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REPORT

Ontogenetic change in the lipid and fatty acid composition of scleractinian coral larvae

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Abstract Some scleractinian coral larvae have an extraordinary capacity to delay metamorphosis, and this is reflected in the large geographic range of many species. Coral eggs typically contain a high proportion of wax esters, which have been hypothesized to provide a source of energy for long-distance dispersal. To better understand the role of lipids in the dispersal of broadcast spawning coral larvae, ontogenetic changes in the lipid and fatty acid composition of *Goniastrea retiformis* were measured from the eggs until larvae were 30 days old. Egg biomass was 78.8 ± 0.5% lipids, 86.3 ± 0.2% of which were wax esters, 9.3 ± 0.0% polar lipids, 4.1 ± 0.2% sterols, and 0.3 ± 0.1% triacylglycerols. The biomass of wax esters declined significantly through time, while polar lipids, sterols and triacylglycerols remained relatively constant, suggesting that wax esters are the prime source of energy for development. The most prevalent fatty acid in the eggs was palmitic acid, a marker of the dinoflagellate *Symbiodinium*, highlighting the importance of symbiosis in coral reproductive ecology. The proportion of polyunsaturated fatty acids declined through time, suggesting that they are essential for larval development. Interestingly, triacylglycerols are only abundant in the propagules that contain *Symbiodinium*, suggesting important differences in the energetic of dispersal among species with vertical and horizontal transmission of symbionts.

Keywords Coral reefs · Development · Dispersal · Fertilization · *Goniastrea* · Metabolism

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Introduction

Larval dispersal is of great importance to the ecology and evolution of sessile marine species because it determines the patterns of connectivity among populations (Jones et al. 2009). Larvae dispersal distance depends on many factors, both physical, such as currents and winds (Siegel et al. 2009), and biological, such as larval energy reserves (Burgess et al. 2009), survival and settlement competence dynamics (Connolly and Baird 2010), buoyancy and swimming ability (Whalan et al. 2008), and a full understanding of dispersal potential requires all these factors to be considered (Jones et al. 2009).

Many coral larvae have an extraordinary capacity to delay metamorphosis; in the absence of suitable settlement cues, a proportion of larvae can remain alive and competent for months (Richmond 1987; Graham et al. 2008; Connolly and Baird 2010). Coral propagules can be either symbiotic or aposymbiotic (symbiont-free), depending on whether or not the zooxanthellae are transmitted into the egg, prior to release. Coral larvae do not feed in the plankton; therefore, aposymbiotic larvae depend exclusively on energy reserves provided by the parent. In contrast, symbiotic larvae can obtain energy from zooxanthellae (Richmond 1987) to survive longer and thus possibly disperse further.

Lipids are the main biochemical component, making up between 34 and 85.5% of the biomass of newly released propagules of scleractinian corals (Table 1). The most abundant lipids in coral propagules are wax esters (WEs), phospholipids (PLs), and triacylglycerols (TAGs) (Table 1). Phospholipids are major constituents of cell membranes but are thought not to be involved in metabolism. Wax esters and triacylglycerols constitute a reserve of fatty acids that can be oxidized to provide energy or that can be incorporated in cell structures. Richmond (1987) hypothesized that a high abundance of wax esters may enable long-distance dispersal, because wax esters take longer to metabolize than triacyglycerols. Lipids also provide buoyancy, particularly wax esters that have a lower density than other lipid classes (Gurr et al. 2002; Lee et al. 2006), and may therefore promote fertilization by transporting gametes to the ocean surface (Harii et al. 2007). Photosynthetic zooxanthellae provide an extra energy source to symbiotic coral larvae, possibly contributing to the extension of larval duration; in the absence of zooxanthellae, coral larvae exhaust their energy reserves more rapidly (Harii et al. 2010).

The fatty acids composition of coral eggs and larvae is still poorly studied (Arai et al. 1993); therefore, the specific role of each fatty acid during coral larval dispersal remains unknown. However, studies on other marine organisms have shown that palmitic (16:0) and vaccenic (18:1ω9) acids are the preferred source of metabolic energy in animals (Bergé and Barnathan 2005), whereas highly unsaturated fatty acids, particularly the essential docosahexaenoic (DHA), eicosapentaenoic (EPA), and arachidonic (ARA) acids, are critical to growth, survival, reproduction, and food conversion efficiency (Brett and Müller-Navarra 1997; Bergé and Barnathan 2005). Additionally, the fatty acids composition of eggs is known to reflect the feeding ecology of the adults (Dalsgaard et al. 2003).

Here, the potential role of lipids in coral larval dispersal was explored by measuring the change in total lipid, lipid classes, and fatty acid composition of the eggs and larvae of the coral *Goniastrea retiformis* species during the first 30 days of development.

