Title: Ocean acidification increases fatty acids levels of larval fish

Authors: Carlos Díaz-Gil[1,2,*], Ignacio A. Catalán[1], Miquel Palmer[1], Cynthia K. Faulk[3] and Lee A. Fuiman[3]

Affiliations:

[1] Instituto Mediterráneo de Estudios Avanzados, IMEDEA (CSIC-UIB), C/Miquel Marqués 21, 07190, Esporles, Illes Balears, Spain

[2] Laboratori d'Investigacions Marines i Aqüicultura, LIMIA (Balearic Government), C/Eng. Gabriel Roca 69, 07157, Port d'Andratx, Illes Balears, Spain

[3] Marine Science Institute, The University of Texas at Austin, Port Aransas, TX 78373, USA

* Corresponding to: cdiaz@imedea.uib-csic.es; phone: 0034 971610829

Keywords: ocean acidification, fatty acids, larval fish, *Sciaenops ocellatus*

Accepted in Biology Letters (22/06/2015) corrected pre-processing copy

Abstract:

Rising levels of anthropogenic carbon dioxide in the atmosphere are acidifying the oceans and producing diverse and important effects on marine ecosystems, including the production of fatty acids (FAs) by primary producers and their transfer through food webs. FAs, particularly essential FAs, are necessary for normal structure and function in animals and influence composition and trophic structure of marine food webs. To test the effect of ocean acidification on the FA composition of fish, we conducted a replicated experiment in which larvae of the marine fish red drum (*Sciaenops ocellatus*) were reared under a climate change scenario of elevated CO_2 levels (2100 µatm) and under current control levels (400 µatm). We found significantly higher whole-body levels of FAs, including 9 of the 11 essential FAs, and altered relative proportions of FAs in the larvae reared under higher levels of CO_2. Consequences of this effect of ocean acidification could include alterations in performance and survival of fish larvae and transfer of FAs through food webs.

Introduction:

Anthropogenic CO_2 in the atmosphere is dissolving into the oceans and acidifying them [1–3]. This decline in pH is expected to be greater in coastal areas where the effects will be especially important because of the high biodiversity, presence of areas of special conservation interest (e.g., coral reefs), or importance to seafood production [2,3]. Ocean acidification (OA) has been demonstrated to affect fundamental processes of early stages of fish, such as growth and survival [4], behaviour [5,6], auditory and olfactory function [7,8], otolith calcification [9], and even cause tissue damage [10]. The effect of OA on the synthesis or metabolic pathways of important biomolecules is less known.

Fatty acids (FAs) are biomolecules that are structural components of cell membranes, metabolized for energy, or stored for future use. FAs are designated as *X*:*Y*ω*Z*, where *X* is the number of carbon atoms, *Y* is the number of double bonds and ω*Z* indicates the position of the first double bond from the methyl terminus [11,12]. Some FAs can be assembled from precursors, but most animals cannot synthesize *de novo* enough of the long-chain (≥ 18 carbon atoms) FAs that contain multiple double bonds to meet their physiological requirements [12]. These highly unsaturated FAs are manufactured by primary producers, and animals obtain them almost exclusively from their diet. For that reason, they are known as essential FAs (EFAs) [11]. Some EFAs (e.g., eicosapentaenoic acid [EPA, 20:5ω3] and arachidonic acid [ARA, 20:4ω6]) are precursors of other important biomolecules, such as eicosanoids and prostaglandins. Moreover, EFAs are indispensable for development of neural and retinal tissues and proper neural functioning in many animals, including humans [13].

Changes in the EFA composition of organisms at lower trophic levels due to ocean acidification are currently under scrutiny [14,15]. For example the majority of primary production in the oceans is expected to shift from diatoms to other microalgae (e.g., *Phaeocystis*) [16], and as a result EFA production in the oceans is expected to decrease [12,16]. Beyond this shift in

availability of EFAs, OA may alter the way that animals process and store FAs obtained from their diet, which would have consequences for the animal's survival and the transfer of FAs to higher trophic levels [17-18]. We selected the marine fish red drum (*Sciaenops ocellatus*) as a model species to conduct the first experiments on the potential effect of ocean acidification on FA composition of fish larvae. Red drum is a species of high economic importance in aquaculture and recreational fisheries, inhabiting estuarine and coastal areas on the east coast of North America which are endangered by global change and ocean acidification [2], and the species has been the subject of intense research on the dynamics and ecological significance of variations in FA composition of eggs and larvae [17-18].

