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Dietary eicosapentaenoic acid and docosahexaenoic acid are linearly retained by common insect crop pests (cabbage looper and bertha armyworm) and alter insect biomass

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Abstract. The long-chain omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are prevalent in aquatic ecosystems and are not part of the natural diet of herbivorous, terrestrial insects, which generally consume alpha-linolenic acid (ALA) and linoleic acid (LNA). However, recent advances in genetic engineering have lead to the development of terrestrial crops that express the novel traits of EPA and DHA production. In the present study, we examine the effects of dietary EPA and DHA on the growth, development and fatty acid content of two crop pest insects: bertha armyworm and cabbage looper. Five experimental diets were formulated to include increasing amounts of pure EPA and DHA (in relation to the total diet lipid level), according to the ratios (EPA + DHA relative to a vegetable oil containing ALA and LNA): 0 (control), 0.25 : 0.75 (lowest), 0.5 : 0.5 (low), 0.75 : 0.25 (medium) and 1 : 0 (high). Dietary EPA and DHA had significant effects on development time, mass and fatty acid content in both species. Dietary treatment (interactive with time) had a significant effect on individual mass of both insects, indicating that, over time, EPA and DHA impacted growth. However, insect mass, development and morphology results are not linearly related with increasing dietary EPA and DHA. Both species retained EPA and DHA in adult form, and the body content of EPA and DHA was significantly, positively correlated with EPA and DHA diet treatments in both the bertha armyworm ($r^2 = 91.3\%$) and cabbage looper ($r^2 = 75.8\%$). Dietary EPA and DHA could have fitness consequences for these organisms and could be nutritionally transferred to higher consumers.

Key words. Bertha armyworm, cabbage looper, docosahexaenoic acid, eicosapentaenoic acid, larval insect development, omega-3 fatty acids, terrestrial food webs.

Introduction

Essential fatty acids play critical roles in metabolic energy storage, cell membrane structure, cell signalling, neurological function and the immune system in both aquatic (Arts *et al.*, 2009) and terrestrial organisms, ranging from insects (Canavoso *et al.*, 2001) to humans (Calder, 2015). The 18-carbon omega-3 (*n*-3) and omega-6 (*n*-6) polyunsaturated fatty acids (PUFA), alpha-linolenic acid (ALA; 18:3*n*-3) and linoleic acid (LNA; 18:2*n*-6), respectively, are essential to most animals (including insects; Canavoso *et al.*, 2001) because they generally cannot be synthesized *de novo*, and therefore must be obtained from the diet. These PUFA are essential at all stages of insect development (Stanley-Samuelson *et al.*, 1988; Stanley & Nelson, 1993; Canavoso *et al.*, 2001). Terrestrial plant lipids provide the essential PUFA (ALA and LNA) that are required by terrestrial invertebrate and vertebrate herbivores.

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However, the *n*-3 long-chain (LC) PUFA, eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3) are also known to be 'physiologically essential' in vertebrates and invertebrates (Arts *et al*., 2001) because they play critical roles in key physiological functions that support cardiovascular health in vertebrates and neurological development, in both groups (Calder, 2015). Both EPA and DHA are produced in aquatic environments, primarily by algae (Brett & Müller-Navarra, 1997; Galloway & Winder, 2015; Colombo *et al.*, 2017), and are selectively retained, in general, by higher trophic-level organisms (e.g. fish; Gladyshev *et al.*, 2013; Twining *et al.*, 2016; Colombo *et al.*, 2017). However, it is unclear how EPA and DHA are used or retained in terrestrial insects.

Dietary EPA and DHA are known to stimulate growth and reproduction in aquatic invertebrates, including arthropods (Wacker *et al.*, 2002; Arendt *et al.*, 2005; Müller-Navarra, 2006; Wacker & Martin-Creuzburg, 2007; Parrish, 2009). Thus, these two fatty acids are often used as a proxy for health, reproductive success and survival in these organisms (Müller-Navarra *et al.*, 2000; Brett *et al.*, 2009). However, because terrestrial plants typically do not produce EPA and DHA (Gladyshev *et al.*, 2013; Hixson *et al.*, 2015), terrestrial herbivorous insects do not normally consume these two bioactive fatty acids. Thus, whether or not EPA and DHA will affect growth, reproduction, physiological performance and/or survival in terrestrial invertebrates remains largely untested.

EPA and DHA are not normally part of the natural diet of terrestrial insects. However, trace quantities of EPA are found in small quantities in phospholipids in specific tissues (e.g. flight muscles, eyes and/or reproductive tissues) (Stanley-Samuelson *et al.*, 1988) of some insects. Furthermore, a more recent study reports finding only 18:3*n*-3 in functional phospholipids (Ziegler *et al.*, 2015). In addition, EPA has an important role as a precursor to eicosanoids, a group of lipid mediators that have functional roles in insect immunity, stress response and reproduction (Stanley & Miller, 2006). Under typical conditions, EPA is most often synthesized in terrestrial invertebrates from its precursor, ALA, and is generally not stored in substantial quantities in insects (Stanley-Samuelson *et al.*, 1988). This is likely because most herbivorous terrestrial insects (except for amphibiotic insects that have an aquatic larval stage (e.g. chironomids, mayflies, stoneflies and dragonflies) (Sushchik *et al.*, 2016) consume terrestrial, higher plants, which do not have the ability to synthesize EPA and DHA (Harwood, 1996).

There is growing interest in understanding the roles that dietary EPA and DHA may play in influencing the growth, development and physiology of terrestrial insects. First, there is increasing interest in incorporating insects as ingredients in human food, as well as livestock and aquaculture feeds (van Huis, 2013). Nutrient uptake by larval insects from their diet, therefore, will be an important consideration because the larva will mediate the transfer of nutrients to the consumer (animal or human). Accordingly, there are recent studies investigating insect larvae dietary requirements and their function as a nutrient vector of EPA and DHA to humans and animals (St-Hilaire *et al.*, 2007; Barroso *et al.*, 2017; Lehtovaara *et al.*, 2017). There are also recent developments with respect to the production of genetically engineered (GE) oilseed crops that produce EPA and DHA, with these crops now being considered as a potentially sustainable source of these fatty acids (Connelly & MacIntosh, 2018; Sottosanto *et al.*, 2018). Although these GE crops are not yet produced commercially, a novel terrestrial source of EPA and DHA, produced on an industrial scale, would make these bioactive fatty acids broadly available to terrestrial herbivorous insects in regions where these crops are grown (Colombo *et al.*, 2018; MacDonald *et al.*, 2018). The novel introduction of these bioactive compounds to terrestrial crops could impact trophic ecology and, as such, this has been considered as a top emerging issue of ecological concern (Sutherland *et al.*, 2019). The development of these technological advancements has piqued an interest in understanding the roles of dietary EPA and DHA in the physiology, behaviour and ecology of terrestrial insects.

In the present study, we examine the effects of dietary EPA and DHA on the growth, development and fatty acid content of two insect species; the cabbage looper (*Tricoplusia ni* Hübner) and the bertha armyworm (*Mamestra configurata Walker*), which are common in many parts of North America and indiscriminately consume the tissues (leaves, stems, seeds) of oilseed crops.