Methods

Gametes of six colonies of *Goniastrea retiformis* were collected at Oku, Okinawa, in June 2002 following Babcock et al. (2003). Five hundred eggs were sampled right after spawn (day 0). Larvae were cultured in the Sesoko Tropical Biosphere Station, Okinawa, Japan, and maintained in three replicate 20-L plastic buckets in 0.2 µm filtered seawater at initial densities of approximately 1,000 larvae L^{-1} under a quantum irradiance of 160 µmol photons m^{-2} s^{-1} with a 12:12 h light/dark photo-period at temperatures between 25 and 27°C. Water was changed on each sampling occasion. Between 200 and 500 larvae were taken from each bucket after 1, 3, 7, 17, and 30 days. Larvae were counted into 25-mL plastic jars modified to form a sieve. The vials were drained, and the 60-micron plankton mesh sieve removed and placed in liquid nitrogen. Larvae were first freeze-dried before lipids were extracted with $CHCl_3$–MeOH (1:2) (Bligh and Dyer 1959). The combined lipid extracts from each sample were partitioned with $CHCl_3$ and H_2O (Meziane and Tsuchiya 2002). Lipids were recovered in the lower $CHCl_3$ phase, the solvents were removed under vacuum, and the lipid extracts were stored in $CHCl_3$. The lipid extract was then weighed to estimate the biomass per larva. To analyze lipid classes, aliquots of lipid extract were applied to 10 cm/10 cm high-performance thin-layer chromatography (HPTLC) plates (Merck Ltd, Tokyo, Japan). The plates were first developed full-length with hexane–ether–acetic acid (80:20:0.1). After drying in a stream of air, plates were immersed in a mixture of phosphoric acid, 33% acetic acid, sulfuric acid, and 0.5% copper sulfate (5:5:0.5:90 v/v) for 40 s then heated at 130°C for 12 min. The chromatograms were scanned on a GT9000 (Seiko Epson Co., Nagano, Japan) in gray scale mode using Adobe Photoshop (Adobe Systems). Percent compositions of lipid classes were determined on the basis of band intensity by an image analysis program of NIH Image (Research Services Branch) that contains the lipid standards band intensity; the total amount of each lipid class was calculated by reporting the obtained surface to the total lipid concentration measured at the beginning of the extraction. The authentic standards used were cholesterol for sterols, oleic acid for free fatty acid (FFA), triolein for triacylglycerol (TAG), and stearyl oleate for wax ester (WE) following Oku et al. (2002).

In order to estimate the proportion of fatty acids methyl esters (FAMEs) in the total lipids, a second plate was prepared after saponification and esterification (Metcalfe and Schmitz 1961). All lipid extracts' bands were scrapped

Table 1 Average lipid content of eggs or newly released planulae (% in dry weight) and their triacylglycerol (TAG) and wax esters (WE) composition (% of total lipids) in coral species with different modes of larval feeding

Species	Mode of development	Symbiotic	Lipid (% in dry weight)	TAG (% of total lipid)	WE (% of total lipid)	TAG/WE ratio
Goniastrea retiformis	Broadcast	No	78.8[a]	0.3[a]	86.3[a]	0.00
Acropora brueggemanni	Brooder	No	58[b]	0.4[b]	72.4[b]	0.01
Acropora millepora	Broadcast	No	69.1[c]	1.1[c]	81.8[c]	0.01
Acropora tenuis	Broadcast	No	62.5–85.2[b,c]	1.9[c]	63–79.8[b,c]	0.02–0.03
Montipora digitata	Broadcast	Yes	67.4[c]	5.1–8.4[c,d]	56.5–69[c,d]	0.09
Favia fragum	Brooder	Yes	34–47[e]	NA	NA	0.12
Pocillopora damicornis	Brooder	Yes	68.1–70[b,f]	16.5–31[b,d]	52–60.3[b,e]	0.32–0.51

NA Not available

[a] This study

[b] Harii et al. (2007)

[c] Arai et al. (1993)

[d] Harii et al. (2010)

[e] Norström and Sandström (2010)

[f] Richmond (1987)

and eluted with $CHCl_3$–MeOH (2:1 v/v) at 40°C for 60 min. The FAMEs were separated and quantified by gas chromatography (GC 14.B, Shimadzu, Japan) equipped with flame ionization detector. Separation was performed with an FFAP-polar capillary column (30 × 0.32 mm internal diameter, 0.25 mm film thickness). Hydrogen was used as a carrier gas. After injection at 60°C, the oven temperature was raised to 150°C at a rate of 40°C min^{-1}, then to 230°C at 3°C min^{-1}, and finally held constant for 30 min. The flame ionization was held at 240°C. FAMEs' peaks were identified by comparing their retention times with those of authentic standards (Supelco Inc.). An image analysis program (NIH 6 image) was used to estimate the relative contribution of the fatty acids in the lipids by integrating the chromatogram. One-way ANOVAs were used to test for significant differences in total lipid, lipid classes, and percent of each fatty acid among samples followed by Tukey's post hoc multiple comparisons. One-way ANOVAs were also used to test for significant differences in triacylglicerol/wax ester ratio between species, in adults, and propagules. Statistical analyses were performed using Statistica 7.0 (StatSoft) with a significance level of 0.05.