Material and Methods:

Ocean acidification experiment

Two batches of fertilized red drum eggs were collected from natural spawns from a single tank of broodstock. Each batch was divided into two treatment levels: control CO_2 level (400 µatm) and high CO_2 level (2100 µatm), and reared at a constant temperature (27.4 ± 0.3°C) and salinity (36.6 ± 0.9 ppt). Both high CO_2 and control groups (2 tanks per group) were fed equally with the same highly enriched FA diet. At day 23 posthatching all the fish surviving in each tank were euthanized and measured. For each tank, all the fish were then combined, lyophilized, and homogenized, and then three samples were analysed for FAs composition using gas chromatography [18], measuring a total of 27 FAs.

Statistical analyses

The number of fish remaining in each tank was compared between groups using a Mann-Whitney U Test; fish length distribution in each tank was compared across treatments using PERMANOVA, and a t-test was used to compare total FA content. For each fatty acid, ANOVA or a Wilcoxon signed-rank test was used on raw or log-transformed FA concentrations and relative % for comparisons of the control and high CO_2 groups (see Tables S1 and S2). A redundancy analysis (RDA) was performed on the complete FA composition for control and high CO_2 groups, including egg batch as a factor. Statistical analyses were performed using the R package (www.r-project.org) [19].

Results:

There was a significant effect of OA treatment on the number of fish remaining (Mann-Whitney U Test, $P \leq 0.01$), with 61.7 % more fish in the high CO_2 group. Mean fish length was significantly smaller (25.3 %) in fish reared under high CO_2 conditions (PERMANOVA, *Pseudo-F*: 26.7; $P \leq 0.001$). Total FA content was significantly higher in the high CO_2 group (T test, $t_{9.77}$: -4.2, $P \leq 0.01$). Analyses of individual FAs (expressed as mg g^{-1} dry weight) showed that 19 of the 27 FAs had significantly higher values under high CO_2 (Fig. 1 *A*; Table S1), including higher levels for 9 of the 11 EFAs. Expressed on a relative basis (% total FAs), 9 FAs were disproportionately higher under elevated CO_2; 9 were disproportionately lower; and 9 remained relatively unchanged (Fig. 1 *B*; Table S2).

The RDA interaction model (model adjusted R^2 = 50%) showed that CO_2 level was a significant factor but egg batch was not (CO_2 level: $F_{1,8}$: 10.1, P = < 0.001; Egg batch: $F_{1,8}$: 1.4, P > 0.05) (Fig. 2, Table S3). Only the first RDA axis was significant, explaining 54.3% of the model variance (Fig. 2, Table S3).

Discussion:

Our results identify a strong effect of elevated CO_2 levels (2100 µatm of CO_2, predicted for the year 2300 [1]) on the FA content of larval fish. This agrees with recent work that shows an increase in total lipid content of cod (*Gadus morhua*) larvae under high levels of CO_2 (4200 µatm) [10]. That prior study reported no differences in the composition of the lipids, while we found 19 of the 27 FAs analysed to be significantly elevated at only 50% of the level of CO_2 used in the prior study. Further, the relative amounts of different fatty acids varied under elevated CO_2, with some FAs increasing significantly and others decreasing significantly. While ocean acidification usually jeopardizes larval survival [4,6], it is worth noting that the increase in some of these FAs may improve ecological performance of the larvae since recent studies of red drum larvae have shown that higher levels of some EFAs are positively correlated with larval escape performance [17,20]. Three EFAs – DHA, EPA, and ARA – are especially important to larval fish physiology [11] and were expected to be closely regulated, regardless of ocean acidification. Surprisingly, DHA and ARA increased on a weight basis and EPA and ARA decreased on a percentage basis under OA conditions. These changes could have significant impacts on physiological functions.

Higher tissue levels of EFAs could potentially result from increased absorption, synthesis, biotransformation, and/or storage. Some marine fish can manufacture EFAs but their capacity is limited [11,18,21]. Increased absorption of ingested EFAs is an unlikely explanation because absorption of other nutrients would have increased as well, leading to more growth, but larval growth decreased while tissue levels of EFAs increased. Rather, we suggest that red drum larvae under this stressor deposit a larger portion of the ingested FAs in tissues. Nevertheless the mechanism through which this response to OA operates is unknown.