Materials and methods

Experimental species

*Cabbage looper (*T. ni*).* The cabbage looper is found throughout North America. It is not only a major pest of crucifer crops, including camelina and canola, but also commonly feeds on other agricultural crops (Natural Resources Canada, 2014). It is a generalist feeder, consuming the foliage of various plants species, as well as their developing seedpods (Buntin, 2017). They normally produce two to three overlapping generations per growing season, completing their life cycle in approximately 1 month. Their growth cycle consists of five larval instars, pupation and, finally, metamorphosis into its adult moth stage (Natural Resources Canada, 2014).

*Bertha armyworm (*M. configurata*).* The bertha armyworm is a substantial pest of *Brassica* crops and is native to North America (Ulmer *et al.*, 2001). Its distribution spans from the west coast throughout the Prairie Provinces, where *Brassica* crops are commonly grown (Ulmer *et al.*, 2001; Canola Council of Canada, 2014). Infestations of 50–200 larvae m^{-2} are commonly observed (Canola Council of Canada, 2014). As a generalist feeder, the larval stage causes significant crop damage to the foliage and developing seedpods of *Brassica* crops and, at high infestation rates, whole oilseed pods can be consumed (Ulmer *et al.*, 2001; Canola Council of Canada, 2014). The life cycle of *M. configurata* consists of five larval instars, pupation and, finally, metamorphosis into its adult moth stage, which occurs in approximately 1 month at 20 °C under laboratory conditions (Bucher & Bracken, 1976).

Experimental design

Cabbage looper. Cabbage looper eggs were obtained from Natural Resources Canada (Great Lakes Forestry Centre, Sault

Ste. Marie, Ontario) and were reared at Ryerson University (Toronto, Ontario) in accordance with the standard operating procedures provided. Prior to and after the handling of the eggs, the working area was disinfected with 10% bleach, and covered with Benchkote® (GE Whatman, U.K.). The eggs (from at least 100 females or more) were shipped on gauze pads, which were cut into portions containing approximately 25–30 eggs each. Using sterile forceps, each portion was transferred to a translucent cup (66 mL) containing the dietary treatment. In total, 1000 eggs were obtained, resulting in approximately 33 cages for the initial stages of the experiment. The eggs were kept under a 12 : 12 h light/dark photocycle at approximately 25 °C and 40% relative humidity. Multiple holes were created on the sides of the cups for ventilation. The eggs hatched within 2 days of arrival (approximately 3 days after the eggs were laid), directly onto their respective diet treatments. When the eggs hatched, the larvae, which are negatively geotropic and natural climbers, distributed themselves over the diet and sides of the cup. Cups were always incubated in an inverted position, which accommodates the strong climbing tendency of larvae, and which also allows the frass to fall to the lid, thus keeping the diet surface clean. The larvae were fed on the same diet until 6 days post hatch (dph), when larvae were approximately in instar III and sufficiently large to handle with a thin-tipped paint brush or blunt forceps and were then randomly housed individually (within the same treatment). At 6 dph, the larvae were transferred to three new diet cups per treatment to ensure fresh feeding. At 9 dph, when larvae are at the end of instar V, the larvae were transferred to individual cups ($n = 240$) and the cups were placed on their side. This allowed for easier cleaning of frass. The experimental units were arranged as a randomized block design to control for potential sources of environmental variability in the room (e.g. light, noise, vibration, etc.), with 40 units per block and with six blocks in total. There were five replicates per treatment in each block and 48 replicates per treatment total.

Bertha armyworm. Bertha armyworm eggs were obtained from Agriculture and Agri-Food Canada (Canada) and were reared as described by Bucher & Bracken (1976). Eggs (from at least 100 females or more) were deposited on green cabbage leaf segments, with approximately 100 eggs per leaf segment and transported to Ryerson University as such. The leaf segments, egg side up, were placed on the inner surface of the lid of a translucent Dixie cup (163 mL) that contained approximately 10 mL of diet per cup. The cups were placed upside down (i.e. diet on top), with the lid containing the eggs on the bottom. Such positioning prevented moisture formation on the eggs and is advantageous for the larvae's natural tendency to climb after hatching, thus allowing for easier feeding (Butcher and Bracken 1976). The eggs were kept under a 12 : 12 h light/dark photocycle at approximately 25 °C and 40% relative humidity. Multiple holes on the sides of the cups ensured ventilation. The eggs hatched within 4 days of arrival (approximately 5 days after the eggs were laid), directly onto their respective diet treatments. The experimental units were arranged as in the cabbage looper design.

Experimental diets

Artificial diets were used to best ensure consumption of dietary treatments and manipulate specific dietary fatty acids (EPA and DHA). The artificial diet formulations were based on published methods specifically for rearing the bertha armyworm (Bucher & Bracken, 1976) and for cabbage loopers (Natural Resources Canada, 2014; modified from Grisdale and McMoran 1975) for experimental purposes. For both experiments, the LC-PUFA (i.e. EPA and DHA) incrementally replaced 25% up to 100% of the control oil (either camelina or linseed) in the experimental diets according to the ratios (EPA + DHA: control oil): 0 : 1 (control), 0.25 : 0.75 (lowest), 0.50 : 0.50 (low), 0.75 : 0.25 (medium) and 1 : 0 (high). An EPA : DHA ratio of 11 : 7 was maintained in all diets because this represented the ratio found in GE camelina oil (Ruiz-Lopez *et al.*, 2014). For the cabbage looper, diet ingredients were purchased from Natural Resources Canada (Great Lakes Forestry Centre) and formulated to meet the nutritional requirements of the cabbage looper (modified from Grisdale & McMorran, 1963). The control diet contained linseed only as the lipid component, and treatment diets replaced linseed oil with EPA + DHA in the increments stated above. For the bertha armyworm, the five dietary treatments were slightly modified from the formulation of Bucher & Bracken (1976) to include EPA and DHA as a dietary lipid source instead of camelina oil, and where the control diet contained camelina oil (Three Farmers Products©, Saskatchewan, Canada) as the main dietary lipid source. All of the dietary ingredients for the bertha armyworm diet were obtained from Frontier Scientific Services (Newark, New Jersey). The pure EPA and DHA in free fatty acid form were isolated from algae and were both certified to be at 99% purity (Matreya LLC, Pleasant Gap, Pennsylvania). Dietary EPA and DHA were included in the diets in the free fatty acid form. The total amount of EPA + DHA in diets for the cabbage looper was higher than the bertha armyworm as a result of different lipid amounts in the diet formulations in Bucher & Bracken (1976) compared with the diets provided by Natural Resources Canada.

Survival, development time, growth and morphology measurements

Bertha armyworm larvae were weighed before being placed in individual cups with fresh diet at 14 dph and weighed again at 21 dph. For the cabbage looper, the larvae were weighed at 9 dph only, aiming to reduce handling and the time from hatch to pupation was shorter. After pupation, the pupae were weighed and transferred to a cup with no diet containing a small piece of dry filter paper to absorb any moisture released at the time of emergence. After an individual emerged as an adult, it was kept in the container for 24 h to ensure full emergence and wing expansion. Fully emerged adults were sacrificed by placing them in a −20 °C freezer for approximately 3 min until movement ceased and mortality was presumed. The adults were then weighed (wet weight) and pinned, with wings flattened and gently pressed, to an insect board. The wing span was then measured (forewing and hindwing; between wing tips), along

with total wing length (forewing base to apex), forewing and hindwing length (partial length), and body length (tip of head to tip of abdomen), according to Chai & Srygley (1990).

