The following supplement accompanies the article

Oyster larvae as a potential first feed for small-mouthed ornamental larval fish

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Effects of parental diet on the fatty acid composition of *Saccostrea glomerata* trochophores

Materials and methods

During December 2016 120 adult *S. glomerata* were sourced from a commercial supplier in Urunga, NSW. These oysters were distributed into fifteen 22 l plastic tanks at a density of eight oysters per tank. Tanks were randomly assigned one of three diet treatments, with five replicate tanks per diet. The three diet treatments were live *Proteomonas sulcata*, *Pavlova* sp. algal paste (Pavlova 1800, Reed Mariculture, Campbell, CA, USA), and *Pavlova* sp. algal paste supplemented with the commercial lipid emulsion Selco S.presso (INVE Aquaculture, Salt Lake City, UT, USA) at a ratio of 4:1 by volume. All tanks were fed 0.8 g dry weight algae per tank three times per day. Tanks were flushed every night with 25ºC FSW at 0.5 l min^{-1} to remove waste.

After 12 wk, 1–4 female and 1–3 male oysters from three replicate tanks per diet treatment were able to be thermally induced to spawn in individual 750 ml plastic containers. Eggs and sperm within each replicate tank were pooled into 5 l containers. Sperm was added incrementally to containers holding eggs and fertilization was checked microscopically. When > 90% of eggs had been fertilized, as evidenced by a fertilization envelope, embryos from each replicate tank were collected and washed in a 15 µm wet sieve before being added to a 1 l cylindro-conical culture tank containing gently aerated FSW (~25°C) for ~8 h to develop to the trochophore stage. Trochophores from each replicate were then condensed into 50 ml falcon tubes and centrifuged at 3000 rpm for 3 min. Excess water was removed and samples were frozen at -80ºC prior to fatty acid analyses. Excess oyster trochophores from wild broodstock (see section 2.2) were harvested for fatty acid analyses using the same method.

To test whether the nutritional quality of oyster larvae could be manipulated by altering the diet of their parents, the fatty acid profiles of *S. glomerata* trochophores from wild and differently fed adults were analysed. Lipids were extracted from samples of 1.5–2.7 × 10^7 oyster trochophores from wild oyster broodstock, and 1.1–3.7 × 10^7 oyster trochophores from the aforementioned three adult diet treatments. Lipids were extracted using a 1:2:0.9 dichloromethane (DCM) – methanol - MilliQ water solution (Bligh & Dyer 1959). Phase was broken by adding a 1:1 DCM - saline water solution and left for 4 h. The

lower DCM layer was then concentrated in a rotary evaporator, and transferred to 2 mL vials where the remaining DCM was evaporated on a heat plate while being blown down under N gas. Samples were then sealed and stored at –80ºC before being transported to the Analytical Research Laboratory (Southern Cross University, Lismore, Australia) for methylation and gas chromatographic analysis as per Kanthilatha et al. (2014) with modifications.

Lipids were methylated by first washing with 2 mL of hexane and transferring into reaction vials. A 1 mL aliquot of 0.5 M solution of NaOH in methanol was added and heated at 100ºC for 10 min. Reaction vials were left to cool to ~40ºC before adding 1 mL of 14% boron triflouride in methanol solution and heating at 100ºC for 30 min. 1 mL of n-hexane and 5 mL of saturated sodium chloride solution was then added to the methylated fatty acids. 1 ml of hexane was removed and transferred to a HPLC injection vial and blown off with N gas. The sample was then reconstituted with 500 µL of hexane. Test samples were analysed for fatty acid methyl esters on an Agilent 6890N Gas Chromatograph (GC), equipped with a Flame Ionisation Detector (FID) and an Agilent 6890 split/splitless injector. The inlet and FID were maintained at 220ºC and 260ºC respectively. Samples were injected onto a BPX70 (70% cyanopropyl polysilphenylenesiloxane, 50 mm × 0.22 mm × 0.25 mm) SGE, capillary column. The GC oven temperature was programmed to commence at 100ºC for 5 min before being increased at a rate of 5ºC min^{-1} up to 240ºC, with He as the carrier gas. One microlitre injections were made, with a split ratio of 200:1, and a column flow of 1.3 mL min^{-1}. Peak retention time and areas were recorded and fatty acid methyl esters were identified by correlation of retention time to a well-characterized comprehensive mixture of fatty acid methyl ester standards (Supelco 37 component FAME mix, Sigma Cat. No. 24056). Fatty acid methyl esters identified by GCFID were further verified by GC mass spectrometry (MS) where the same chromatographic conditions for the GCFID method were used. The GCMS employed includes an Agilent 6890 GC equipped with an Agilent 5973 Mass Selective Detector. Mass Spectra were recorded at 70 eV ionization voltage over the mass range of 35e550 amu. Compound identification was verified using Mass spectra libraries (Wiley 275 and NIST98).

