Supporting Information

Quantitative Response-response Relationships Linking Aromatase Inhibition to Decreased Fecundity are Conserved Across Three Fishes with Asynchronous Oocyte Development

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Water Fadrozole Analysis. Concentrations of fadrozole were determined in the stock solution and in each aquaria a minimum of twice weekly. Water (900 µL) was collected from each aquaria and transferred to a deep well 96-well plate, followed by the addition of 100 µL baclofen (150 ng/mL) in water for use as an internal standard. A 2 µL injection was injected on an Agilent 1260 high performance liquid chromatograph (LC) coupled to an Agilent 6410 mass spectrometer (MS) with an electrospray ionization source (Agilent, Santa Clara, CA). Separations were achieved using an Agilent Poroshell EC-C18 (3.0 x 50 mm, 2.7 µm) column under isocratic conditions of 78:22 water:acetonitrile (ACN) at a flow rate of 0.4 mL/min. The retention time of fadrozole was 1.1 min monitoring ion transitions of 224 m/z to 82.1 m/z (quantifier ion) and 224 m/z to 81.1 m/z (qualifier ion) for fadrozole and 214 m/z to 151 m/z for baclofen. Fadrozole was quantified by an internal standard method using Agilent MassHunter Quantitative Analysis software (v. B.06.00). Limit of detection was approximately 0.50 µg/L of water. Mean (with standard deviation) recovery of matrix spiked samples across both studies was 101% (0.05%, n = 45) and duplicate agreement across both studies was 96.1% (0.03%, n = 77).

Plasma Fadrozole Analysis. Concentrations of fadrozole were determined in plasma samples consisting of pools of females from each aquaria. Fadrozole was extracted from plasma (ranging from 5 to 20 µL per sample) using Novum (Phenomenex, Torrance, CA) supported liquid extraction (SLE) cartridges. Briefly, plasma was mixed with 0.5 M ammonium hydroxide to a total volume of 200 µL. Samples were then loaded onto SLE cartridges, allowed to sit for 5 min, eluted with 1200 µL ethyl acetate, then evaporated to dryness under a stream of nitrogen. Extracts were resuspended in 100 µL of 30:70 ACN:water containing 0.5 µg/mL letrozole (for use as an internal standard) and analyzed by LC/MS using the system described for water

analysis, with minor modification. To better separate fadrozole from potential matrix interferences, a gradient separation of water:ACN (solvent A:solvent B) was used with a 20 µL injection volume. Gradient conditions began at 25% B, ramping to 75% B at 3.5 min, held at 95% B for 1 min, then equilibrated at 25% B for 2.5 min. The retention time of fadrozole was 2.6 min monitoring ion transitions of 224 m/z to 82.1 m/z (quantifier ion) and 224 m/z to 81.1 m/z (qualifier ion) for fadrozole and 286 m/z to 217 m/z for letrozole. Fadrozole was quantified by an internal standard method using Agilent MassHunter Quantitative Analysis software (v. B.06.00). Limit of detection was approximately 0.25 µg/L of plasma. Mean (with standard deviation) recovery of matrix spiked fetal bovine serum was 64.6% (11.6%, n = 10).

Table S1. Average measured concentrations of fadrozole in water and plasma.[a,b]

Species	Nominal Water (µg/L)	Measured Water (µg/L)	Measured Plasma (µg/L)	Bioconcentration Factor (BCF)
Japanese Medaka	0	< 0.50	< 0.25	-
	2	2.2 (± 0.02)	1.4 (± 0.3)	0.6
	10	11.5 (± 0.1)	11.5 (± 4)	1.0
	30	34.7 (± 0.4)	33 (± 3)	1.0
Zebrafish	0	< 0.50	< 0.25	-
	2	2.4 (± 0.08)	0.28 (± 0.05)	0.1
	10	11.9 (± 0.2)	2.5 (± 0.4)	0.2
	30	34.0 (± 0.7)	8.6 (± 0.6)	0.3

[a] Standard deviation shown in brackets.

[b] Detection limit was 0.5 µg fadrozole/L for water and 0.25 µg fadrozole/L for plasma.