Lipid and fatty acid analysis

After measurements were taken, adults were placed into individual 2.5-mL cryogenic vials and frozen at −80 °C, followed by freeze drying. Whole freeze-dried adults were individually ground to a fine powder in liquid nitrogen using a mortar and pestle. The ensuing powder was weighed to the nearest microgram. Total lipid was extracted using a modified Folch *et al.* (1957) method, as described in McMeans *et al.* (2012) and Hixson *et al.* (2016). In brief, each sample was extracted three times, using 2 mL of chloroform/methanol (2 : 1, v/v) and then pooled (total 6 mL). Polar impurities were removed by adding 1.6 mL of KCl solution (0.9% w/v); this layer was discarded after centrifugation. The resulting lipid-containing solvent was concentrated to 2 mL and two aliquots (100 μL each) were removed and evaporated to dryness to determine total lipid (gravimetric analysis). The lipid extract was then prepared for gas chromatography by derivatizing to FA methyl esters (FAME) using sulphuric acid as the catalyst. FAME were extracted twice using hexane: diethyl ether (1 : 1; v/v), then individual FAME were separated using a gas chromatograph (GC-2010 Plus; Shimadzu, Japan) equipped with a Supelco SP-2560 column (Sigma-Aldrich, St Louis, Missouri). All solvents used in the extraction and FAME derivatization procedure were of high purity high-performance liquid chromatography grade (> 99%). FAME in samples were identified by comparison of their retention times with a known standard (Supelco-37 Component; Sigma-Aldrich) and quantified with a five-point calibration curve using this same standard. A known concentration of 5-alpha-cholestane (C8003; Sigma-Aldrich) was added to each sample prior to extraction to act as a surrogate internal standard to estimate extraction and instrument recovery efficiency.

Statistical analysis

The experimental units ($n = 48$ per treatment) were arranged in six blocks ($n = 40$ individuals per block) in accordance with a general, randomized, block design for both species (Minitab, version 16; Minitab Inc., State College, Pennsylvania). Using multivariate analysis of variance (MANOVA), we tested the hypothesis that dietary treatment (fixed factor) had an effect on multiple response variables, including development (time to pupation, emergence and pupation to emergence) and morphology [adult mass (for bertha armyworm data), and wing span and length], with block included as a random factor. Prior to running the MANOVA, response variables were *Z*-transformed to scale variables and Box-Cox transformed, if necessary, to meet assumptions of normality; analyses were run in R-STUDIO (R Foundation for Statistical Computing, Austria). To determine whether mass varied during an individual's lifetime across diets, we ran a repeated measures analysis of variance (ANOVA) on bertha armyworm and cabbage looper data. For the bertha armyworm analysis, diet (one through five) and larval stage post hatch (12, 21 and 30 dph), as well as their interaction, were run as fixed effects and block was included as a random effect. Adult mass of the bertha armyworm could not be included in the repeated measures ANOVA as a result of the number of mortalities over time; however, adult mass of the bertha armyworm was included in the MANOVA as described above. For cabbage looper data, diet (one through five) and growth stage (larval, pupal and adult stage) were run as fixed effects and block was included as a random effect. In both analyses, the block factor was not significant and was therefore omitted in successive iterations of the analysis. The response variable 'mass' (larvae, pupae or adult mass for cabbage loopers and bertha armyworm) for each species analyses were Box-Cox transformed to meet assumptions of normality and analyses were run in SAS ENTERPRISE, version 6.1 (SAS Institute Inc., Cary, North Carolina). Finally, ANOVA tests were used to test the hypothesis that dietary treatment (fixed factor) affected various fatty acid amounts in the whole moths. Because the eggs were obtained from large colonies and laid from at least 100 females or more, the statistical analyses used assumes all individuals reared from eggs are independent.

Chi-square goodness-of-fit tests were used to analyze the survival and mortality data and determine whether the number of mortalities were equal among all treatments. Linear regression analyses were used to relate whole insect fatty acid content (response) with diet fatty acid content (predictor) (SigmaPlot, version 11.0; Systat Software Inc., Chicago, Illinois). A treatment effect was considered significant at $P < 0.05$ and, where significant differences occurred, treatment means were differentiated using Tukey's honestly significant difference multiple comparison test. The normality, homogeneity and independence of residuals were considered to evaluate the data and appropriateness of the statistical model used.

Results

Diet composition

The cabbage looper diets contained between 4.3% and 5.3% total lipid (Table 1). The total EPA + DHA amount increased from 0 μg mg^{-1} in the control diet to 17.5 μg mg^{-1} in the high-level diet. The medium level and high-level diets had similar EPA + DHA amounts, although this was not as planned in the formulation. The proportion of dietary fatty acids (%) is provided in the Supporting information (Table S1).

The bertha armyworm diets contained between 4.8% and 5.3% total lipid (Table 2). The fatty acid content of the diets varied in the amount of EPA and DHA, which increased incrementally from the control to the high-level diet (Table 2). The total EPA + DHA amount increased from 0 μg mg^{-1} in the control diet to 2.5 μg mg^{-1} in the high-level diet. The proportion of dietary fatty acids (%) is provided in the Supporting information (Table S2).

Survival, growth, development and morphology

Cabbage looper. There were no significant differences in the number of mortalities or the mortality rate among levels of

Table 1. Total lipid and fatty acid content of cabbage looper diets (μg mg^{-1}, dry weight)

	Diet treatment				
Fatty acid	Control	Lowest	Low	Medium	High
Total lipid (% dw)	4.28	5.10	5.17	5.28	4.95
16:0	16.1	17.5	18.9	19.2	15.3
18:0	3.84	3.82	3.94	3.99	2.94
18:1*n*-9	20.3	21.3	22.5	22.9	17.0
18:2*n*-6 (LNA)	37.2	41.8	46.9	47.9	36.6
18:3*n*-3 (ALA)	39.1	38.8	44.4	45.0	29.7
20:5*n*-3 (EPA)	0.00	2.46	6.39	11.1	11.5
22:6*n*-3 (DHA)	0.00	1.34	3.82	6.28	6.01
∑ EPA + DHA	0.00	3.80	10.2	17.4	17.5
∑ SFA	20.7	22.2	23.9	24.1	19.0
∑ MUFA	22.5	24.0	25.4	25.6	19.3
∑ PUFA	76.3	84.4	101.6	110.6	83.9
∑ *n*-3	39.1	42.6	54.6	62.4	47.2
∑ *n*-6	37.2	41.8	46.9	48.2	36.7

ALA, alpha-linolenic acid; dw, dry weight; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LNA, linolenic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

Table 2. Total lipid and fatty acid content of bertha armyworm diets (μg mg^{-1}, dry weight)

	Diet label				
Fatty acid	Control	Lowest	Low	Medium	High
Total lipid (% dw)	5.08	4.97	4.79	5.25	5.24
16:0	6.19	6.64	6.42	6.23	6.79
18:0	1.43	1.55	1.50	1.48	1.59
18:1*n*-9	6.05	6.92	6.61	6.25	6.95
18:2*n*-6 (LNA)	16.7	18.5	17.8	17.0	18.7
18:3*n*-3 (ALA)	9.18	10.5	10.0	9.29	10.3
20:1*n*-9	2.70	3.17	3.00	2.80	3.12
20:5*n*-3 (EPA)	0.00	0.28	0.82	1.23	1.69
22:6*n*-3 (DHA)	0.00	0.24	0.49	0.72	0.80
∑ EPA + DHA	0.00	0.52	1.31	1.95	2.49
∑ SFA	8.00	8.77	8.46	8.30	9.08
∑ MUFA	8.97	10.4	9.90	9.29	10.5
∑ PUFA	27.3	31.2	30.7	29.5	32.9
∑ *n*-3	15.2	17.5	16.7	15.5	17.2
∑ *n*-6	19.5	21.7	20.8	19.8	21.8

ALA, alpha-linolenic acid; RPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; dw, dry weight; LNA, linolenic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

the dietary treatments imposed (Table 3). However, diet significantly influenced both development time and wing morphology (Pillai's value: $F_{36,464} = 2.027$, $P < 0.001$). Cabbage looper larvae that fed on low EPA + DHA diets were approximately 2–3 days slower to pupate (i.e. time from hatch to pupation) and emerge as moths (i.e. time from hatch to emergence) relative to larvae that fed on all other diets (Table 3). For wing morphology, hindwing length was significantly longer (by 11%) in adults that were fed the high EPA + DHA diet compared with the control ($F_{4,121} = 2.4364$, $P = 0.05079$). There were no other significant differences in wing morphology among treatments, including total forewing span, total hindwing span, forewing span, hindwing span, forewing length and hindwing length.

