

Understanding Fish Larvae's Feeding Biology to Improve Aquaculture Feeding Protocols

Supplementary Material

Table S1. Feeding incidence (FInc) of *Sparus aurata* larvae from different experimental conditions ¹. Values indicate the percentage of larvae with food in the gut for each experimental unit (n = 15).

Trial	Age	Time (min)	FInc %	Trial	Age	Time (min)	FInc %
Time	27	0	0.00	0 lux	38	10	13.33
Time	27	0	0.00	0 lux	38	10	40.00
Time	27	0	0.00	0 lux	38	10	33.33
Time	27	10	66.67	0 lux	38	30	40.00
Time	27	10	46.67	0 lux	38	30	93.30
Time	27	10	66.67	0 lux	38	30	80.00
Time	27	20	73.33	0 lux	49	10	100.00
Time	27	20	73.33	0 lux	49	10	100.00
Time	27	20	86.67	0 lux	49	10	100.00
Time	27	30	86.67	0 lux	49	30	100.00
Time	27	30	73.33	0 lux	49	30	100.00
Time	27	30	40.00	0 lux	49	30	100.00
Time	27	40	46.67	400 lux	38	10	100.00
Time	27	40	86.67	400 lux	38	10	100.00
Time	27	40	80.00	400 lux	38	10	100.00
Time	27	80	100.00	400 lux	38	30	100.00
Time	27	80	73.33	400 lux	38	30	100.00
Time	27	80	86.67	400 lux	38	30	100.00
Time	42	0	0.00	400 lux	49	10	100.00
Time	42	0	0.00	400 lux	49	10	100.00
Time	42	0	0.00	400 lux	49	10	100.00
Time	42	10	100.00	400 lux	49	30	100.00
Time	42	10	100.00	400 lux	49	30	100.00
Time	42	10	100.00	400 lux	49	30	100.00
Time	42	20	100.00	1000 lux	38	10	100.00
Time	42	20	100.00	1000 lux	38	10	100.00
Time	42	20	100.00	1000 lux	38	10	100.00
Time	42	30	100.00	1000 lux	38	30	100.00
Time	42	30	100.00	1000 lux	38	30	100.00
Time	42	30	100.00	1000 lux	38	30	100.00
Time	42	40	100.00	1000 lux	49	10	100.00

Time	42	40	100.00	1000 lux	49	10	100.00
Time	42	40	100.00	1000 lux	49	10	100.00
Time	42	80	100.00	1000 lux	49	30	100.00
Time	42	80	100.00	1000 lux	49	30	100.00
Time	42	80	100.00	1000 lux	49	30	100.00

¹ Temperature trial was not included in the table since FInc was 100% for all larvae.

Table S2. Kruskal–Wallis non-parametric test statistical analysis of *Sparus aurata* larvae feeding intensity under different experimental conditions. Bold and italicized values indicate significant differences.

FI (n prey)			
1. TIME AFTER FEEDING		27 DPH	42 DPH
Chi-Square		38,756	123,661
df		4	4
Asymp. Sig.		0.000	0.000
LIGHT	Time (minutes)	38 DPH	49 DPH
Chi-Square	10	91.403	91.403
df		2	2
Asymp. Sig.		0.000	0.000
Chi-Square	30	87.901	29.844
df		2	2
Asymp. Sig.		0.000	0.000
TEMPERATURE		41 DPH	
Chi-Square	10	40.602	
df		2	
Asymp. Sig.		0.000	
Chi-Square	30	29.120	
df		2	
Asymp. Sig.		0.000	

Table S3. Paired comparisons using Mann–Whitney U test when significant differences were identified with Kruskal–Wallis test; A) time after feeding at 27 and 42 days post-hatching (DPH); B) light levels (0, 400, and 1000 lux) at 38 and 49 DPH, 10 and 30 min after feeding; C) temperature (17, 19, and 25 °C) at 41 DPH, 10 and 30 min after feeding. Bold and italicized values indicate significant differences.