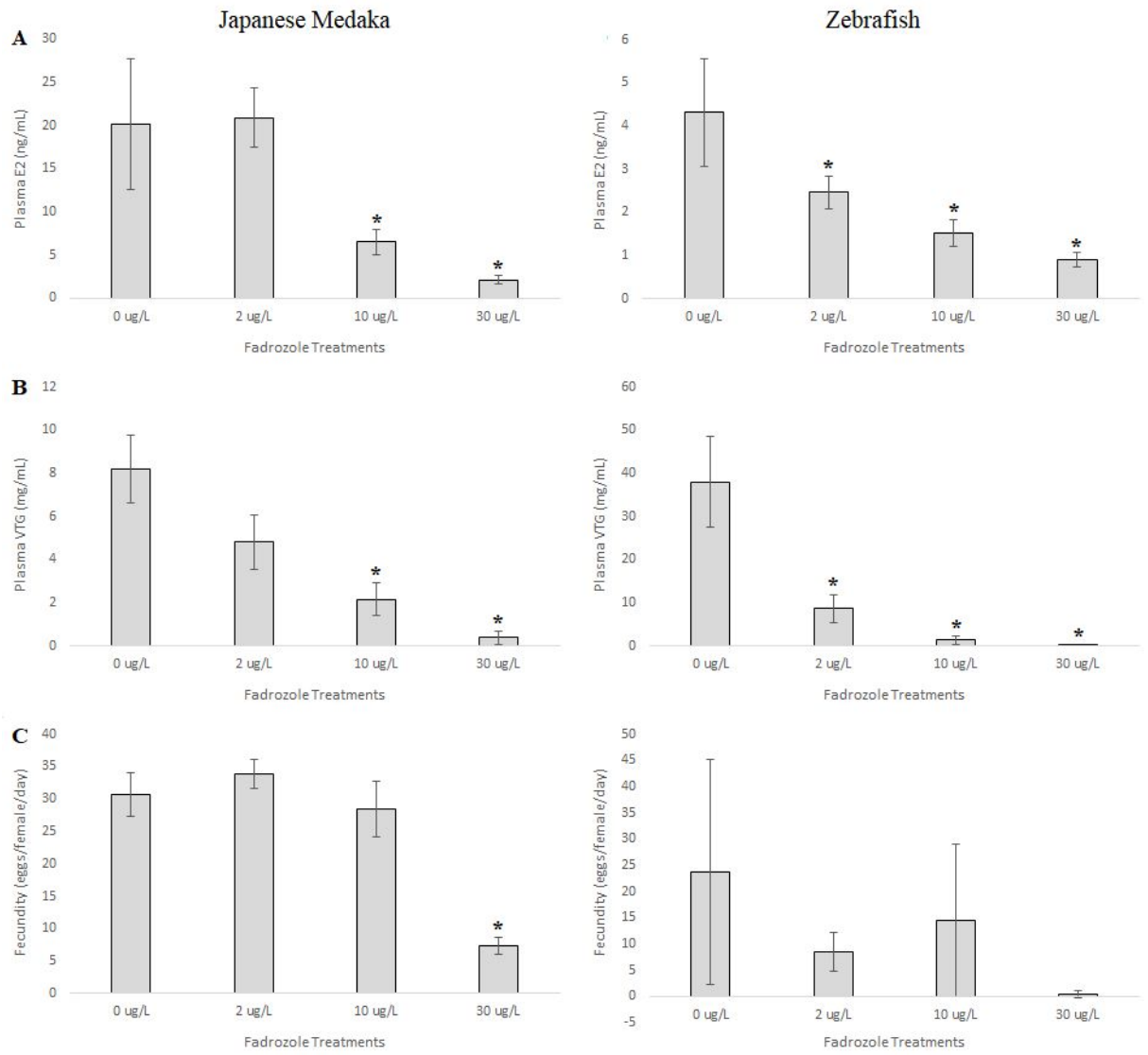

Figure S1. Measured plasma E2 (A), plasma VTG (B), and fecundity (C) for Japanese medaka or zebrafish exposed to one of four concentrations of fadrozole for 21 days. Data represent the mean ± standard deviation for Japanese medaka (n = 4) and zebrafish (n = 3). Statistical difference from 0 ug/L treatment group by use of analysis of variance (ANOVA) followed by Dunnet's post-hoc test ($p \leq 0.05$) is indicated (*). Normality of each dataset was evaluated by use of the Kolmogorov-Smirnov test and homogeneity of variance was determined by use of Levene's test. Data were log-transformed when necessary to meet assumptions, but measured values are shown.