Results

The eggs of *G. retiformis* have 78.8 ± 0.49% lipid by dry weight, which is similar to that of other broadcast spawning coral species (Harii et al. 2010, Table 1). In comparison, adult *Goniastrea* contain between 11 and 42% lipid (Harland et al. 1993), suggesting that lipids are important for larval biology. While the triacylglycerol/wax ester (TAG/WE) ratio in the tissue of adult corals is not significantly different between brooders and broadcast spawners ($F_{1,15} = 0.0316$, $p = 0.96$, Table 2), however, the TAG/WE ratio of propagules is dependent upon whether or not they contain symbionts ($F_{1,7} = 8.177$, $p = 0.02$, Table 1). While all propagules have a high proportion of WEs (23–86.3%), propagules that lack symbionts have much less TAGs (0.3–1.9%, Table 1) than propagules that possess symbionts (5.1–31%, Table 1). In *G. retiformis*, wax esters are the main class of lipid in the eggs (86.34 ± 0.23%, Table 3) followed by polar lipids (9.25 ± 0.00%), sterols (4.11 ± 0.15%), and TAGs (0.30 ± 0.08%). This pattern is similar to the eggs of other corals that lack zooxanthellae, which typically contain a very high content of WEs (58–81.8%), polar lipids (9.3–22.6%), and little TAGs (0.3–1.9%) (Table 1). In contrast, larvae with symbionts such as those of *Pocillopora damicornis* and *Montipora digitata* have a high proportion of TAG (5.1–31%, Table 1).

Total lipid decreased as larvae aged ($F_{5,7} = 10.72$, $p = 0.004$), ranging from 8.4 ± 0.7 µg in the egg to 3 ± 0.5 µg in a 30-day-old larva (Table 3; Fig. 1). During early development (until day 3), lipids decreased rapidly; from day 4 to days 17–21, there was a decrease in lipid composition, but later in development, lipids decreased more rapidly again. WEs remained the larvae's main lipid type throughout development, but declined significantly ($F_{5,7} = 14.27$, $p = 0.001$, Fig. 1). Polar lipids and sterols also decreased with larval age ($F_{5,7} = 8.47$, $p = 0.007$ and $F_{5,7} = 18.19$, $p = 0.001$, respectively, Table 3) but not as rapidly as WEs. Free fatty acids changed significantly over larval development ($F_{5,7} = 8.86$, $p = 0.006$), initially

Table 2 Average lipid content (% in dry weight) and TAG and WE composition (% in total lipids) in the tissue of adult corals

Species	Mode of development	Lipids (%)	TAGs (%)	WEs (%)	TAG/WE ratio
Acropora microphthalma	Broadcast	28.6[a]	16.2[a]	17.4[a]	0.93
Cyphastrea ocelina	Brooder	41[b]	NA	NA	NA
Fungia fungites	Broadcast	23.2[a]	15[a]	31.0[a]	0.48
Goniastrea aspera	Broadcast	29.6[a]	16.1[a]	31.4[a]	0.51
Galaxea fascicularis	Broadcast	37.0[a]	30.4[a]	21.3[a]	1.43
Goniastrea retiformis	Broadcast	10.9[c]	22.6[c]	41.96[c]	0.54
Montastrea annularis	Broadcast	24–31.9[c,d]	22.5[c]	42.49[c]	0.53
Montipora aequituberculata	Broadcast	22.5[a]	22.7[a]	10[a]	2.27
Montipora verrucosa	Broadcast	42–47[b]	NA	NA	NA
Oulastrea crispata	Broadcast	19.3[a]	22.2[a]	20[a]	1.11
Pocillopora capitata	NA	34.5[e]	38[e]	31[e]	1.22
Pocillopora damicornis	Brooder	30.8–41[a,b]	21.2[a]	26.4[a]	0.8
Pocillopora meandrina	Broadcast	32–34[b]	NA	NA	NA
Pocillopora verrucosa	Broadcast	10.7–14.1[a,c]	14.9–36.6[a,c]	15.8–22.3[a,c]	0.94–1.64
Porites compressa	Broadcast	30–33[b]	NA	NA	NA
Porites cylindrica	Broadcast	21.1[a]	22.8[a]	17.6[a]	1.3
Porites lutea	Broadcast	20.1[a]	21[a]	18.0[a]	1.17
Porites porites	Brooder	9–12.4[c,d]	18.1[c]	27.84[c]	0.65
Siderastrea siderea	Broadcast	26–35[d]	NA	NA	NA
Stylophora pistillata	Brooder	17–20.8[a,c]	19.2–24.6[a,c]	21.8–48.6[a,c]	0.51–0.88
Tubastrea sp.	Brooder	15.6[a]	21.9[a]	9.1[a]	2.41