(A)

Age (DPH)	Time (min)	Statistical descriptor	20	30	40	80
27	10	Mann–Whitney U	727.5	771.5	713	341.5
		Z	-2.343	-1.992	-2.47	-5.473
		Asymp. Sig. (2-tailed)	0.019	0.046	0.014	0.000
	20	Mann–Whitney U		1006	974.5	480.5
		Z		-0.053	-0.31	-4.31
		Asymp. Sig. (2-tailed)		0.958	0.757	0.000
	30	Mann–Whitney U			980.5	525.5
		Z			-0.261	-3.951
		Asymp. Sig. (2-tailed)			0.794	0.000
	40	Mann–Whitney U				506
		Z				-4.107
		Asymp. Sig. (2-tailed)				0.000
42	10	Mann–Whitney U	427.5	176.5	128	34.5
		Z	-4.722	-6.747	-7.138	-7.893
		Asymp. Sig. (2-tailed)	0.000	0.000	0.000	0.000
	20	Mann–Whitney U		543.5	448.5	143.5
		Z		-3.785	-4.31	-7.013
		Asymp. Sig. (2-tailed)		0.000	0.000	0.000
	30	Mann–Whitney U			802.5	307.5
		Z			-1.695	-5.69
		Asymp. Sig. (2-tailed)			0.09	0.000
	40	Mann–Whitney U				447.5
		Z				-4.559
		Asymp. Sig. (2-tailed)				0.000

Table S3. (cont) Paired comparisons using Mann–Whitney U test when significant differences were identified with Kruskal–Wallis test; A) time after feeding at 27 and 42 days post-hatching (DPH); B) light levels (0, 400, and 1000 lux) at 38 and 49 DPH, 10 and 30 min after feeding; C) temperature (17, 19, and 25 °C) at 41 DPH, 10 and 30 min after feeding. Bold and italicized values indicate significant differences.

(B)

				10 min	
Age (DPH)	Time (min)	Lux		400	1000
38	10	0	Mann–Whitney U	3.5	0
			Z	-8.334	-8.362
			Asymp. Sig. (2-tailed)	0.000	0.000
		400	Mann–Whitney U		818.5
			Z		-1.567
			Asymp. Sig. (2-tailed)		0.117
49	10	0	Mann–Whitney U	168	237
			Z	-6.815	-6.258
			Asymp. Sig. (2-tailed)	0.000	0.000
		400	Mann–Whitney U		744.5
			Z		-2.163
			Asymp. Sig. (2-tailed)		0.031
30 min					
38	30	0	Mann–Whitney U	16.5	2
			Z	-8.055	-8.171
			Asymp. Sig. (2-tailed)	0.000	0.000
		400	Mann–Whitney U		967
			Z		-0.367
			Asymp. Sig. (2-tailed)		0.713
49	30	0	Mann–Whitney U	475.5	387
			Z	-4.334	-5.048
			Asymp. Sig. (2-tailed)	0.000	0.000
		400	Mann–Whitney U		939.5
			Z		-0.589
			Asymp. Sig. (2-tailed)		0.556

Minutes		38 and 49 (age)		
		0	400	1000
10	Mann–Whitney U	1	0	0
	Z	-8.353	-8.172	-8.172
	Asymp. Sig. (2-tailed)	0.000	0.000	0.000
30	Mann–Whitney U	0	0.000	0
	Z	-8.187	-8.172	-8.172
	Asymp. Sig. (2-tailed)	0.000	0.000	0.000
age		10 and 30 (minutes)		
		0	400	1000
38	Mann–Whitney U	467.5	360.5	461.5
	Z	-4.708	-5.264	-4.449
	Asymp. Sig. (2-tailed)	0.000	0.000	0.000
49	Mann–Whitney U	65.5	17.000	7.5
	Z	-7.642	-8.034	-8.11
	Asymp. Sig. (2-tailed)	0.000	0.000	0.000

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Table S3 (cont.) Paired comparisons using Mann–Whitney U test when significant differences were identified with Kruskal–Wallis test; A) time after feeding at 27 and 42 days post-hatching (DPH); B) light levels (0, 400, and 1000 lux) at 38 and 49 DPH, 10 and 30 min after feeding; C) temperature (17, 19, and 25 °C) at 41 DPH, 10 and 30 min after feeding. Bold and italicized values indicate significant differences.

