

The following supplement accompanies the article

Use of DNA metabarcoding for stomach content analysis in the invasive lionfish *Pterois volitans* in Puerto Rico

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Marine Ecology Progress Series 000: 000–000 (2016)

Table S1. Metrics reported for all lionfish. Gonadal stages are IA = inactive, ED = early development, SM = sexually mature and GRAVID = females containing eggs. For sex, F = female, M = male, and IM = immature, where visual inspection could not identify sex. Weight is recorded with the stomach but without venomous spines. Length of the fish is recorded as total length (TL) and standard length (SL).

Lionfish ID	DATE COLLECTED	LOCATION	TL (mm)	SL (mm)	Weight (g)	Sex	Gonad Stage
PL001	1/JUN/2013	INSHORE	252	190	179	IM	IA
PL003	1/JUN/2013	INSHORE	222	170	115	IM	IA
PL004	1/JUN/2013	INSHORE	240	175	171	IM	IA
PL005	1/JUN/2013	INSHORE	255	193	201	IM	IA
PL006	1/JUN/2013	INSHORE	274	207	209	IM	IA
PL007	1/JUN/2013	INSHORE	290	221	302	IM	IA
PL008	1/JUN/2013	INSHORE	225	171	112	IM	IA
PL009	1/JUN/2013	INSHORE	165	122	41	IM	IA
PL010	1/JUN/2013	INSHORE	228	171	145	IM	IA
PL011	15/JUN/2013	INSHORE	237	179	131	IM	IA
PL012	15/JUN/2013	INSHORE	267	204	176	IM	IA
PL013	15/JUN/2013	INSHORE	255	196	199	IM	IA
PL014	15/JUN/2013	INSHORE	219	168	117	IM	IA
PL015	15/JUN/2013	INSHORE	172	134	57	IM	IA
PL016	15/JUN/2013	INSHORE	246	186	139	IM	IA
PL017	15/JUN/2013	INSHORE	256	196	163	IM	IA
PL018	15/JUN/2013	INSHORE	262	204	179	IM	IA
PL020	15/JUN/2013	INSHORE	232	179	148	IM	IA
PL021	29/JUN/2013	INSHORE	99	74	9	IM	IA
PL022	29/JUN/2013	INSHORE	134	95	25	IM	IA
PL-N-01	30/JUN/2013	INSHORE	N/A	71	8	IM	IA
PL-N-02	30/JUN/2013	INSHORE	N/A	60	5	IM	IA
PL-N-03	9/JUL/2013	INSHORE	275	193	239	IM	IA
PL-N-04	9/JUL/2013	INSHORE	221	162	111	IM	IA
PL-N-05	9/JUL/2013	INSHORE	237	174	153	F	ED