NA not available

[a] Yamashiro et al. (1999)

[b] Stimson (1987)

[c] Harland et al. (1993)

[d] Harland et al. (1992)

[e] Patton et al. (1977)

Table 3 Average (±SE) lipid content and composition (μg larva^{-1}) during larval development (from egg to 30-day-old larva) of *Goniastrea retiformis*

Time (days after spawning)	Total lipids	Polar lipids	Sterols	FFA	TAG	Wax esters
0	8.40 ± 0.70[a]	0.78 ± 0.07[a]	0.34 ± 0.02[ac]	0.00 ± 0.00[a]	0.02 ± 0.00	7.25 ± 0.62[a]
1	7.80 ± 0.60[ab]	0.93 ± 0.05[a]	0.54 ± 0.01[b]	0.03 ± 0.01[ab]	0.00 ± 0.00	6.29 ± 0.54[ab]
3	7.00 ± 0.58[ab]	0.66 ± 0.07[a]	0.48 ± 0.03[bc]	0.13 ± 0.04[bc]	0.46 ± 0.22	5.27 ± 0.48[abc]
7	6.17 ± 0.60[ab]	0.69 ± 0.06[a]	0.54 ± 0.00[b]	0.19 ± 0.01[c]	0.84 ± 0.09	4.45 ± 0.43[bcd]
17	5.00 ± 0.50[bc]	0.49 ± 0.15[ab]	0.26 ± 0.07[a]	0.00 ± 0.00[a]	0.72 ± 0.27	3.28 ± 0.28 [cd]
30	3.00 ± 0.50[c]	0.23 ± 0.03[b]	0.19 ± 0.04[a]	0.04 ± 0.01[ab]	0.33 ± 0.09	2.20 ± 0.33[d]

For each column, superscript letters indicate significant differences over time (Tukey's test). (*FFA* free fatty acids; *TAG* triacylglycerols)

increasing and later decreasing (Table 3). TAGs were conserved through development ($F_{5,7} = 3.69$, $p = 0.059$).

Goniastrea retiformis eggs were rich in palmitic acid (16:0, 37.51 ± 1.07% of the total fatty acids). Other saturated fatty acids in the eggs were 14:0 and 18:0 (Table 4). The major monounsaturated fatty acids were oleic and vaccenic acids (18:1ω7/9 and 16:1ω7/9, respectively) (Table 4). Polyunsaturated fatty acids (PUFAs) were abundant (36.76 ± 1.22%). The most abundant PUFAs in the propagules were 18:3ω6 (8.78 ± 0.41%), 20:4ω6 (8.54 ± 0.06%), and 22:5ω3 (8.17 ± 1.18%), followed by 18:2ω6, 20:3ω6, 22:4ω3, 20:5ω3 (EPA), 22:6ω3 (DHA), and 18:4ω3 (Table 4). The proportion of most PUFAs, for example 18:2ω6, 18:3ω6, 20:3ω6, and 20:4ω6, declined significantly with larval age ($p < 0.05$, Table 4). The proportion of the fatty acids 14:0, 16:1ω7/9, 20:1ω9, and the

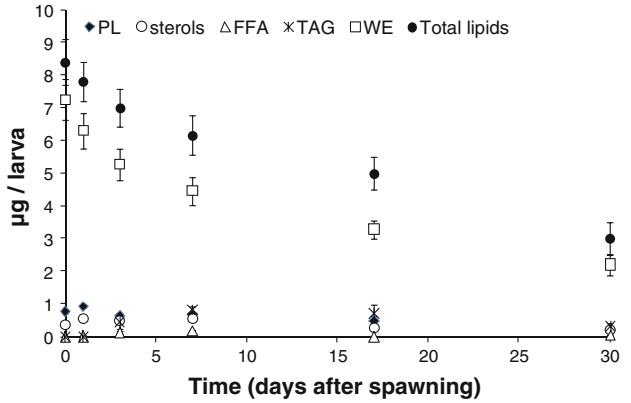

Fig. 1 Lipid content and composition (average ± SE) during larval development of *Goniastrea retiformis* (*PL* polar lipids; *FFA* free fatty acids; *TAG* triacylglycerols; *WE* wax esters)

PUFAs 18:4ω3, 22:4ω3, 22:5ω3, and 22:6ω3 remained constant.