Mass significantly differed among diets ($F_{4,147} = 3.29$, $P = 0.0128$), across developmental stages ($F_{2,294} = 553.3$, $P < 0.001$), and with the interaction of diet and developmental stage ($F_{8,294} = 2.17$, $P = 0.0297$). Overall, the mass of cabbage loopers differed significantly among diets, where individuals fed on the high EPA + DHA diet were 43% larger than individuals fed the low and medium diets. Not surprisingly, pupae were significantly heavier than adults, which were heavier than larvae (Table 3). Finally, cabbage loopers exhibited a significant interaction of diet and developmental stage. Specifically, the mass of pupae fed the control and lowest diets were 2% and 12% smaller, respectively, than pupae fed on the medium diets, with no other biologically significant pairwise differences.

Bertha armyworm. There were no significant differences among the number of mortalities or the mortality rate of bertha armyworms among diets (Table 4). Development time, morphology and adult mass, as well, did not differ significantly in response to dietary treatment (Pillai's value: $F_{44,144} = 1.14$, $P = 0.2797$) (Table 4). Diet ($F_{4,191} = 4.31$, $P = 0.0023$), time ($F_{2,382} = 46.6$, $P < 0.001$), and the interaction of diet and time (at 14, 21 and 30 dph), significantly influenced larval mass ($F_{8,382} = 2.31$, $P = 0.0199$). Bertha armyworm larvae that fed on the control, low, and medium diets (and larvae fed on the lowest diet, marginally), weighed significantly less than larvae fed on the high diet, regardless of time. Additionally, over time, regardless of diet, larval mass increased, from 14 to 30 dph. Finally, the interaction revealed that larvae at 21 dph that fed on the high diet weighed 89%, 82%, and 49% more than larvae that fed on the lowest, low, and medium diets, respectively.

Adult moth fatty acid content

Cabbage looper. Adult cabbage loopers reared on the high EPA + DHA diet had lower total lipid content than the other treatments, although the remaining treatments did not differ among one another (Table 5). Generally, adults reared on the high EPA + DHA diet also showed significantly lower amounts of 16:0, 18:1*n*-9, 18:2*n*-6 and 18:3*n*-3; however, they had significantly higher amounts of EPA and DHA. Saturated fatty acids were lowest in adults fed high EPA + DHA diets but did not differ among the remaining treatments. Monounsaturated fatty acids were lowest in adults in the control and the high EPA + DHA treatments. Total PUFA did not differ among treatments. Total n-3 PUFA was lowest in the high EPA + DHA treatments; however, this treatment did not differ from the control. Adults reared on the high EPA + DHA diet had lower *n*-6 PUFA contents than those on the medium EPA + DHA diet. Adult EPA + DHA content was significant related to diet EPA + DHA content, as analyzed by linear regression (Fig. 1). Retention coefficients (slope) of linear regression for whole insect fatty acid content versus diet fatty content showed positive significant relationships for most fatty acids, with the exception of sums of PUFA, *n*-3 and *n*-6 PUFA (Table 6). The proportion of fatty acids is provided in the Supporting information (Table S3).

Table 3. Survival number of mortalities (*n*) and mortality rate (%), development time (days), mass (mg) and morphology (cm) of larvae and adult cabbage loopers that were fed artificial diets containing eicosapentaenoic acid and docosahexaenoic acid during the larval stage (mean ± SD)

Survival and Mortalities	Control	Lowest	Low	Medium	High	Chi-square	d.f.	*P*-value
Larvae mortalities (*n*)	25	34	47	36	39	3.78	4, 207	0.441
Pupae mortalities (*n*)	9	4	3	4	6	7.04	4, 181	0.134
Total mortalities (*n*)	34	38	50	40	45	4.38	4, 26	0.356
Overall mortality rate (%)	47	53	69	56	63	5.12	4, 288	0.275
Development time[1]						***F*-value**	**d.f.**	***P*-value**
Hatch to pupation (days)	16.7 ± 2.2^{ab}	16.8 ± 2.3^{ab}	19.0 ± 1.5^{c}	17.1 ± 2.1^{b}	15.8 ± 1.7^{a}	9.00	4, 121	**< 0.001**
Hatch to emergence (days)	23.8 ± 2.2^{a}	23.6 ± 2.1^{a}	26.3 ± 1.0^{b}	24.5 ± 1.6^{a}	23.7 ± 2.1^{a}	10.2	4, 121	**< 0.001**
Mass[2,3] **and morphology**[1]								
Larvae mass (9 dph) (mg)	7.9 ± 2.2	10.1 ± 2.2	7.9 ± 1.7	8.0 ± 2.0	14.0 ± 9.0	2.17	8, 284	**0.0297**
Pupae mass (mg)	197 ± 26	177 ± 30	164 ± 25	201 ± 25	174 ± 38			
Adult mass (mg)	93.1 ± 21	82.9 ± 19	78.2 ± 15	92.7 ± 21	91.7 ± 21			
Total forewing span (cm) (λ = 1.7099)	3.3 ± 0.3	3.4 ± 0.2	3.3 ± 0.3	3.4 ± 0.2	3.3 ± 0.2	0.2748	4, 121	0.8937
Total hindwing span (cm)	2.3 ± 0.2	2.2 ± 0.2	2.2 ± 0.3	2.2 ± 0.4	2.3 ± 0.3	0.0847	4, 121	0.9870
Forewing width (cm) (λ = 1.4166)	1.5 ± 0.3	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	0.2299	4, 121	0.9211
Hindwing width (cm)	0.9 ± 0.2	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.2	0.8363	4, 121	0.5047
Forewing length (cm) (λ = 0.9729)	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.3494	4, 121	0.8441
Hindwing length (cm) (λ = 1.1338)	0.93 ± 0.2^{a}	0.95 ± 0.1^{ab}	0.97 ± 0.2^{ab}	0.98 ± 0.1^{ab}	1.05 ± 0.1^{b}	2.4364	4, 121	**0.05079**
Total body length (cm) (λ = 0.5709)	1.3 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	1.5080	4, 121	0.20408

[1]Differences are considered significant at are $P < 0.05$ determined by analysis of variance as a function of mulivariate analysis of variance. Different superscripts indicate significant pairwise differences determined by Tukey's pairwise comparison; means that share the same lowercase letters are not significantly different.
[2]Significant differences ($P < 0.05$) for mass measurements were analyzed by repeated measures (diet, developmental stage over time, diet × time). The statistical analysis shows the results for the interactive effect (diet × time).
[3]Mass, collectively (i.e. larvae, pupae and adult mass), was Box-Cox transformed by λ = 0.3969.
The lambda (λ) value is presented for response variables that required a Box-Cox transformation.