(C)

Min			19 °C	25 °C
10	17 °C	Mann–Whitney U	945	301
		Z	-0.545	-5.743
		Asymp. Sig. (2-tailed)	0.586	0.000
	19 °C	Mann–Whitney U		.000
		Z		-8.172
		Asymp. Sig. (2-tailed)		0.000
30	17 °C	Mann–Whitney U	959.5	447
		Z	-0.428	-4.564
		Asymp. Sig. (2-tailed)	0.669	0.000
	19 °C	Mann–Whitney U		424.500
		Z		-4.746
		Asymp. Sig. (2-tailed)		0.000

10 and 30 min			
	17 °C	19 °C	25 °C
Mann–Whitney U	193	288.5	302.500
Z	-6.615	-5.844	-5.730
Asymp. Sig. (2-tailed)	0.000	0.000	0.000

Table S4. Summary of the statistical analysis of one-way ANOVA or two-way ANOVA used for comparisons of CCK levels of *Sparus aurata* larvae under different experimental conditions: A) time after feeding at 27 and 42 days post-hatching (DPH); B) light levels (0, 400, and 1000 lux) at 38 and 49 DPH, 10 and 30 min after feeding; C) temperature (17, 19, and 25 °C) at 41 DPH, 10 and 30 min after feeding. Bold and italicized values indicate significant differences.

(A) Feeding over time

Feeding over time		ANOVA Sig.
Comparing among different times at 27		0.189
Comparing among different times at 42		0.008
Comparing between 27 and 42 DPH at:	0	0.055
	10	0.559
	20	0.051
	30	0.002
	80	0.139

(B) Light intensity

Light intensity	Minutes after feeding	Age	Light	Age * Light
Two-way ANOVA	10	0.000	0.125	0.358
	30	0.000	0.000	0.000 *

* Since significant interaction was observed between factors, a one-way ANOVA was used.

Light intensity			ANOVA sig.
Among different treatments	38	10 min	0.847
		30 min	0.000
Among different treatments	49DPH	10 min	0.136
		30 min	0.032
At 0 lux	38	10 and 30 min	0.001
At 400 lux			0.568
At 1000 lux			0.909
At 0 lux	49DPH	10 and 30 min	0.672
At 400 lux			0.000
At 1000 lux			0.006

(C) Temperature

Trial	Temperature	Time after feeding	T*t
Two-way ANOVA	0.900	0.677	0.911

Table S5. Two-way ANOVA results for digestive enzyme activities of *Sparus aurata* fed at different temperatures: T – temperature; t – time in minutes. Bold and italicized values indicate significant differences.

Digestive enzymes	T °C	Time min	T x t
Trypsin	0.441	0.130	0.475
Amylase	0.460	0.736	0.630
Alkaline phosphatase	0.001	0.085	0.377
Aminopeptidase	0.240	0.201	0.512