Discussion

The eggs of corals typically contain a high content of wax esters (WEs), but larvae with symbionts have a much higher proportion of triacylglycerols (TAGs) than propagules that lack zooxanthellae (Table 1).This suggests that the TAGs are located in the cells of the symbiont and not in the host tissue. Secondly, wax esters are used to power the dispersal of lecithotrophic larvae, being a prime source of fatty acids either for oxidation to provide energy or for incorporation in phospholipids in cell membranes (Lee et al. 2006). In contrast, symbiotic larvae may not need as much WEs because they have zooxanthellae that can provide them with other sources of carbon to power dispersal. Thirdly, we speculate that WEs may also contribute to fertilization ecology (Harii et al. 2007). Their low density may allow the gametes of broadcast spawners to remain at the surface and thus maximize fertilization. The reduction in wax esters relative to other lipids though larval development will also result in reduced buoyancy, thus facilitating larval settlement. On the other hand, propagules that have symbionts may have low WEs because many of these species are able to settle shortly after release (Harii et al. 2002; Nishikawa et al. 2003), and therefore, buoyancy may not be advantageous.

The pattern of lipid consumption observed in *G. retiformis* was similar to that in *Acropora tenuis* (Harii et al. 2007): rapid decrease in lipids during early development (until days 2–3), followed by period where lipids are not considerably used (until days 17–21), and again a rapid consumption later in development. The rapid decrease during early development might reflect lipid oxidation

Table 4 Average (±SE) percent of composition of fatty acids during larval development of *Goniastrea retiformis* (from egg to 30-day-old larvae) and *p* value (one-way ANOVA)

Fatty acid (% total FA)	Time (days after spawning)						
	0	1	3	7	17	30	*p*
14:0	2.00 ± 0.38	2.86 ± 0.27	2.58 ± 0.11	2.42 ± 0.17	2.71 ± 0.21	1.97 ± 0.27	0.187
16:0	37.51 ± 1.07^{ab}	35.31 ± 2.78^{a}	38.17 ± 1.12^{abc}	38.43 ± 0.50^{abc}	44.77 ± 2.02^{bc}	46.66 ± 0.42^{c}	0.011
18:0	1.77 ± 0.62^{a}	1.48 ± 0.98^{a}	2.80 ± 0.24^{ab}	1.50 ± 0.24^{a}	3.97 ± 0.29^{ab}	5.42 ± 0.15^{b}	0.006
Σ SFA	41.28 ± 1.31^{a}	39.64 ± 2.08^{a}	43.55 ± 1.24^{ab}	42.35 ± 0.91^{a}	51.44 ± 2.09^{bc}	54.04 ± 0.54^{c}	0.002
16:1ω7/9	5.47 ± 0.37	8.47 ± 3.33	6.29 ± 0.08	4.95 ± 0.11	5.62 ± 0.34	5.89 ± 0.96	0.616
18:1ω7/9	15.52 ± 0.28^{a}	15.45 ± 1.38^{a}	14.35 ± 0.04^{a}	19.06 ± 0.13^{b}	14.94 ± 0.17^{a}	16.39 ± 0.03^{ab}	0.012
20:1ω9	0.99 ± 0.17	1.02 ± 0.20	0.93 ± 0.02	1.24 ± 0.01	0.99 ± 0.01	1.14 ± 0.03	0.455
Σ MUFA	21.98 ± 0.08	24.93 ± 1.74	21.57 ± 0.10	25.24 ± 0.02	21.55 ± 0.49	23.41 ± 0.90	0.057
18:2ω6	4.68 ± 0.09^{a}	3.70 ± 0.02^{abc}	3.94 ± 0.03^{ab}	3.91 ± 0.01^{ab}	3.12 ± 0.45^{bc}	2.67 ± 0.16^{c}	0.004
18:3ω6	8.78 ± 0.41^{a}	7.63 ± 0.52^{abc}	7.97 ± 0.13^{ab}	6.66 ± 0.04^{bc}	6.03 ± 0.40 ^{cd}	4.40 ± 0.07^{d}	0.001
18:4ω3	0.58 ± 0.02	0.56 ± 0.03	0.52 ± 0.05	0.46 ± 0.04	0.32 ± 0.03	0.20 ± 0.20	0.100
20:3ω6	1.92 ± 0.06^{a}	1.84 ± 0.03^{ab}	1.91 ± 0.08^{ab}	1.79 ± 0.01^{ab}	1.70 ± 0.06^{ab}	1.60 ± 0.07^{b}	0.037
20:4ω6	8.54 ± 0.06^{a}	8.34 ± 0.54^{a}	8.28 ± 0.06^{a}	7.24 ± 0.04^{ab}	6.57 ± 0.29^{bc}	5.62 ± 0.33^{c}	0.002
20:5ω3	1.85 ± 0.11^{a}	2.82 ± 0.09^{b}	2.46 ± 0.21^{ab}	2.13 ± 0.00^{ab}	2.03 ± 0.17^{ab}	1.69 ± 0.14^{a}	0.010
22:4ω3	1.57 ± 0.16	1.56 ± 0.05	1.57 ± 0.03	1.57 ± 0.03	1.34 ± 0.01	1.21 ± 0.11	0.075
22:5ω3	8.17 ± 1.18	7.97 ± 0.64	7.41 ± 0.78	6.97 ± 0.05	5.35 ± 0.17	4.75 ± 0.54	0.050
22:6ω3	0.70 ± 0.14	1.04 ± 0.18	0.86 ± 0.12	0.86 ± 0.01	0.59 ± 0.01	0.44 ± 0.11	0.148
Σ PUFA	36.76 ± 1.22^{a}	35.44 ± 0.34^{a}	34.89 ± 1.35^{a}	31.57 ± 0.06^{ab}	27.03 ± 1.59^{bc}	22.56 ± 1.43^{c}	0.001