Bertha armyworm. Adult bertha armyworms reared on the control diet had a significantly higher total body lipid than those reared on the diets containing EPA and DHA (Table 7). There was a significant difference in the fatty acid content (μg mg^{-1}) in adults for all fatty acids except for 18:0, 18:1*n*-9 and total monounsaturated fatty acids. The amount of EPA and DHA (independently) was significantly higher in the medium and high treatments than the control and lowest treatments. The EPA and DHA content of adult insects was highest in those reared on the medium and high EPA + DHA diets. Adult EPA + DHA content was significant related to diet EPA + DHA content, as analyzed by linear regression ($r = 0.946$; $P < 0.001$) (Fig. 2). Retention coefficients (slope) of linear regression for whole insect fatty acid content versus diet fatty content did not show significant relationships for most fatty acids (Table 8). However, EPA, DHA, and the sum of EPA + DHA showed a significant positive relationship between whole insect fatty acid content versus diet fatty acid content. There was also a significant negative relationship between whole insect ALA and diet ALA content. The proportion of fatty acids is provided in the Supporting information (Table S4).

Discussion

The *n*-3 LC-PUFA (EPA and DHA) are not normally available to terrestrial insects that do not inhabit (as larval stages) and/or live in proximity to aquatic ecosystems where they may prey on insects emerging from aquatic ecosystems. Thus, until recently, the effects of *n*-3 LC-PUFA consumption on terrestrial insects have been largely unknown (St-Hilaire *et al.*, 2007; Hixson *et al.*, 2016; Barroso *et al.*, 2017; Lehtovaara *et al.*, 2017). This is important in the context of the potential large-scale cultivation of novel transgenic crops engineered to produce EPA and DHA (Colombo *et al.*, 2018; MacDonald *et al.*, 2018) and for culturing insects as a food source (van Huis, 2013). Dietary fatty acids can exert significant effects on the life cycle and physiology of insects, including, for example, changes in mating success, fertility, survival and also in the production of morphological abnormalities (Grau and Terriere, 1971; Andrews & Miskus, 1972; Dadd, 1983; Wang *et al.*, 2006; Hixson *et al.*, 2016). In the present study, dietary EPA and DHA had significant effects on development time, body mass and fatty acid content in the cabbage looper and bertha armyworm. Dietary treatment (interactive with time) had a significant effect on individual mass of both insects. Fatty acids in the diet were retained in adult insects. There was a clear positive relationship between the amount of dietary EPA + DHA and the amount of EPA and DHA subsequently retained in adult insects. It is worth noting, however, that the EPA + DHA content in the cabbage looper diets is higher than in the bertha armyworm diets (i.e., 17.5 μg mg^{-1} in the high treatment for cabbage loopers, 2.49 μg mg^{-1} in the high treatment for bertha armyworms).

Survival, growth, development and morphology

Dietary EPA and DHA did not impact survival in cabbage loopers or bertha armyworms. However, development time was affected by dietary treatment. In both the cabbage looper and bertha armyworm, the time from hatch to pupation was longer for larvae that were fed a low amount of EPA + DHA compared with larvae that were fed the high amount of EPA + DHA or

Table 4. Survival, number of mortalities (*n*) and mortality rate (%), development time (days), mass (mg) and morphology (cm) of larvae and adult bertha armyworms that were fed artificial diets containing eicosapentaenoic acid and docosahexaenoic acid during the larval stage (mean ± SD)

Survival and mortalities	Control	Lowest	Low	Medium	High	Chi-square	d.f.	*P*-value
Larvae mortalities (*n*)	10	15	18	20	17	3.89	4, 80	0.421
Pupae mortalities (*n*)	10	9	10	6	10	1.46	4, 45	0.834
Total mortalities (*n*)	20	24	28	26	27	1.66	4, 125	0.799
Overall mortality rate (%)	42	50	58	54	56	7.82	4, 260	0.098
Development time[1]						***F*-value**	**d.f.**	***P*-value**
Hatch to pupation (days) ($\lambda = 0.5163$)	32.7 ± 3.4^{b}	36.2 ± 4.3^{a}	35.9 ± 5.6^{a}	34.3 ± 3.6^{abc}	32.6 ± 5.3^{bc}	1.14	44, 144	0.2797
Hatch to emergence	52.1 ± 3.8	55.2 ± 3.6	53.4 ± 5.2	54.5 ± 3.2	51.6 ± 3.6			
Pupation to emergence (days) ($\lambda = 0.7935$)	19.9 ± 1.6	20.1 ± 3.9	19.6 ± 1.9	20.5 ± 2.0	19.4 ± 3.1			
Mass[2,3] **and morphology**[1]								
Larvae mass (14 dph) (mg)	42.3 ± 25	36.6 ± 25	40.2 ± 21	44.3 ± 32	50.6 ± 30	2.31	8, 382	**0.0199**
Larvae mass (21 dph) (mg)	163 ± 114	145 ± 119	151 ± 135	185 ± 132	275 ± 185			
Larvae mass (30 dph) (mg)	296 ± 133	275 ± 145	194 ± 142	225 ± 85	288 ± 190			
Adult mass (mg) ($\lambda = 0.5648$)	185 ± 41	200 ± 42	169 ± 33	163 ± 23	176 ± 53	1.14	44, 144	0.2797
Total forewing span (cm)	3.75 ± 0.3	3.85 ± 0.2	3.66 ± 0.4	3.79 ± 0.3	3.83 ± 0.3			
Total hindwing span (cm)	2.35 ± 0.3	2.33 ± 0.2	2.36 ± 0.2	2.53 ± 0.3	2.21 ± 0.3			
Forewing span (cm) ($\lambda = 0.1097$)	1.66 ± 0.1	2.63 ± 3.8	1.71 ± 0.2	2.65 ± 3.5	1.73 ± 0.1			
Hindwing span (cm)	1.14 ± 0.3	1.14 ± 0.1	1.17 ± 0.2	1.11 ± 0.2	1.2 ± 0.2			
Forewing length (cm)	0.94 ± 0.1	0.91 ± 0.1	0.89 ± 0.1	0.91 ± 0.1	0.95 ± 0.1			
Hindwing length (cm) ($\lambda = 1.1077$)	0.90 ± 0.1	0.94 ± 0.1	0.87 ± 0.2	0.96 ± 0.1	0.93 ± 0.1			
Total body length (cm)	1.91 ± 0.3	1.98 ± 0.2	1.94 ± 0.1	1.96 ± 0.2	1.96 ± 0.2			

[1]Differences are considered significant at are $P < 0.05$ determined by analysis of variance as a function of multivariate analysis of variance.
[2]Significant differences ($P < 0.05$) for mass measurements were analyzed by repeated measures (diet, larvae mass over time (days post hatch, dph), diet × time). The statistical analysis shows the results for the interactive effect (diet × time).
[3]Mass, collectively (i.e., larvae 14 dph, larvae 21 dph, and larvae 30 dph), was Box-Cox transformed by $\lambda = 0.6362$.
The lambda (λ) value is presented for response variables that required a Box-Cox transformation.