Ocean acidification can affect organisms and ecosystems by altering FA production (e.g., changes in communities of primary producers [22]) or through effects on uptake of FAs by

higher trophic levels [23]. We showed that storage of many FAs by red drum larvae increases and that FA proportions differ under ocean acidification. The consequences of these changes in FAs in tissues on ecological performance of fish larvae and on food web structure and function need to be explored for a more complete understanding of the impacts of OA on marine ecosystems.

Author contributions:

C.D.G., I.A.C., M.P., C.F., and L.A.F. designed the experiment; C.D.G., performed the experiment. C.D.G., and C.F., carried out the fatty acid composition analysis; L.A.F., M.P., and C.D.G., analyzed data. C.D.G., I.A.C., M.P., and L.A.F., wrote the manuscript. All authors discussed the results and contributed to the final version of the manuscript.

Ethical guidelines:

Procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Texas at Austin under Animal Use Protocols AUP-2013-00041, AUP-2013-00155, and AUP-2012-00133.

Acknowledgements:

We thank Andrew J. Esbaugh, Benjamin Walther, John Mohan, Jeff Kaiser, Andrew Kang and Genesok Oh for their assistance.

Funding statement:

CDG was funded by FPI-INIA-2012, this manuscript was financed by the research project REC^2 (grant#CTM2011-23835). Contribution 1705 of the University of Texas Marine Science Institute. Authors declare no competing interest.

References:

1. Caldeira, K. & Wickett, M. E. 2005 Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *J. Geophys. Res.* **110**. (doi:10.1029/2004JC002671)

2. Rhein, M. et al. 2013 Observations: Ocean. In *IPCC Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (eds T. F. Stocker D. Qin G.-K. Plattner M. Tignor S. K. Allen J. Boschung A. Nauels Y. Xia V. Bex & P. M. Midgley), pp. 255–316. Cambridge, United Kingdom and New York, USA: Cambridge University Press.

3. Doney, S. C. et al. 2012 Climate change impacts on marine ecosystems. *Ann. Rev. Mar. Sci.* **4**, 11–37. (doi:10.1146/annurev-marine-041911-111611)

4. Baumann, H., Talmage, S. C. & Gobler, C. J. 2012 Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. *Nat. Clim. Chang.* **2**, 38–41. (doi:10.1038/nclimate1291)

5. Welch, M. J., Watson, S.-A., Welsh, J. Q., McCormick, M. I. & Munday, P. L. 2014 Effects of elevated CO2 on fish behaviour undiminished by transgenerational acclimation. *Nat. Clim. Chang.* **4**, 1086–1089. (doi:10.1038/nclimate2400)

6. Dixson, D. L., Munday, P. L. & Jones, G. P. 2010 Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. *Ecol. Lett.* **13**, 68–75. (doi:10.1111/j.1461-0248.2009.01400.x)

7. Simpson, S. D., Munday, P. L., Wittenrich, M. L., Manassa, R., Dixson, D. L., Gagliano, M. & Yan, H. Y. 2011 Ocean acidification erodes crucial auditory behaviour in a marine fish. *Biol. Lett.* **7**, 917–20. (doi:10.1098/rsbl.2011.0293)

8. Munday, P. L., Dixson, D. L., Donelson, J. M., Jones, G. P., Pratchett, M. S., Devitsina, G. V & Døving, K. B. 2009 Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 1848–52. (doi:10.1073/pnas.0809996106)

9. Checkley, D. M., Dickson, A. G., Takahashi, M., Radich, J. A., Eisenkolb, N. & Asch, R. 2009 Elevated CO2 enhances otolith growth in young fish. *Science* **324**, 1683. (doi:10.1126/science.1169806)

10. Frommel, A. Y., Maneja, R., Lowe, D., Malzahn, A. M., Geffen, A. J., Folkvord, A., Piatkowski, U., Reusch, T. B. H. & Clemmesen, C. 2011 Severe tissue damage in Atlantic cod larvae under increasing ocean acidification. *Nat. Clim. Chang.* **2**, 42–46. (doi:10.1038/nclimate1324)

11. Tocher, D. R. 2003 Metabolism and functions of lipids and fatty acids in teleost fish. *Rev. Fish. Sci.* **11**, 107–184.