For each row, superscript letters indicate significant differences over time (Tukey's test). (*FA* fatty acids; *SFA* saturated fatty acids; *MUFA* monounsaturated fatty acids; *PUFA* polyunsaturated fatty acids)

fuelling intensive cell division during embryogenesis and motility (Okubo et al. 2008). However, the proportion of lipids consumed during the first days after spawning differs between aposymbiotic and symbiotic larvae. Aposymbiotic larvae of the coral *G. retiformis* consume 64.3% of its lipids over 30 days; however, 26% of these are consumed in the first 3 days (Fig. 1). Similarly, of all lipids consumed by *A. tenuis* larvae over a period of 30 days, 58% of the consumption happens in the first 5 days (Harii et al. 2007). The larvae of corals with zooxanthellae also consume lipids, but their rate of consumption is lower: in the first 6 days of development, *Pocillipora damicornis* only consumes 16% of the total lipids consumed in 30 days (Harii et al. 2010).

The fatty acid composition of the eggs of *G. retiformis* (Table 4) was similar to those of the adults of this species (Harland et al. 1993) and of the eggs of *A. millepora*, *A. tenuis*, and *M. digitata* (Arai et al. 1993). Palmitic acid, the most abundant fatty acid in *G. retiformis* eggs, is a trophic marker of zooxanthellae (Papina et al. 2003; Zhukova and Titlyanov 2003). Even though the eggs of *G. retiformis* do not contain zooxanthellae, zooxanthellae provide palmitic acid to the adult corals (Papina et al. 2003), and therefore, palmitic acid becomes highly represented in their tissue and eggs (Arai et al. 1993; Yamashiro et al. 1999). When adult corals are exposed to a combination of high temperatures and irradiance that disrupt symbiosis, they become deprived of a significant amount of palmitic acid (Oku et al. 2003). Because this is the main fatty acid in coral eggs, the larval quantity and quality following a bleaching event is likely to be negatively impacted, as has been observed on the broadcast spawning soft coral *Lobophytum compactum* (Michalek-Wagner and Willis 2001). The high abundance of polyunsaturated fatty acids (PUFAs) (36.76 ± 1.22%) is probably due to the fact that symbiotic zooxanthellae in cnidarians contain a lot of PUFAs (Zhukova and Titlyanov 2003; Mortillaro et al. 2009) and the symbionts transfer them to the host tissue (Harland et al. 1993; Papina et al. 2003, 2007; Pitt et al. 2009), after which they are incorporated into their eggs. The EPA/DHA ratio (2.64) suggests that *G. retiformis* has a medium trophic level, similar to other marine invertebrates such as shrimps and crabs (Rosa et al. 2007). The eggs of *G. retiformis* contain several fatty acid trophic markers such as 16:0 (zooxanthellae), 14:0 (copepods), 18:1ω9 (marine animals), and 16:1ω7 (diatoms), a high percentage of C_{18} and C_{20} PUFA (26.35%, which indicates herbivory), including 18:2ω6 (green algae), and long-chain fatty acids $>C_{22}$ (microalgae) (Auel et al. 2002; Scott et al. 2002; Dalsgaard et al. 2003). This fatty acid profile indicates that adult corals have a diverse diet and do not rely exclusively on their symbionts. Specifically, several corals, including *G. retiformis*, have significant amounts of arachidonic acid (ARA) in their tissues and eggs (Arai et al. 1993; Yamashiro et al. 1999; this study). This fatty acid cannot be produced de novo and does not seem to be provided by the symbiont (Papina et al. 2003; Zhukova and Titlyanov 2003). Therefore, ARA is likely to be obtained by heterotrophic feeding on plankton. This indicates that, in order for corals to reproduce in captivity, the provision of live food rich in this essential fatty acid is key. Often corals in captivity are fed with *Artemia* nauplii that naturally have a low DHA and ARA content (Figueiredo et al. 2009) and therefore need to be previously enriched with these fatty acids to constitute a suitable diet for corals.