Table 5. Total lipid and fatty acid content of emerged cabbage looper adults (μg mg^{-1} dry weight)

Fatty acid	Control	Lowest	Low	Medium	High	*F*-value	d.f.	*P*-value
Total lipid (% dw)	10.1 ± 1.1^{ab}	12.1 ± 1.8^{a}	10.8 ± 2.1^{a}	12.1 ± 1.9^{a}	8.6 ± 1.1^{b}	8.5	4, 54	0.000
16:0	3.1 ± 0.4^{ab}	3.7 ± 0.6^{a}	3.2 ± 0.2^{ab}	3.8 ± 0.6^{a}	2.6 ± 0.4^{b}	7.6	4, 54	0.000
18:0	0.21 ± 0.05	0.21 ± 0.04	0.17 ± 0.08	0.22 ± 0.04	0.16 ± 0.02	3.5	4, 54	0.013
18:1*n*-9	3.8 ± 0.5^{b}	5.0 ± 0.8^{a}	4.2 ± 0.9^{ab}	4.8 ± 0.9^{a}	3.4 ± 0.4^{b}	8.9	4, 54	0.000
18:2*n*-6	1.3 ± 0.1^{ab}	1.4 ± 0.2^{a}	1.2 ± 0.4^{ab}	1.4 ± 0.2^{a}	1.1 ± 0.1^{b}	9.8	4, 54	0.000
18:3*n*-3	0.81 ± 0.2^{b}	0.76 ± 0.3^{b}	1.1 ± 0.1^{a}	$0.75 \pm 0.3b^{c}$	0.49 ± 0.1^{c}	3.7	4, 54	0.009
20:5*n*-3	0.05 ± 0.008^{c}	0.09 ± 0.03^{bc}	0.12 ± 0.05^{b}	0.21 ± 0.05^{a}	0.21 ± 0.05^{a}	32.5	4, 45	0.000
22:6*n*-3	0.00 ± 0.0^{a}	0.003 ± 0.001^{a}	0.008 ± 0.003^{a}	0.01 ± 0.006^{a}	0.09 ± 0.05^{b}	27.9	4, 54	0.000
∑ EPA + DHA	0.0 ± 0.0^{d}	0.09 ± 0.03^{cd}	0.13 ± 0.05^{c}	0.22 ± 0.05^{b}	0.30 ± 0.06^{a}	51.6	4, 54	0.000
∑ SFA	3.4 ± 0.5^{ab}	4.0 ± 0.6^{a}	3.5 ± 0.7^{ab}	4.1 ± 0.7^{a}	2.8 ± 2.5^{b}	7.6	4, 54	0.000
∑ MUFA	4.5 ± 0.5^{b}	5.8 ± 0.9^{a}	5.1 ± 1.3^{ab}	5.7 ± 1.0^{a}	4.3 ± 1.0^{b}	5.9	4, 54	0.000
∑ PUFA	2.1 ± 0.3	2.2 ± 0.4	2.2 ± 0.8	2.4 ± 0.5	1.9 ± 0.2	1.7	4, 54	0.158
∑ *n*-3	4.6 ± 0.5^{bc}	5.7 ± 0.9^{b}	5.1 ± 1.2^{ab}	5.6 ± 1.1^{ab}	3.9 ± 0.5^{c}	7.9	4, 54	0.000
∑ *n*-6	1.3 ± 0.1^{ab}	1.4 ± 0.2^{ab}	1.2 ± 0.5^{ab}	1.5 ± 0.2^{a}	1.1 ± 0.1^{b}	3.6	4, 54	0.000

Different superscripts indicate significant pairwise differences determined by Tukey's pairwise comparison; means that share the same lowercase letters are not significantly different. Differences are considered significant at $P < 0.05$, as determined by analysis of variance.
EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

none at all (control). It is possible that, at low concentrations, EPA and DHA does not meet a threshold for physiological activity, whereas higher amounts of EPA and DHA exceed our threshold for measureable physiological activity (in terms of the metrics employed here). Notably, cabbage loopers fed the high EPA + DHA diet reach pupation 1 day earlier than those fed the control diet, although this result is not statistically significant. However, insect mass, development and morphology results are not linearly related with increasing dietary EPA and DHA. Insect mass compared among treatment groups is also not consistent over time. For example, cabbage looper larvae that are fed the high EPA + DHA diet are heaviest at 9 dph larvae compared with larvae in all other treatments, although they are no longer heaviest at the pupae or adult stage compared with other treatments. Similarly, bertha armyworm larvae fed the high EPA + DHA diet are heaviest at 14 and 21 dph compared with larvae in any other treatment, although not at 30 dph. As such, the direct impacts of EPA and DHA on these measurements are

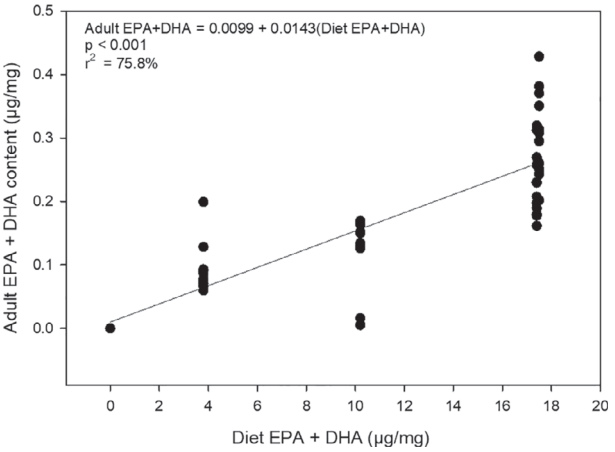

Fig. 1. Linear relationship between the amounts of dietary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and adult EPA + DHA content in newly emerged cabbage looper adults. Data points represent the EPA + DHA content (µg mg^{-1} dry weight) in a single individual.

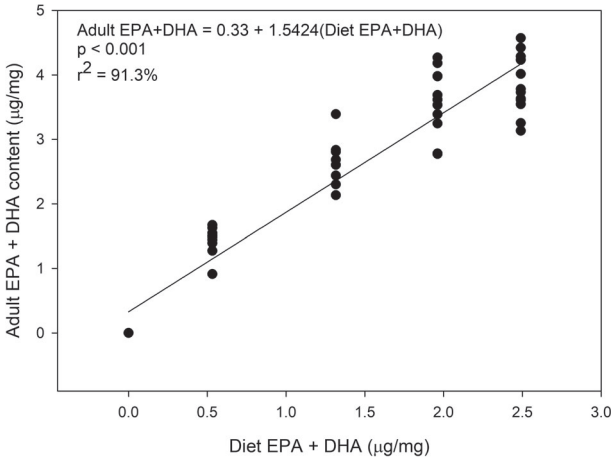

Fig. 2. Linear relationship between the amounts of dietary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and adult EPA + DHA content in newly emerged bertha armyworm adults. Data points represent the EPA + DHA content (µg mg^{-1} dry weight) in a single individual.