12. Brett, M. T. & Müller-Navarra, D. C. 1997 The role of highly unsaturated fatty acids in aquatic foodweb processes. *Freshw. Biol.* **38**, 483–499. (doi:10.1046/j.1365-2427.1997.00220.x)

13. Crawford, M. A. & Broadhurst, C. L. 2012 The role of docosahexaenoic and the marine food web as determinants of evolution and hominid brain development: the challenge for human sustainability. *Nutr. Health* **21**, 17–39. (doi:10.1177/0260106012437550)

14. Torstensson, A., Hedblom, M., Andersson, J., Andersson, M. X. & Wulff, A. 2013 Synergism between elevated pCO2 and temperature on the Antarctic sea ice diatom Nitzschia lecointei. *Biogeosciences* **10**, 6391–6401. (doi:10.5194/bg-10-6391-2013)

15. Leu, E., Daase, M., Schulz, K. G., Stuhr, A. & Riebesell, U. 2013 Effect of ocean acidification on the fatty acid composition of a natural plankton community. *Biogeosciences* **10**, 1143–1153. (doi:10.5194/bg-10-1143-2013)

16. Desvilettes, C. & Bec, A. 2009 Formation and transfter of fatty acids in aquatic microbial food webs: role of heterotrophic protists. In *Lipids in Aquatic Organisms* (eds M. T. Arts M. T. Brett & M. J. Kainz), pp. 25–42. New York: Springer. (doi:10.1007/978-0-387-89366-2)

17. Fuiman, L. A. & Ojanguren, A. F. 2011 Fatty acid content of eggs determines antipredator performance of fish larvae. *J. Exp. Mar. Bio. Ecol.* **407**, 155–165. (doi:10.1016/j.jembe.2011.06.004)

18. Faulk, C. K. & Holt, G. J. 2008 Biochemical composition and quality of captive-spawned cobia Rachycentron canadum eggs. *Aquaculture* **279**, 70–76. (doi:10.1016/j.aquaculture.2008.03.050)

19. Team, R. D. C. 2011 *R: A language and environment for statistical computing.* Vienna, Austria.

20. Perez, K. O. & Fuiman, L. A. 2015 Maternal diet and larval diet influence survival skills of larval red drum Sciaenops ocellatus. *J. Fish Biol.* **86,** 1286-1304**.**. (doi:10.1111/jfb.12637)

21. Norambuena, F., Morais, S., Estévez, A., Bell, J. G., Tocher, D. R., Navarro, J. C., Cerdà, J. & Duncan, N. 2013 Dietary modulation of arachidonic acid metabolism in senegalese sole (Solea senegalensis) broodstock reared in captivity. *Aquaculture* **372-375**, 80–88. (doi:10.1016/j.aquaculture.2012.10.035)

22. Wynn-Edwards, C., King, R., Davidson, A., Wright, S., Nichols, P., Wotherspoon, S., Kawaguchi, S. & Virtue, P. 2014 Species-Specific Variations in the Nutritional Quality of Southern Ocean Phytoplankton in Response to Elevated pCO2. *Water* **6**, 1840–1859. (doi:10.3390/w6061840)

23. Litzow, M. A., Bailey, K. M., Prahl, F. G. & Heintz, R. 2006 Climate regime shifts and reorganization of fish communities: The essential fatty acid limitation hypothesis. *Mar. Ecol. Prog. Ser.* **315**, 1–11. (doi:10.3354/meps315001)

Figure captions:

Fig. 1. Mean concentrations of FAs (mg FA g^{-1} dry weight) (*A*) and mean relative % of FAs (*B*); in red drum larvae reared under control (red) and high CO_2 (blue) conditions. Error bars are one standard error (s.e.). Asterisks indicate significant differences (see Tables S1 and S2) (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Fig. 2: Ordination plot of RDA results. Letters and numbers identify CO_2 level and replicate number (C = control (400 µatm); T = high CO_2 (2100 µatm)). Circles represent the FAs measured in red drum larvae. Coloured polygons enclose each treatment group. Arrow shows the direction and intensity of the effect of high CO_2 on FA composition.