Results

S. glomerata trochophores from parents fed *P. sulcata* had lower levels of arachidonic acid (ARA) ($F_{3,8} = 6.646$, $p = 0.015$) and total n-6 PUFA ($F_{3,8} = 54.055$, $p < 0.001$) than those in all other treatments (Table S1). Trochophores from wild parents had higher levels of eicosapentaenoic (EPA) ($F_{3,8} = 5.335$, $p = 0.026$) and total n-3 PUFA ($F_{3,8} = 6.751$, $p = 0.014$) than those from parents fed *P. sulcata*, while EPA and n-3 PUFA in trochophores from parents fed *Pavlova* sp. paste and *Pavlova* supplemented with Selco S.presso was not different to all other treatments (Table S1). Trochophores from parents fed *Pavlova* sp. paste supplemented with Selco S.presso had the highest levels of docosahexaenoic acid (DHA), followed by trochophores from wild parents, then parents fed *P. sulcata* ($F_{3,8} = 36.179$, $p < 0.001$) (Table 1). DHA in trochophores from parents fed *Pavlova* sp. was not different to wild parents and parents fed *Pavlova* sp. paste supplemented with Selco S.presso. Trochophores from wild parents and parents fed *P. sulcata* had lower DHA:EPA ratios than those from parents fed *Pavlova* sp. paste or *Pavlova* supplemented with Selco S.presso ($F_{3,8} = 28.783$, $p < 0.001$) (Table S1). There was no difference in the EPA:ARA ratio among treatments ($F_{3,8} =$

1.494, p = 0.288) (Table S1). The remaining effects of the four different adult *S. glomerata* diet treatments on the fatty acid composition within trochophore offspring are summarised in Table S1.

Table S1. Fatty acid composition of *Saccostrea glomerata* trochophores from wild parents (Wild), and broodstock fed *Proteomonas sulcata* (*P. sulcata*), *Pavlova* sp. algal paste (Pavlova), or *Pavlova* sp. paste supplemented with Selco S.presso (Pav. + Selco). Fatty acid data are means ± SE, $n = 3$ and expressed as mg g lipid^{-1}. For each fatty acid, concentrations with the same letters are not significantly different according to 1-way ANOVA in SPSS v24.0 followed by Tukey's HSD test ($p < 0.05$).