Table 6. Coefficients of linear regressions between whole insect fatty acid content (µg mg^{-1}) and diet fatty acid content (µg mg^{-1}) for the cabbage looper (d.f. = 1,54)

Fatty acid	Slope (mean ± SE)	*F*-value	*P*-value
16:0	0.219 ± 0.056	15.2	< 0.001
18:0	0.041 ± 0.018	4.93	0.030
18:1*n*-9	0.223 ± 0.051	18.7	< 0.001
18:2*n*-6	0.015 ± 0.007	4.37	0.031
18:3*n*-3	0.168 ± 0.054	9.58	< 0.001
20:5*n*-3	0.014 ± 0.001	124	< 0.001
22:6*n*-3	0.009 ± 0.002	18.2	< 0.001
∑ EPA + DHA	0.014 ± 0.001	182	< 0.001
∑ SFA	0.225 ± 0.052	18.2	< 0.001
∑ MUFA	0.213 ± 0.059	13.1	< 0.001
∑ PUFA	0.009 ± 0.005	2.94	0.092
∑ *n*-3	0.027 ± 0.017	2.37	0.129
∑ *n*-6	0.012 ± 0.008	2.15	0.148

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

nonlinear but have an impact relative to insects fed a control diet.

Dietary lipids can have positive or negative effects on herbivore performance in insects, including development time; however, the specific relationship with individual fatty acids and development time has not been thoroughly documented. Insects fed low-quality diets, with inadequate levels of the essential fatty acid ALA, exhibit poor performance. For example, the Asian lady beetle (*Harmonia axrydis*) reared on an artificial diet with low ALA showed poor emergence rates and reductions in other quality parameters (including daily weight gain, the development times, the weights of the newly-emerged adults, the female fecundity and fertility) compared with individuals that were fed a control diet of Zeller eggs (*Ephestia kuehniella*), which is assumed to be of higher quality (Sighinolfi *et al.*, 2013). Plants of poor (or perhaps novel) nutritional quality can adversely affect the larval growth and development of herbivorous insects, which essentially becomes a paradox because the insects need to feed more to reach the adult stage, potentially causing more damage to the host plant (Clancy & Price, 1987).

Varying development times associated with EPA and DHA consumption could have other ecological consequences, which could impact the fitness and survival in these crop pests. Slower development (observed in low dietary amounts of EPA and DHA) or faster development (high dietary amounts of EPA and DHA in the cabbage looper) could misalign ideal pupation timing with food availability, environmental stressors and/or predator demography. For many *Brassica* pests, such as bertha armyworm, their life cycle coincides with crop/nutrient availability and pupation is in early autumn for overwintering (Canola Council of Canada, 2014). Increasing the duration of the larval stage could mean more crop damage because the larvae will consume the crop for longer periods prior to pupation, although delayed pupation could be fatal with somewhat unpredictable first frost dates and generally falling temperatures in autumn. Early pupation could mean better preparation for overwintering. The cabbage looper normally produces two or three overlapping generations per season (Natural Resources Canada, 2014) and potentially shorter development times could mean that these overlapping generations could be completed earlier.

Both diet and development determine insect body mass in the present study, meaning that, over time, EPA and DHA impacts growth. Black soldier fly larvae also show increased larval mass specifically in response to dietary lipid containing EPA and DHA. Black soldier fly larvae (*Hermetia illucens*) reared on a mixture of cow manure and either 10%, 25%, or 50% fish offal (from rainbow trout, which contains EPA and DHA) weigh significantly more than the control larvae fed cow manure (St-Hilaire *et al.*, 2007). Larvae fed as little as 10% fish offal have 43% more than the control group fed cow manure (30%

Table 7. Total lipid and fatty acid content of emerged bertha armyworm adults (μg mg^{-1} dry weight)

Fatty acid	Control	Lowest	Low	Medium	High	*F*-value	d.f.	*P*-value
Total lipid (% dw)	26.9 ± 3.8^{a}	22.5 ± 2.9^{b}	20.8 ± 2.5^{b}	20.5 ± 2.7^{b}	20.4 ± 5.7^{b}	5.32	4, 51	0.001
16:0	5.98 ± 2.1^{a}	5.31 ± 1.4^{ab}	2.99 ± 0.4^{b}	4.34 ± 1.0^{ab}	4.75 ± 1.8^{ab}	3.07	4, 51	0.026
18:0	0.53 ± 0.1	0.52 ± 0.1	0.38 ± 0.03	0.44 ± 0.2	0.45 ± 0.1	1.68	4, 51	0.172
18:1*n*-9	10.7 ± 4.1	9.75 ± 2.7	5.50 ± 0.7	8.11 ± 0.1	9.11 ± 3.4	2.58	4, 51	0.051
18:2*n*-6	5.26 ± 1.5^{a}	5.23 ± 0.9^{a}	3.27 ± 0.5^{b}	4.66 ± 0.9^{ab}	4.41 ± 1.1^{ab}	3.69	4, 51	0.012
18:3*n*-3	2.90 ± 0.6^{a}	2.49 ± 0.4^{a}	1.84 ± 0.2^{b}	2.32 ± 0.3^{ab}	1.99 ± 0.4^{b}	8.23	4, 51	< 0.001
20:1*n*-9	0.25 ± 0.1^{a}	0.23 ± 0.1^{ab}	0.14 ± 0.0^{b}	0.21 ± 0.0^{ab}	0.18 ± 0.1^{b}	4.71	4, 51	0.003
20:5*n*-3	0.0 ± 0.0^{a}	0.14 ± 0.0^{a}	0.25 ± 0.0^{b}	0.34 ± 0.0^{c}	0.37 ± 0.0^{c}	119	4, 51	< 0.001
22:6*n*-3	0.0 ± 0.0^{a}	0.007 ± 0.0^{ab}	0.01 ± 0.0^{bc}	0.02 ± 0.0^{d}	0.017 ± 0.0^{cd}	16.9	4, 51	< 0.001
∑ EPA + DHA	0.0 ± 0.0^{a}	0.15 ± 0.0^{a}	0.26 ± 0.0	0.36 ± 0.0	0.39 ± 0.0	111.4	4, 51	< 0.001
∑ SFA	6.74 ± 2.3^{a}	6.06 ± 1.5^{ab}	3.54 ± 0.5^{b}	5.00 ± 1.1^{ab}	5.43 ± 0.1^{ab}	3.01	4, 51	0.029
∑ MUFA	12.0 ± 4.7	10.9 ± 3.1	6.2 ± 0.8	9.9 ± 3.2	10.1 ± 3.7	2.41	4, 51	0.064
∑ PUFA	8.41 ± 2.1^{a}	8.00 ± 1.2^{ab}	5.47 ± 0.7^{b}	6.69 ± 1.5^{ab}	6.88 ± 1.5^{ab}	3.65	4, 51	0.012
∑ *n*-3	13.5 ± 4.7^{a}	12.2 ± 3.0^{ab}	7.34 ± 0.8^{b}	10.3 ± 1.8^{ab}	11.9 ± 0.4^{ab}	2.91	4, 51	0.003
∑ *n*-6	5.59 ± 1.6^{a}	5.51 ± 0.9^{ab}	3.4 ± 061^{b}	4.57 ± 1.8^{ab}	4.63 ± 1.1^{ab}	3.19	4, 51	0.022

Different superscripts indicate significant pairwise differences determined by Turkey's pairwise comparison; means that share the same lowercase letters are not significantly different. Differences are considered significant at $P < 0.05$, as determined by analysis of variance.
EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

Table 8. Coefficients of linear regressions between whole insect fatty acid content (μg mg^{-1}) and diet fatty acid content (μg mg^{-1}) for the bertha armyworm (d.f. = 1, 51)