Lionfish ID	DATE COLLECTED	LOCATION	TL (mm)	SL (mm)	Weight (g)	Sex	Gonad Stage
PL-N-06	9/JUL/2013	INSHORE	252	190	218	IM	IA
PL-N-07	9/JUL/2013	INSHORE	244	174	154	IM	IA
PL-N-08	9/JUL/2013	INSHORE	230	165	137	IM	IA
PL-N-09	9/JUL/2013	INSHORE	320	239	438	IM	IA
PL-N-10	19/JUL/2013	OFFSHORE	270	214	251	F	GRAVID
PL-N-11	19/JUL/2013	OFFSHORE	242	180	154	F	GRAVID
PL-N-12	19/JUL/2013	OFFSHORE	255	189	184	M	ED
PL-N-13	19/JUL/2013	OFFSHORE	240	181	168	F	GRAVID
PL-N-14	19/JUL/2013	OFFSHORE	241	180	158	F	GRAVID
PL-N-15	19/JUL/2013	OFFSHORE	238	178	143	F	GRAVID
PL-N-16	19/JUL/2013	OFFSHORE	230	174	152	F	GRAVID
PL-N-17	30/JAN/2014	OFFSHORE	250	190	170	F	GRAVID
PL-N-18	30/JAN/2014	OFFSHORE	273	204	224	F	GRAVID
PL-N-19	30/JAN/2014	OFFSHORE	280	215	255	M	SM
PL-N-20	30/JAN/2014	OFFSHORE	265	195	224	F	GRAVID
PL-N-21	30/JAN/2014	OFFSHORE	370	284	541	M	SM
PL-N-22	30/JAN/2014	OFFSHORE	310	245	383	M	SM
PL-N-23	30/JAN/2014	OFFSHORE	250	192	166	F	GRAVID
PL-N-24	30/JAN/2014	OFFSHORE	230	175	146	F	GRAVID
PL-N-25	30/JAN/2014	OFFSHORE	335	260	428	M	SM
PL-N-26	30/JAN/2014	OFFSHORE	305	235	324	M	SM
PL-N-27	30/JAN/2014	OFFSHORE	255	195	186	F	GRAVID
PL-N-28	30/JAN/2014	OFFSHORE	290	230	309	M	SM
PL-N-29	30/JAN/2014	OFFSHORE	275	215	252	F	GRAVID
PL-N-30	30/JAN/2014	OFFSHORE	270	205	195	F	GRAVID
PL-N-31	30/JAN/2014	OFFSHORE	265	198	191	M	SM
PL-N-32	30/JAN/2014	OFFSHORE	235	173	139	F	GRAVID
PL-N-33	30/JAN/2014	OFFSHORE	240	183	175	F	GRAVID
PL-N-34	30/JAN/2014	OFFSHORE	245	185	177	F	GRAVID
PL-N-35	30/JAN/2014	OFFSHORE	250	195	171	F	GRAVID
PL-N-36	30/JAN/2014	OFFSHORE	275	205	256	F	GRAVID
PL-N-37	30/JAN/2014	OFFSHORE	243	175	153	F	GRAVID
PL-N-39	30/JAN/2014	OFFSHORE	295	224	278	M	SM
PL-N-40	30/JAN/2014	OFFSHORE	260	197	200	F	GRAVID
PL-N-41	30/JAN/2014	OFFSHORE	265	205	235	F	GRAVID
PL-N-42	30/JAN/2014	OFFSHORE	220	170	117	F	GRAVID
PL-N-43	30/JAN/2014	OFFSHORE	255	195	196	F	GRAVID
PL-N-44	30/JAN/2014	OFFSHORE	210	163	105	F	GRAVID

Table S2. Barcodes for all samples, both liquid and tissue portions of the stomach. Lionfish ID contains an “L” or “T” denoting liquid or tissue, respectively. The F# and R# refer to an identification label specific to this project. F & R tag identifies the three base tag attached to both ends of the sequence. The Forward and Reverse tags are the barcodes suggested by the Scripps Genomic facilities, and this project’s combinations of those codes specific to each sample.

Lionfish ID	F#	R#	F Tag	R Tag	Forward Tag	Reverse Tag
PLN06L	F2	R2	ATG	ATG	CGATGT	CGATGT
PL004T	F3	R5	ATG	ATG	TTAGGC	ACAGTG
PL022T	F1	R1	ATG	ATG	ATCACG	ATCACG
PLN41T	F1	R2	ATG	ATG	ATCACG	CGATGT
PLN34L	F1	R4	ATG	ATG	ATCACG	TGACCA
PLN39L	F1	R5	ATG	ATG	ATCACG	ACAGTG
PL022L	F1	R6	ATG	ATG	ATCACG	GCCAAT
PLN30T	F1	R7	ATG	ATG	ATCACG	CAGATC
PLN30L	F2	R1	ATG	ATG	CGATGT	ATCACG
PL021T	F2	R3	ATG	ATG	CGATGT	TTAGGC
PL004L	F2	R5	ATG	ATG	CGATGT	ACAGTG
PL005T	F2	R6	ATG	ATG	CGATGT	GCCAAT
PL005L	F2	R7	ATG	ATG	CGATGT	CAGATC
PL012L	F3	R4	ATG	ATG	TTAGGC	TGACCA
PL006T	F3	R6	ATG	ATG	TTAGGC	GCCAAT
PL019T	F3	R7	ATG	ATG	TTAGGC	CAGATC
PL019L	F4	R1	ATG	ATG	TGACCA	ATCACG
PL020T	F4	R2	ATG	ATG	TGACCA	CGATGT
PL021L	F4	R4	ATG	ATG	TGACCA	TGACCA
PL017T	F4	R5	ATG	ATG	TGACCA	ACAGTG
PL017L	F4	R6	ATG	ATG	TGACCA	GCCAAT
PL018L	F4	R7	ATG	ATG	TGACCA	CAGATC
PL018T	F5	R1	ATG	ATG	ACAGTG	ATCACG
PL013T	F5	R2	ATG	ATG	ACAGTG	CGATGT
PL001T	F5	R4	ATG	ATG	ACAGTG	TGACCA
PL008L	F5	R5	ATG	ATG	ACAGTG	ACAGTG
PLN43T	F5	R6	ATG	ATG	ACAGTG	GCCAAT
PLN44T	F5	R7	ATG	ATG	ACAGTG	CAGATC
PLN44L	F6	R1	ATG	ATG	GCCAAT	ATCACG
PL012T	F6	R2	ATG	ATG	GCCAAT	CGATGT
PL008T	F6	R4	ATG	ATG	GCCAAT	TGACCA
PL001L	F6	R5	ATG	ATG	GCCAAT	ACAGTG
PL003T	F6	R6	ATG	ATG	GCCAAT	GCCAAT
PL003L	F6	R7	ATG	ATG	GCCAAT	CAGATC
PL007T	F7	R1	ATG	ATG	CAGATC	ATCACG
PL007L	F7	R2	ATG	ATG	CAGATC	CGATGT