Fatty Acid	Wild	*P. sulcata*	Pavlova	Pav. + Selco
C12:0	0.03 ± 0.00^{ac}	0.05 ± 0.00^{a}	0.01 ± 0.01^{b}	0.03 ± 0.00^{c}
C14:0	3.15 ± 0.27^{a}	4.63 ± 0.04^{b}	2.45 ± 0.08^{c}	3.27 ± 0.12^{a}
C15:0	0.52 ± 0.02^{a}	0.57 ± 0.01^{ab}	0.70 ± 0.02^{c}	0.62 ± 0.01^{bc}
C16:0	21.85 ± 0.41	21.47 ± 0.15	20.75 ± 0.41	21.68 ± 0.20
C17:0	1.49 ± 0.04^{a}	1.61 ± 0.00^{b}	1.80 ± 0.01^{c}	1.63 ± 0.02^{b}
C18:0	6.34 ± 0.17^{a}	6.56 ± 0.08^{a}	7.96 ± 0.16^{b}	6.99 ± 0.15^{a}
C20:0	0.14 ± 0.01^{a}	0.19 ± 0.01^{b}	0.13 ± 0.02^{a}	0.10 ± 0.01^{a}
C21:0	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.02	0.04 ± 0.00
C22:0	0.11 ± 0.01	0.07 ± 0.00	0.11 ± 0.01	0.11 ± 0.01
C24:0	0.22 ± 0.02	0.22 ± 0.00	0.25 ± 0.01	0.20 ± 0.01
Total SFA	33.89 ± 0.90	35.40 ± 0.27	34.18 ± 0.35	34.67 ± 0.12
C14:1	0.27 ± 0.03^{a}	0.47 ± 0.01^{b}	0.34 ± 0.05^{a}	0.25 ± 0.02^{a}
C15:1	0.13 ± 0.00^{a}	0.11 ± 0.00^{b}	0.15 ± 0.00^{c}	0.14 ± 0.00^{ac}
C16:1	2.43 ± 0.38^{a}	5.08 ± 0.04^{b}	1.99 ± 0.12^{a}	2.52 ± 0.20^{a}
C17:1	0.10 ± 0.05^{a}	0.30 ± 0.00^{b}	0.14 ± 0.01^{a}	0.15 ± 0.01^{a}
C18:1n-9 (Trans)	0.23 ± 0.05^{ab}	0.15 ± 0.09^{a}	0.40 ± 0.01^{b}	0.33 ± 0.03^{ab}
C18:1n-9	3.30 ± 0.08^{ab}	2.91 ± 0.02^{a}	3.70 ± 0.13^{b}	3.65 ± 0.09^{b}
C18:1	1.75 ± 0.07^{a}	1.60 ± 0.03^{a}	2.19 ± 0.12^{b}	1.68 ± 0.03^{a}
C20:1	0.29 ± 0.01^{a}	0.29 ± 0.00^{a}	0.33 ± 0.01^{b}	0.35 ± 0.01^{b}
C22:1n-9	1.00 ± 0.02^{a}	1.18 ± 0.00^{b}	1.02 ± 0.01^{a}	1.01 ± 0.01^{a}
C24:1	0.04 ± 0.00^{a}	0.04 ± 0.00^{a}	0.06 ± 0.00^{b}	0.03 ± 0.00^{c}
Total MUFA	9.54 ± 0.49^{a}	12.14 ± 0.19^{b}	10.33 ± 0.29^{a}	10.12 ± 0.18^{a}
C18:2n-6 (Trans)	0.05 ± 0.00^{a}	0.03 ± 0.01^{a}	0.16 ± 0.01^{b}	0.06 ± 0.00^{a}
C18:2n-6	2.40 ± 0.08^{a}	1.85 ± 0.01^{b}	2.39 ± 0.03^{a}	2.39 ± 0.05^{a}
C18:3n-3	5.92 ± 0.05^{a}	6.14 ± 0.01^{a}	4.16 ± 0.12^{b}	4.38 ± 0.17^{b}
C18:3n-6	0.15 ± 0.01^{a}	0.11 ± 0.00^{b}	0.13 ± 0.01^{b}	0.13 ± 0.00^{b}
C20:2	0.25 ± 0.01	0.24 ± 0.00	0.26 ± 0.00	0.26 ± 0.01
C20:3n-3	0.25 ± 0.02^{a}	0.44 ± 0.00^{b}	0.18 ± 0.00^{c}	0.21 ± 0.01^{ac}
C20:3n-6	0.13 ± 0.00	0.12 ± 0.00	0.13 ± 0.00	0.13 ± 0.01
C20:4n-6 ARA	1.24 ± 0.11^{a}	0.91 ± 0.01^{b}	1.22 ± 0.04^{a}	1.20 ± 0.03^{a}
C20:5n-3 EPA	10.25 ± 0.91^{a}	7.82 ± 0.09^{b}	8.38 ± 0.20^{ab}	8.21 ± 0.10^{ab}
C22:2	0.16 ± 0.01^{a}	0.26 ± 0.00^{b}	0.14 ± 0.01^{a}	0.16 ± 0.01^{a}
C22:6n-3 DHA	13.00 ± 0.50^{a}	10.79 ± 0.08^{b}	13.70 ± 0.12^{ac}	14.37 ± 0.03^{c}
Total PUFA	33.81 ± 1.29^{a}	28.72 ± 0.15^{b}	30.86 ± 0.23^{ab}	31.49 ± 0.21^{ab}
Total n-3 PUFA	29.42 ± 1.32^{a}	25.20 ± 0.16^{b}	26.43 ± 0.21^{ab}	27.17 ± 0.24^{ab}
Total n-6 PUFA	3.98 ± 0.11^{a}	3.02 ± 0.01^{b}	4.02 ± 0.04^{a}	3.91 ± 0.06^{a}
Total Fatty Acids	77.24 ± 0.12^{a}	76.25 ± 0.31^{ab}	75.36 ± 0.13^{b}	76.28 ± 0.35^{ab}
DHA : EPA	1.28 ± 0.06^{a}	1.38 ± 0.01^{a}	1.64 ± 0.05^{b}	1.75 ± 0.02^{b}
EPA : ARA	8.55 ± 1.61	8.56 ± 0.03	6.87 ± 0.07	6.83 ± 0.09

LITERATURE CITED

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