Fatty acid	Slope (mean ± SE)	*F*-value	*P*-value
16:0	−0.360 ± 1.02	0.12	0.726
18:0	−0.221 ± 0.30	0.54	0.465
18:1*n*-9	−0.730 ± 1.23	0.32	0.574
18:2*n*-6	−0.197 ± 0.21	0.88	0.352
18:3*n*-3	−0.387 ± 0.135	8.26	0.006
20:5*n*-3	0.213 ± 0.010	424	< 0.001
22:6*n*-3	0.024 ± 0.003	55.5	< 0.001
∑ EPA + DHA	0.127 ± 0.006	426	< 0.001
∑ SFA	−0.509 ± 0.674	0.57	0.454
∑ MUFA	−0.743 ± 0.856	0.75	0.389
∑ PUFA	−0.224 ± 0.124	3.26	0.077
∑ *n*-3	−0.479 ± 0.559	0.79	0.379
∑ *n*-6	−0.104 ± 0.213	0.24	0.627

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

lipid versus 21% lipid, in larvae). Our results show that cabbage looper larvae at (9 dph) that are fed on the high EPA + DHA diet are 77% larger than individuals fed the low and medium diets. Furthermore, the omega-3 fatty acid content of larvae increase from negligible amounts, when fed cow manure, to approximately 3% of the lipid consisting *n*-3 LC-PUFA, when fed fish offal (St-Hilaire *et al.*, 2007). This evidence is consistent with our results that dietary lipid content predicts the nutritional content of the insect; however, this does not necessarily directly predict the insect mass.

Ultimately, insect growth rates (including both rate of development and final weight at pupation) can significantly impact overall fitness (Slansky, 1982). Indeed, fecundity in most insects is positively correlated with female body size, under constant environmental conditions (Honěk, 1993). In many herbivorous insects, measurements of size, such as pupal or adult weight (Leather, 1988a, b), and allometric measurements (related to size), such as hind tibia length (Carter *et al.*, 1991), are strongly correlated with potential fecundity (Amwack & Leather, 2002). Host plant quality, especially the nutrient composition, is known to affect the fecundity of herbivorous insects (Amwack & Leather, 2002). Thus, EPA + DHA stored in adults, as observed in the present study, may influence both fecundity, as it relates to larger body size, and also transfer to offspring, which could consequently affect larval growth and development time of the next generation. Future research should explore the impact of EPA and DHA on insect fecundity.

Fatty acid content

Dietary EPA + DHA are directly incorporated in the tissues of the cabbage looper and bertha armyworm larvae and are retained during pupation and in the emerged adults (Figs. 1 and 2). The positive linear relationship accounts for > 90% of the variation between diet and whole-body EPA and DHA content in adult bertha armyworm. It is well known that insect PUFA content is related to dietary PUFA content (Stanley-Samuelson & Dadd, 1983; Stanley-Samuelson *et al.*, 1988; Wang *et al.*, 2006; Komprda *et al.*, 2013; Sighinolfi *et al.*, 2013; Barroso *et al.*, 2017; Lehtovaara *et al.*, 2017; Rutaro *et al.*, 2018). The retention coefficient (slope of linear regression) is less than 20% for PUFA (and all fatty acids), which suggests a maximum or limited uptake of EPA + DHA into the adult insect. Given that terrestrial insects have not evolved with access to dietary EPA + DHA, there has not been strong selection pressure to highly retain LC-PUFA and, as such, high retention efficiencies for LC-PUFA are not expected for terrestrial insects. That said, the positive and significant linear regression for EPA and DHA demonstrates that these LC-PUFA are retained in a dose-dependent manner. Dietary EPA and DHA retention

is also observed in adult cabbage white butterflies (another brassicaceous crop pest) after they are fed diets containing increasing levels of EPA + DHA (Hixson *et al.*, 2016), and there is a similarly positive, linear relationship ($r^2 > 90\%$). Black soldier fly larvae fed fish oil also retain a sum of 3% of ALA, EPA and DHA (St-Hilaire *et al.*, 2007). LC-PUFA retention is also observed in the grasshopper *Ruspolia differens* when providing them with fish feed containing EPA and DHA (Lehtovaara *et al.*, 2017). Retention of EPA specifically is observed in the black soldier fly larvae (Barroso *et al.*, 2017), as well as the malarial vector mosquito, *Anopheles stephensi* (Moribayashi *et al.*, 2004). It is possible that this retention could indicate a role of EPA in insect function. This was prevalent in a study demonstrating that, after a few hours of the start of a feeding trial, the *n*-3 LC-PUFA reaches the highest concentration, indicating that the larvae retain these PUFA from the diet into tissue storage, mainly membrane phospholipids (Barroso *et al.*, 2017). There is also evidence of bioenhancement of PUFA from the diet to the larva and butterfly in the blue morpho *Morpho peleides* (Wang *et al.*, 2006). The high unsaturation of membranes may have functional importance in providing membrane fluidity useful in flight. This could suggest that the enzymes involved in the synthetic pathways for phospholipids and triacylglycerol synthesis may be selective for EPA incorporation.

In terms of using insects as a nutritional resource, we suggest that future investigations might focus on assessing the bioaccessibility of the *n*-3 LC-PUFA present in the larvae as a source of food, as well as assess the degree of storage of EPA and DHA in insects by using other foodstuffs (e.g. microalgae, transgenic yeast and/or fishery industry byproducts). Although the retention efficiency of fatty acids is under 20% in adult insects in the present study, retention may be different in larvae. This also has implications for the trophic transfer of EPA and DHA from transgenic oilseeds to terrestrial herbivores, as well as to higher-order consumers. This should be considered carefully when assessing the ecological consequences of commercially grown terrestrial crops that produce EPA and DHA, given that, in aquatic ecosystems, trophic transfer efficiency is higher for LC-PUFA (9.3%) than for bulk carbon (4.8%; Gladyshev *et al.*, 2013). In addition, the potential for PUFA retention is documented in terrestrial insects (Moribayashi *et al.*, 2004; Wang *et al.*, 2006; Barroso *et al.*, 2017; Lehtovaara *et al.*, 2017). This has important ecological implications for commercially grown crops that produce EPA and DHA, considering the subsequent consumption of crops by insect pests will result in the assimilation of these compounds in their tissues.

Conclusions

In the insects investigated in the present study, dietary EPA + DHA incrementally added to the diets shows interactive effects on the development time and biomass and storage of EPA and DHA in the whole body of emerged adults. In terms of nutritional ecology in insects, variation from the optimum set of conditions (body size, timing of life cycle events, etc.) can impact maximal fitness for the individual (Slansky, 1982). The changes observed in the present study in terms of biomass, development time and body nutritional content could have fitness consequences for these organisms. Direct incorporation of EPA and DHA in both species, transferred from larvae to adult, demonstrates that there could be trophic transfer of EPA and DHA from primary producers (via transgenic oilseeds oilseeds) to primary consumers (e.g. cabbage looper and bertha armyworm) and, thus, this is potentially available to higher trophic level consumers. This also has great potential for accumulating EPA and DHA in insect larvae that are cultivated as a nutritional source for livestock, aquaculture species and humans. Thus, in terms of commercializing transgenic oilseed crops, the potential for introducing EPA and DHA in the agroecosystem may have broad consequences for the growth and survival of many terrestrial organisms.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Total lipid and fatty acid content of cabbage looper diets (percentage of total FAME dry weight)

Table S2. Total lipid and fatty acid content of bertha armyworm diets (percentage of total FAME dry weight)

Table S3. Total lipid and fatty acid content of cabbage looper adults (% total FAME dry weight)

Table S4. Total lipid and fatty acid content of emerged bertha armyworm adults (% total FAME dry weight)

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