In an aposymbiotic larva such as those of *G. retiformis,* the total biomass of fatty acids can only decrease or remain constant through time because animals lack the biochemical pathways to produce them de novo (Moran and Manahan 2003). Consequently, when the proportion of a particular fatty acid decreases, it must either have been consumed or converted into another fatty acid, but when the proportion increases, it is not possible to infer whether the fatty acid has been produced through conversion or has simply been consumed at a lower rate than other fatty acids (or not consumed at all) (Rønnestad 1995). In *G. retiformis*, larval development seems to depend on PUFAs because the proportion of most PUFAs declined significantly with larval age. Polyunsaturated fatty acids confer fluidity, flexibility, and selective permeability to cellular membranes, affecting many cellular and physiological processes, including cold adaptation and survival, modulation of ion channels, endocytosis/exocytosis, defense against pathogens, and activities of membrane-associated enzymes that are sensitive to the biophysical properties of lipid membranes (Bergé and Barnathan 2005).

In conclusion, the high lipid content of eggs and larvae, particularly wax esters, potentially provides a source of energy to support long periods in the plankton. Additionally, the high abundance of wax esters in the eggs makes them buoyant, suggesting that selective pressure to increase fertilization success may also shape the biochemical composition of coral eggs.

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References

Arai T, Kato M, Heyward A, Ikeda Y, Iizuka T, Maruyama T (1993) Lipid composition of positively buoyant eggs of reef building corals. Coral Reefs 12:71–75

Auel H, Harjes M, Rocha R, Stübing D, Hagen W (2002) Lipid biomarkers indicate different ecological niches and trophic relationships of the Arctic hyperiid amphipods *Themisto abyssorum* and *T. libellula*. Polar Biol 25:374–383

Babcock RC, Baird AH, Piromvaragorn S, Thomson DP, Willis BL (2003) Identification of scleractinian coral recruits from Indo-Pacific Reefs. Zool Stud 42:211–226

Bergé JP, Barnathan G (2005) Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. Adv Biochem Eng Biotech 96:49–105

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Brett MT, Müller-Navarra DC (1997) The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshw Biol 38:483–499

Burgess SC, Harti SP, Marshall DJ (2009) Pre-settlement behavior in larval bryozoans: the roles of larval age and size. Biol Bull 216:344–354

Connolly SR, Baird AH (2010) Estimating dispersal potential for marine larvae: dynamic models applied to scleractinian corals. Ecology 91:3572–3583

Dalsgaard J, St. John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. Adv Mar Biol 46:225–340

Figueiredo J, van Woesik R, Lin J, Narciso L (2009) *Artemia franciscana* enrichment model—How to keep them small, rich and alive? Aquaculture 294:212–220

Graham EM, Baird AH, Connolly SR (2008) Survival dynamics of scleractinian coral larvae and implications for dispersal. Coral Reefs 27:529–539

Gurr MI, Harwood JL, Frayn KN (2002) Lipid biochemistry. Blackwell Science

Harii S, Kayanne H, Takigawa H, Hayashibara T, Yamamoto M (2002) Larval survivorship, competency periods and settlement of two brooding corals, *Heliopora coerulea* and *Pocillopora damicornis*. Mar Biol 141:39–46

Harii S, Nadaoka K, Yamamoto M, Iwao K (2007) Temporal changes in settlement, lipid content and lipid composition of larvae of the spawning hermatypic coral *Acropora tenuis*. Mar Ecol Prog Ser 346:89–96

Harii S, Yamamoto M, Hoegh-Guldberg O (2010) The relative contribution of dinoflagellate photosynthesis and stored lipids to the survivorship of symbiotic larvae of reef-building corals. Mar Biol 157:1215–1224

Harland AD, Spencer Davies P, Fixter L (1992) Lipid content of some Caribbean corals in relation to depth and light. Mar Biol 113:357–361

Harland AD, Navarro JC, Spencer Davies P, Fixter LM (1993) Lipids of some Caribbean and Red Sea corals: total lipid, wax esters, triglycerides and fatty acids. Mar Biol 117:113–117

Jones GP, Almany GR, Russ GR, Sale PF, Steneck RS, van Oppen MJH, Willis BL (2009) Larval retention and connectivity among populations of corals and reef fishes: history, advances and challenges. Coral Reefs 28:307–325