Figure 1

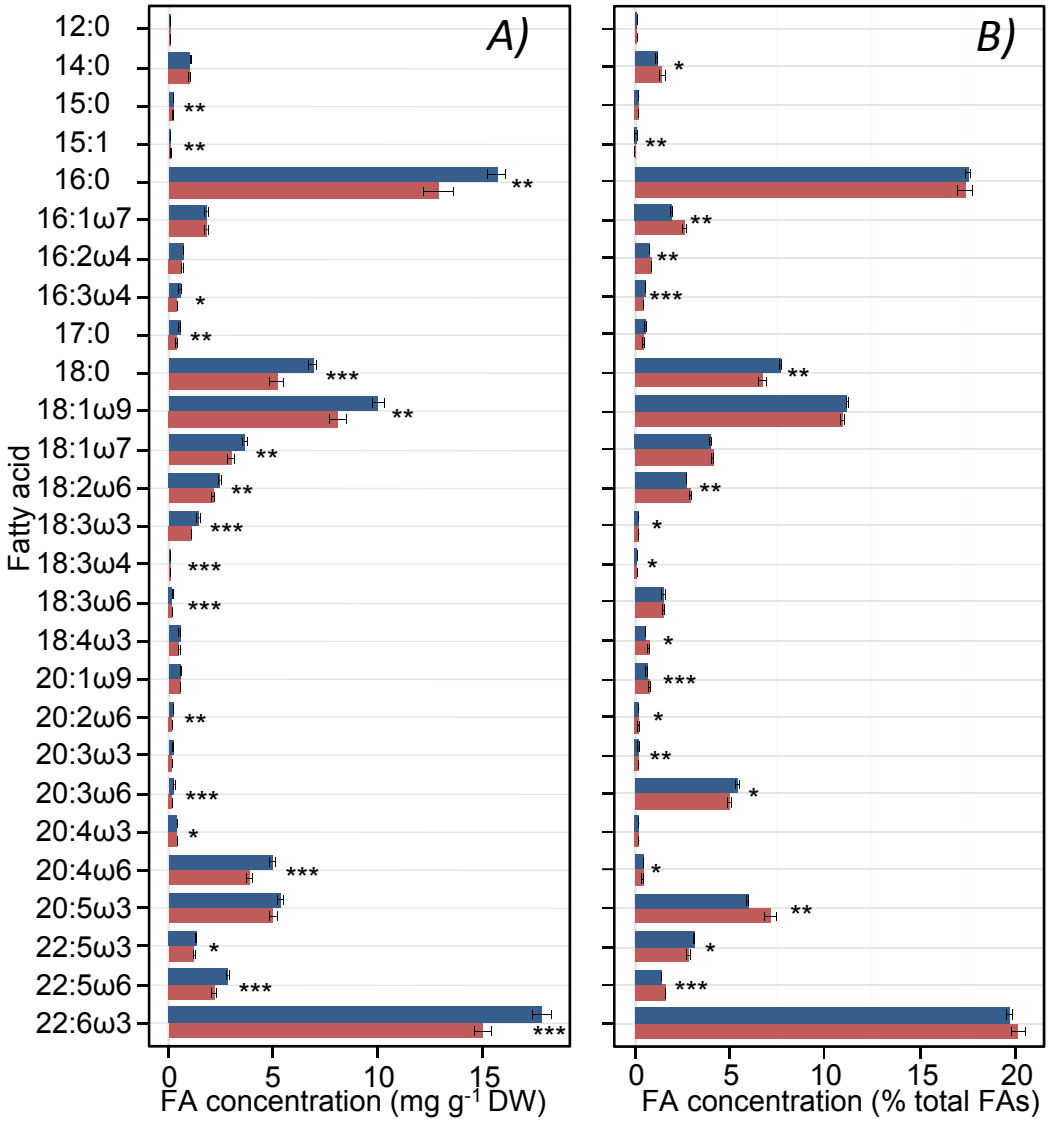
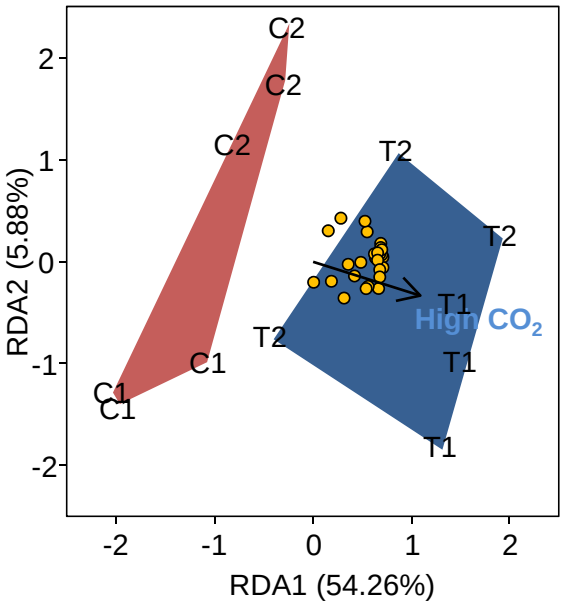