Table S2. Summary of basal levels of plasma E2, plasma VTG, and fecundity measured in adult female fishes with asynchronous oocyte development.[a]

Family Name	Common Name	Species Name	Plasma E2 (ng/mL)	Plasma VTG (mg/mL)	Fecundity (egg/female/day)	Reference
Cyprinidae	Fathead Minnow	*Pimephales promelas*	4.4	15	21	This study
			6.1 (0.2 – 21)	17 (4.3 – 48)	21 (0 – 72)	1-3
	Zebrafish	*Danio rerio*	4.3	38	24	This study
			3.2 (1.0 – 8.0)	8.1 (0.5 – 25)	33 (25 – 45)	4-14
	Chinese Rare Minnow	*Gobiocypris rarus*	2.6 (1.2 – 4.0)	3.2 (0.5 – 7.5)	29 (22 – 32)	15-21
Adrianichthyidae	Japanese Medaka	*Oryzias latipes*	20	8.2	31	This study
			112 (29 – 230)	1.8 (0.03 – 5.0)	18 (6 – 78)	22-33
	Java Ricefish	*Oryzias javanicus*	-	-	6	34
	Marine Medaka	*Oryzias melastigma*	-	-	30	35
Cyprinodontidae	Sheepshead Minnow	*Cyprinodon variegatus*	-	7.7 (4 – 12)	82 (40 – 110)	36,37
Fundulidae	Mummichog	*Fundulus heteroclitus*	6	2	42	38
Melanotaeniidae	Murray Rainbowfish	*Melanotaenia fluviatilis*	2.6 (0.3 – 5.0)	-	-	39,40
Labridae	Cunner	*Tautogolabrus adspersus*	-	10	2000	41

[a] Stated values represent the mean across published studies with the range of individual study means indicted in brackets. Values with only one published study have no range indicated. No available information is indicated (-).

Table S3. Families of fishes containing one or more species with demonstrated record of asynchronous oocyte development.

Order	Family	Common Names	Reference
Atheriniformes	Melanotaeniidae	rainbowfishes	42
	Telmatherinidae	sail-fin silversides	43
Beloniformes	Adrianichthyidae	ricefishes	44
	Hemiramphidae	halfbeaks	45
Characiformes	Serrasalmidae	piranhas, silver dollars	46
Clupeiformes	Clupeidae	herrings, shads, sardines	47
	Engraulidae	anchovies	47
Cypriniformes	Cyprinidae	carps, minnows	2
Cyprinodontiformes	Cyprinodontidae	pupfishes	48
Gadiformes	Gadidae	cods	48
	Merluccidae	hakes	47
Gobiiformes	Eleotridae	sleeper gobies	49
	Oxudercidae	mudskippers	50
Istiophoriformes	Istiohoridae	marlins	51
	Xiphiidae	swordfishes	47
Myctophiformes	Myctophidae	lanternfishes	52
Perciformes	Blenniidae	combtooth blennies	48
	Caproidae	boarfishes	53
	Carangidae	jacks, pompanos	47
	Cheilodactylidae	morwongs	54
	Cyclopteridae	lumpfishes	48
	Emmelichthyidae	rovers	55
	Labridae	wrasses	56
	Lutjanidae	snappers	57
	Mullidae	goatfishes	58
	Sciaenidae	drums, croakers	59
	Scorpaenidae	scorpionfishes	60
	Sebastidae	rockfishes	61
	Sparidae	sea breams	62
Pleuronectiformes	Pleuronectidae	righteye flounders	63
	Soleidae	true soles	47
Scombriformes	Scombridae	mackerels, tunas	47
Siluriformes	Loricariidae	suckermouth catfishes	64
	Sisoridae	Asian catfishes	48
	Trichomycteridae	parasitic catfishes	65
Syngnathiformes	Syngnathidae	seahorses, pipefishes	48

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