Lee RF, Hagen W, Kattner G (2006) Lipid storage in marine zooplankton. Mar Ecol Prog Ser 307:273–306

Metcalfe LD, Schmitz AA (1961) The rapid preparation of fatty acids esters for gas chromatography analysis. Anal Chem 33: 363–364

Meziane T, Tsuchiya M (2002) Organic matter in a subtropical mangrove-estuary subjected to wastewater discharge: Origin and utilisation by two macrozoobenthic species. J Sea Res 47:1–11

Michalek-Wagner K, Willis BL (2001) Impacts of bleaching on the soft coral *Lobophytum compactum*. I. Fecundity, fertilization and offspring viability. Coral Reefs 19:231–239

Moran AL, Manahan DT (2003) Energy metabolism during larval development of green and white abalone, *Haliotis fulgens* and *H. sorenseni*. Biol Bull 204:270–277

Mortillaro JM, Pitt KA, Lee SY, Meziane T (2009) Light intensity influences the production and translocation of fatty acids by zooxanthellae in the jellyfish *Cassiopea sp*. J Exp Mar Biol Ecol 378(1–2):22–30

Nishikawa A, Katoh M, Sakai K (2003) Larval settlement rates and gene flow of broadcast spawning (*Acropora tenuis*) and planula-brooding (*Stylophora pistillata*) corals. Mar Ecol Prog Ser 256:87–97

Norström A, Sandström M (2010) Lipid content of *Favia fragum* larvae: changes during planulation. Coral Reefs 29:793–795

Oku H, Yamashiro H, Onaga K, Iwasaki H, Takara K (2002) Lipid distribution in branching coral *Montipora digitata*. Fish Sci 68:517–522

Oku H, Yamashiro H, Onaga K, Sakai K, Iwasaki H (2003) Seasonal changes in the content and composition of lipids in the coral *Goniastrea aspera*. Coral Reefs 22:83–85

Okubo N, Yamamoto HH, Nakaya F, Okaji K (2008) Oxygen consumption of a single embryo/planula in the reef-building coral *Acropora intermedia*. Mar Ecol Prog Ser 366:305–309

Papina M, Meziane T, van Woesik R (2003) Symbiotic zooxanthellae provide the host-coral *Montipora digitata* with polyunsaturated fatty acids. Comp Biochem Physiol B 135:533–537

Papina M, Meziane T, van Woesik R (2007) Acclimation effect on fatty acids of the coral *Montipora digitata* and its symbiotic algae. Comp Biochem Physiol B 147:583–589

Patton JS, Abraham S, Benson AA (1977) Lipogenesis in the intact coral *Pocillopora capitata* and its isolated zooxanthelae: evidence for a light-driven carbon cycle between symbiont and host. Mar Biol 44:235–247

Pitt KA, Connolly RM, Meziane T (2009) Stable isotope and fatty acid tracers in energy and nutrient studies of jellyfish: a review. Hydrobiologia 616:119–132

Richmond RH (1987) Energetics, competency, and long-distance dispersal of planula larvae of the coral *Pocillopoa damicornis*. Mar Biol 93:527–533

Rønnestad I (1995) Interpretation of ontogenic changes in composition studies of fish embryos and larvae: presenting proportional data can lead to erroneous conclusions. Aquac Nutr 1:199

Rosa R, Calado R, Narciso L, Nunes ML (2007) Embryogenesis of decapod crustaceans with different life history traits, feeding ecologies and habitats: a fatty acid approach. Mar Biol 151: 935–947

Scott CL, Kwasniewski S, Falk-Petersen S, Sargent JR (2002) Species differences, origins and functions of fatty alcohols and fatty acids in the wax esters and phospholipids of *Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus* from Arctic waters. Mar Ecol Prog Ser 235:127–134

Siegel DA, Mitarai S, Costello CJ, Gaines SD, Kendall BE, Warner RR, Winters KB (2009) The stochastic nature of larval connectivity among nearshore marine populations. Proc Natl Acad Sci USA 105:8974–8979

Stimson JS (1987) Location, quantity and rate of change in quantity of lipids in tissue of Hawaiian hermatypic corals. Bull Mar Sci 41:889–904

Whalan S, Ettinger-Epstein P, Battershill C, de Nys R (2008) Larval vertical migration and hierarchical selectivity of settlement in a brooding marine sponge. Mar Ecol Prog Ser 368:145–154

Yamashiro H, Oku H, Higa H, Chinen I, Sakai K (1999) Composition of lipids, fatty acids and sterols in Okinawan corals. Comp Biochem Physiol B 122:397–407

Zhukova NV, Titlyanov EA (2003) Fatty acid variation in symbiotic dinoflagellates from Okinawan corals. Phytochemistry 62: 191–195