histological sections of ovaries) as well as decreases in the circulating levels of vitellogenin and a trend of decreased ERα mRNA levels at sites A and B compared to D (Table 2).

Taken together, the results of the laboratory and field studies demonstrate that chronic exposure of Atlantic croaker to moderate hypoxia causes marked impairment of ovarian growth and the production of fully grown oocytes, which in turn is associated with dysfunction of the estrogen signaling cascade regulating the production of the yolk precursor lipoprotein, vitellogenin. Several of the reproductive endocrine responses that show a strong correlation with ovarian growth are potentially useful as early warning indicators of reduced fecundity and long term hazards to the maintenance of fish population size.

Acknowledgement

Supported by US EPA-STAR Grant No. R-82945801 to P.T.

References

Bell, G.W., Eggleston, D.B., 2005. Marine Biology 146, 761–770.
Copeland, P.A., Thomas, P., 1989. General and Comparative Endocrinology 7, 425–441.
Diaz, R.J., 2001. Journal of Environmental Quality 30, 275–281.
Eby, L.A., Crowder, L.B., 2002. Canadian Journal of Fisheries and Aquatic Sciences 59, 952–965.
Hawkins, M.B., Thornton, J.W., Crews, D., Skipper, J.K., Dotte, A., Thomas, P., 2000. Proceedings of the National Academy of Sciences, USA 97, 10751–10756.
Khan, I.A., Thomas, P., 1998. Ovarian cycle, teleost fish. In: Knobil, E., Neill, J.D. (Eds.), Encyclopedia of Reproduction, vol. 3. Academic Press, San Diego, CA, pp. 552–564.