A)
B)
Fatty acid
12:0
14:0
15:0
15:1
16:0
16:1ω7
16:2ω4
16:3ω4
17:0
18:0
18:1ω9
18:1ω7
18:2ω6
18:3ω3
18:3ω4
18:3ω6
18:4ω3
20:1ω9
20:2ω6
20:3ω3
20:3ω6
20:4ω3
20:4ω6
20:5ω3
22:5ω3
22:5ω6
22:6ω3
0
5
10
15
FA concentration (mg g-1 DW)
0
5
10
15
20
FA concentration (% total FAs)

Figure 2


C2
C2
C2
T2
T2
T1
High CO_2
T2
T1
T1
C1
C1
C1
2
1
0
-1
-2
-2
-1
0
1
2
RDA2 (5.88%)
RDA1 (54.26%)

Ocean acidification increases fatty acids levels of larval fish

Supplementary material

Supplementary Table 1: Univariate differences in fatty acid composition on a weight basis (mg FA g^{-1} dry weight). Effect of treatment was assessed using ANOVA (log-transformed data when needed) or Wilcoxon tests. Significant results are denoted in bold and marked with * P <0.05; ** P < 0.01; and *** P < 0.001.

Fatty acid	Control		High CO_2		Transformation/ Analysis	P	
	Mean	s.e.	Mean	s.e.			
12:0	0.048	0.003	0.059	0.006	ANOVA	0.114	
14:0	1.007	0.050	1.064	0.037	ANOVA	0.384	
15:0	0.206	0.010	0.262	0.009	ANOVA	**0.002**	**
15:1	0.041	0.009	0.100	0.020	Log/ANOVA	**0.010**	**
16:0	12.910	0.728	15.719	0.420	ANOVA	**0.007**	**
16:1ω7	1.804	0.096	1.818	0.051	ANOVA	0.904	
16:2ω4	0.648	0.037	0.717	0.026	ANOVA	0.164	
16:3ω4	0.390	0.019	0.548	0.059	Log/ANOVA	**0.016**	*
17:0	0.382	0.027	0.521	0.014	Log/ANOVA	**0.003**	**
18:0	5.172	0.340	6.920	0.199	Wilcoxon test	**0.004**	***
18:1ω9	8.116	0.407	10.016	0.265	ANOVA	**0.003**	**
18:1ω7	3.000	0.137	3.655	0.104	ANOVA	**0.003**	**
18:2ω6	2.114	0.068	2.464	0.073	ANOVA	**0.005**	**
18:3ω3	1.104	0.017	1.421	0.061	ANOVA	**0.001**	***
18:3ω4	0.071	0.004	0.102	0.003	ANOVA	**0.001**	***
18:3ω6	0.150	0.007	0.199	0.006	ANOVA	**0.001**	***
18:4ω3	0.508	0.030	0.521	0.017	ANOVA	0.710	
20:1ω9	0.555	0.028	0.573	0.024	ANOVA	0.649	
20:2ω6	0.164	0.019	0.245	0.011	ANOVA	**0.005**	**
20:3ω3	0.185	0.011	0.221	0.023	ANOVA	0.196	
20:3ω6	0.185	0.013	0.270	0.013	ANOVA	**0.001**	***
20:4ω3	0.365	0.006	0.407	0.012	ANOVA	**0.013**	*
20:4ω6	3.879	0.178	4.959	0.145	ANOVA	**0.001**	***
20:5ω3	5.001	0.172	5.376	0.161	ANOVA	0.142	
22:5ω3	1.194	0.038	1.318	0.035	ANOVA	**0.038**	*
22:5ω6	2.181	0.101	2.828	0.084	ANOVA	**0.001**	***
22:6ω3	15.047	0.422	17.850	0.482	ANOVA	**0.001**	***