Lionfish ID	F#	R#	F Tag	R Tag	Forward Tag	Reverse Tag
PLN009L	F7	R3	ATG	ATG	CAGATC	TTAGGC
PLN14T	F7	R4	ATG	ATG	CAGATC	TGACCA
PLN04L	F7	R6	ATG	ATG	CAGATC	GCCAAT
PLN18L	F7	R7	ATG	ATG	CAGATC	CAGATC
PLN33L	F8	R1	ATG	ATG	ACTTGA	ATCACG
PLN18T	F8	R2	ATG	ATG	ACTTGA	CGATGT
PL010T	F8	R4	ATG	ATG	ACTTGA	TGACCA
PL010L	F8	R5	ATG	ATG	ACTTGA	ACAGTG
PL015T	F8	R6	ATG	ATG	ACTTGA	GCCAAT
PL015L	F8	R7	ATG	ATG	ACTTGA	CAGATC
PL016T	F9	R1	ATG	ATG	GATCAG	ATCACG
PL016L	F9	R2	ATG	ATG	GATCAG	CGATGT
PLN32L	F9	R3	ATG	ATG	GATCAG	TTAGGC
PLN19T(B)	F9	R4	ATG	ATG	GATCAG	TGACCA
PLN19L(A)	F9	R5	ATG	ATG	GATCAG	ACAGTG
PLN20L	F9	R6	ATG	ATG	GATCAG	GCCAAT
PLN25L	F9	R7	ATG	ATG	GATCAG	CAGATC
PLN40L	F10	R1	ATG	ATG	TAGCTT	ATCACG
PLN20T	F10	R2	ATG	ATG	TAGCTT	CGATGT
PLN36T	F10	R4	ATG	ATG	TAGCTT	TGACCA
PLN16L	F10	R6	ATG	ATG	TAGCTT	GCCAAT
PLN31T	F11	R2	ATG	ATG	GGCTAC	CGATGT
PLN34T	F11	R4	ATG	ATG	GGCTAC	TGACCA
PLN37T	F11	R5	ATG	ATG	GGCTAC	ACAGTG
PLN37L	F11	R6	ATG	ATG	GGCTAC	GCCAAT
PLN39T	F11	R7	ATG	ATG	GGCTAC	CAGATC
PLN42T	F12	R1	ATG	ATG	CTTGTA	ATCACG
PLN42L	F12	R2	ATG	ATG	CTTGTA	CGATGT
PLN10T	F12	R3	ATG	ATG	CTTGTA	TTAGGC
PLN29T	F12	R4	ATG	ATG	CTTGTA	TGACCA
PLN02L	F12	R5	ATG	ATG	CTTGTA	ACAGTG
PLN03L	F12	R6	ATG	ATG	CTTGTA	GCCAAT
PLN07L	F12	R7	ATG	ATG	CTTGTA	CAGATC
PLN10L	F13	R1	ATG	ATG	AGTCAA	ATCACG
PLN02T	F13	R2	ATG	ATG	AGTCAA	CGATGT
PLN24L	F13	R4	ATG	ATG	AGTCAA	TGACCA
PLN15T	F13	R5	ATG	ATG	AGTCAA	ACAGTG
PLN15L	F13	R6	ATG	ATG	AGTCAA	GCCAAT
PLN16T	F13	R7	ATG	ATG	AGTCAA	CAGATC
PLN17T	F14	R1	ATG	ATG	AGTTCC	ATCACG
PLN17L	F14	R2	ATG	ATG	AGTTCC	CGATGT
PLN19T(A)	F14	R3	ATG	ATG	AGTTCC	TTAGGC