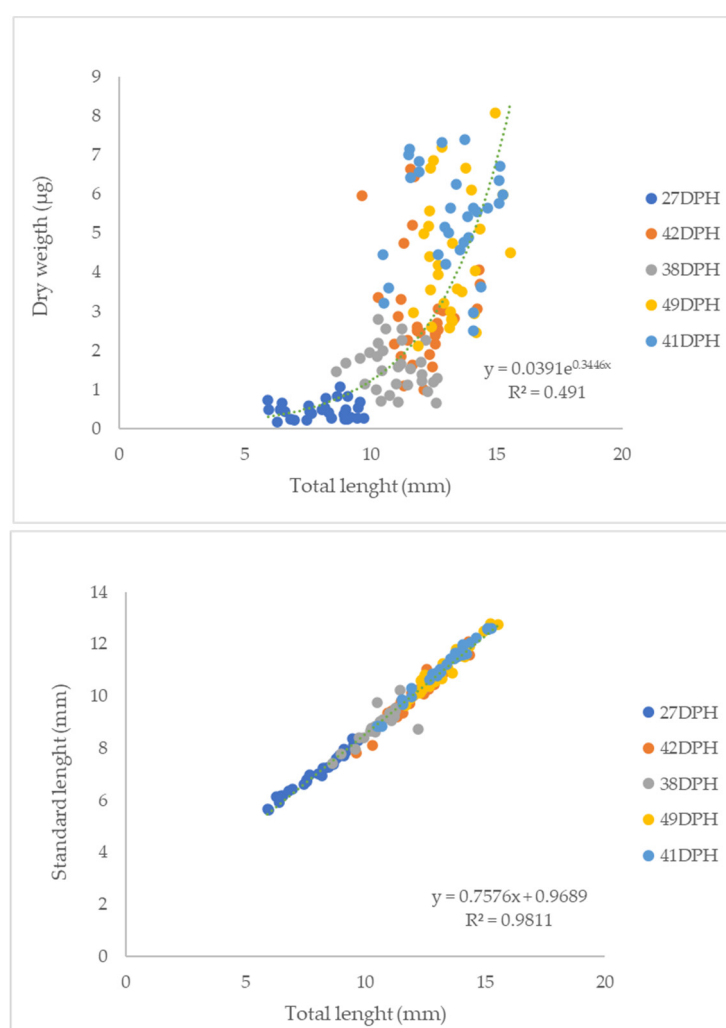


Figure S1. Plot of dry weight with total length (A) and standard length and total length (B) for *Sparus aurata* larvae in the short-term trials (time—27 and 42 DPH; light—38 and 49 DPH; temperature—41 DPH).

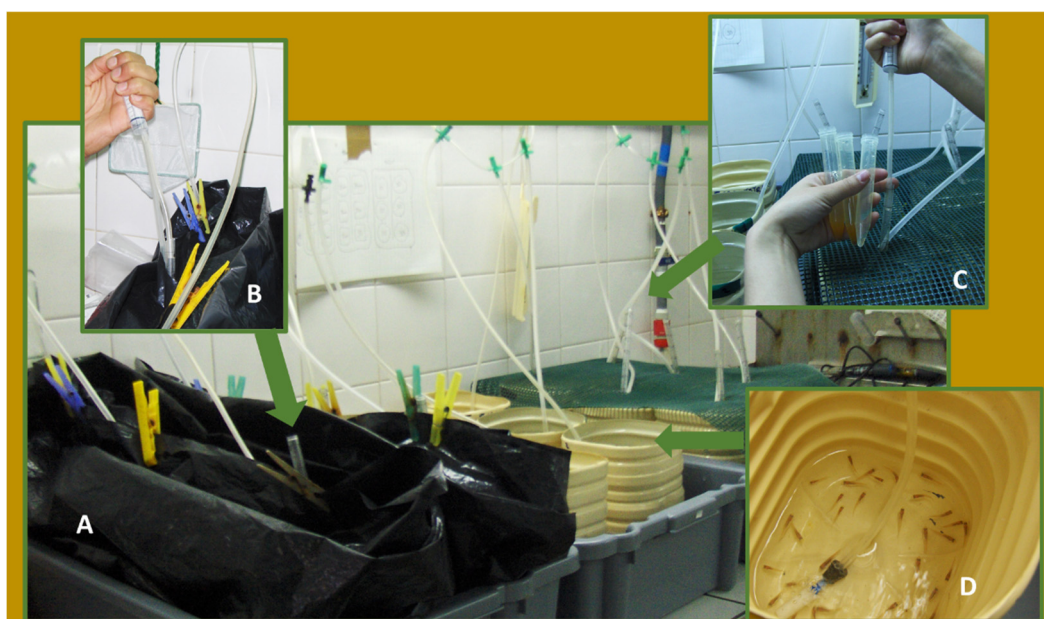


Figure S2. Experimental set-up for light trial (A); fish larvae were fed using a syringe, to maintain light treatment (B,C). Detail of gilthead seabream post-larvae in experimental unit during sampling.