Supplementary Table 2: Univariate differences in relative fatty acid composition (% total fatty acids). Effect of treatment was assessed using ANOVA (log-transformed data when needed) or Wilcoxon tests. Significant results are denoted in bold and marked with * P <0.05; ** P < 0.01; and *** P < 0.001 with higher proportions identified by ↑ and lower proportions by ↓.

Fatty acid	Control		High CO_2		Transformation/ Analysis	P		
	Mean	s.e.	Mean	s.e.				
12:0	0.067	0.006	0.063	0.006	ANOVA	0.696		
14:0	1.492	0.121	1.182	0.023	ANOVA	**0.031**	*	↓
15:0	0.277	0.004	0.292	0.007	ANOVA	0.097		
15:1	0.045	0.008	0.110	0.023	Log/ANOVA	**0.005**	**	↑
16:0	17.398	0.381	17.537	0.138	ANOVA	0.740		
16:1ω7	2.600	0.118	2.018	0.017	Wilcoxon test	**0.005**	**	↓
16:2ω4	0.907	0.019	0.803	0.017	ANOVA	**0.002**	**	↓
16:3ω4	0.498	0.018	0.582	0.005	ANOVA	**0.001**	***	↑
17:0	0.515	0.030	0.607	0.065	Wilcoxon test	0.199		
18:0	6.767	0.223	7.747	0.063	ANOVA	**0.002**	**	↑
18:1ω9	10.953	0.155	11.168	0.054	ANOVA	0.220		
18:1ω7	4.093	0.044	4.080	0.019	ANOVA	0.789		
18:2ω6	2.928	0.041	2.733	0.027	ANOVA	**0.003**	**	↓
18:3ω3	0.202	0.002	0.223	0.002	Wilcoxon test	**0.003**	*	↑
18:3ω4	0.095	0.002	0.113	0.002	Wilcoxon test	**0.004**	*	↑
18:3ω6	1.520	0.053	1.557	0.062	ANOVA	0.663		
18:4ω3	0.753	0.063	0.578	0.010	ANOVA	**0.021**	*	↓
20:1ω9	0.817	0.033	0.643	0.017	Log/ANOVA	**0.001**	***	↓
20:2ω6	0.207	0.023	0.277	0.009	Log/ANOVA	**0.014**	*	↑
20:3ω3	0.242	0.014	0.303	0.010	ANOVA	**0.006**	**	↑
20:3ω6	5.027	0.117	5.468	0.089	ANOVA	**0.013**	*	↑
20:4ω3	0.272	0.014	0.247	0.021	ANOVA	0.344		
20:4ω6	0.512	0.021	0.450	0.007	ANOVA	**0.017**	*	↓
20:5ω3	7.132	0.337	5.940	0.071	ANOVA	**0.006**	**	↓
22:5ω3	2.817	0.086	3.125	0.051	ANOVA	**0.012**	*	↑
22:5ω6	1.637	0.026	1.463	0.013	ANOVA	**0.001**	***	↓
22:6ω3	20.168	0.368	19.717	0.179	ANOVA	0.295		

Supplementary Table 3: Results of RDA interaction model by factors and constrained axis computed for fatty acid composition of red drum larvae exposed to control and high levels of CO_2 (*Treatment*) for n = 2 replicate experiments (*Batch*). Only *Treatment* and axis *RDA1* explained a significant amount of the variance of multivariate fatty acid composition.

		Eigenvalue	Proportion explained	Variance	F	P	
Factors	Treatment			12.36	10.06	< 0.001	***
	Batch			1.68	1.37	0.23	
	Treatment × Batch			3.15	2.56	0.08	
	Residual			9.82			
Axis	RDA1	14.65	0.54	14.65	11.93	0.005	**
	RDA2	1.59	0.06	1.59	1.29	0.280	
	RDA3	0.94	0.03	0.94	0.77	0.550	
	Residual			9.82			