Lionfish ID	F#	R#	F Tag	R Tag	Forward Tag	Reverse Tag
PLN21T	F14	R4	ATG	ATG	AGTTCC	TGACCA
PLN21L	F14	R5	ATG	ATG	AGTTCC	ACAGTG
PLN23T	F14	R6	ATG	ATG	AGTTCC	GCCAAT
PLN23L	F14	R7	ATG	ATG	AGTTCC	CAGATC
PLN24T	F15	R1	ATG	ATG	ATGTCA	ATCACG
PLN26T	F15	R2	ATG	ATG	ATGTCA	CGATGT
PLN26L	F15	R3	ATG	ATG	ATGTCA	TTAGGC
PLN05T	F15	R4	ATG	ATG	ATGTCA	TGACCA
PLN01T	F15	R5	ATG	ATG	ATGTCA	ACAGTG
PLN01L	F15	R6	ATG	ATG	ATGTCA	GCCAAT
PLN08T	F15	R7	ATG	ATG	ATGTCA	CAGATC
PLN08L	F3	R1	ATG	ATG	TTAGGC	ATCACG

Table S3. Barcodes for all samples, both liquid and tissue portions of the stomach. Lionfish ID contains an “L” or “T” denoting liquid or tissue, respectively. The F# and R# refer to an identification label specific to this project. F & R tag identifies the three base tag attached to both ends of the sequence in some cases. The Forward and Reverse tags are the barcodes suggested by Scripps, and the use of the original PCR primers for tagging purposes. Combinations are specific to each sample.

Lionfish ID	F #	R#	F Tag	R Tag	Forward Tag	Reverse Tag
PLN11T	F	R2	-	ATG	GGWACWGGWTGAACWGT WTAYCCYCC	CGATGT
PLN12T	F	R4	-	ATG	GGWACWGGWTGAACWGT WTAYCCYCC	TGACCA
PLN12L	F	R5	-	ATG	GGWACWGGWTGAACWGT WTAYCCYCC	ACAGTG
PLN13T	F	R6	-	ATG	GGWACWGGWTGAACWGT WTAYCCYCC	GCCAAT
PLN13L	F	R7	-	ATG	GGWACWGGWTGAACWGT WTAYCCYCC	CAGATC
PLN22T	F	R1	-	ATG	GGWACWGGWTGAACWGT WTAYCCYCC	ATCACG
PLN27T	F13	R	ATG	-	AGTCAA	TAIACYTCIGGRTGICCRAA RAAYCA
PLN28T	F14	R	ATG	-	AGTTCC	TAIACYTCIGGRTGICCRAA RAAYCA
PLN35T	F15	R	ATG	-	ATGTCA	TAIACYTCIGGRTGICCRAA RAAYCA
PLN35L	F3	R	ATG	-	TTAGGC	TAIACYTCIGGRTGICCRAA RAAYCA
PL013L	F1	R	ATG	-	ATCACG	TAIACYTCIGGRTGICCRAA RAAYCA
PLN04T	F2	R	ATG	-	CGATGT	TAIACYTCIGGRTGICCRAA RAAYCA
PLN07T	F4	R	ATG	-	TGACCA	TAIACYTCIGGRTGICCRAA RAAYCA
PLN09T	F5	R	ATG	-	ACAGTG	TAIACYTCIGGRTGICCRAA RAAYCA
PL014L	F6	R	ATG	-	GCCAAT	TAIACYTCIGGRTGICCRAA RAAYCA
PLN14L	F7	R	ATG	-	CAGATC	TAIACYTCIGGRTGICCRAA RAAYCA
PLN22L	F8	R	ATG	-	ACTTGA	TAIACYTCIGGRTGICCRAA RAAYCA
PLN25T	F9	R	ATG	-	GATCAG	TAIACYTCIGGRTGICCRAA RAAYCA
PLN27L	F10	R	ATG	-	TAGCTT	TAIACYTCIGGRTGICCRAA RAAYCA
PLN29L	F12	R	ATG	-	CTTGTA	TAIACYTCIGGRTGICCRAA RAAYCA