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Rotifers enriched with a mixed algal diet promote survival, growth and development of barramundi larvae, *Lates calcarifer* (Bloch)

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ABSTRACT

Fulfilling the nutritional requirements of marine fish larvae, including barramundi (*Lates calcarifer*), remains a bottleneck for optimal commercial hatchery production. This study investigated the effect of mixed algal diets for the enrichment of rotifers on the survival, growth, development and stress resistance of barramundi larvae. Four groups of 2–13 days-post-hatch (dph) barramundi larvae were fed rotifers enriched with either *Nannochloropsis oculata, Chlorella vulgaris*, or a blend of 50:50 or 75:25 of *N. oculata* to *C. vulgaris* (based on dry weight, DW), respectively. The highly unsaturated fatty acids (HUFAs) docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA) were used as qualitative proxies of the feed. The barramundi larvae fed with the 50:50 diet outperformed all other treatments, with significantly greater larval length ($P<0.05$), eye diameter ($P<0.05$) and body depth ($P<0.05$) at 10 dph, a trend conserved over the whole growth period. The barramundi larvae fed the 50:50 diet also achieved the highest flexion percentage at 10 dph ($P<0.05$) and scored the highest survival past the stress test (85%; $P<0.05$). The best performing treatment had the second highest level of DHA which suggests that while DHA is an important fatty acid for barramundi larvae it is likely its interaction with other EFA and monounsaturated fatty acid also plays a critical role in their development. Overall, blending microalgae species can enhance the balance of dietary fatty acid for barramundi larvae which in turn can lead to better growth, faster development and higher stress resistance when compared to monospecific microalgal diets.

1. Introduction

The development of a diet fulfilling the nutritional requirements of fish larvae is critical for optimal hatchery production. Currently there is little known regarding the nutritional requirements of marine fish larvae compared to their freshwater counterparts (Hamre et al., 2013). The knowledge gaps are especially true for the exogenous feeding period until weaning onto artificial diets. The vast majority of freshwater fish larvae can be produced on a commercial scale using exclusively formulated diets from the start of exogenous feeding; however, most marine fish larvae cannot be reared with such diets and have to be fed live feed (Mæhre et al., 2013). Such incapacity of marine fish larvae is mainly due to their small size, undeveloped digestive system and poor acceptance of artificial diets (Tocher, 2010). Although weaning protocols have been developed for some marine species (Dhert et al., 1992; Suzer et al., 2007; Ballagh et al., 2010), the use of live feed is still required for the commercial production of marine fish. Common live feeds in commercial marine fish hatcheries include rotifers (*Brachionus* sp.) and brine shrimp (*Artemia* sp.) which are respectively fed to larvae at early (2–30 dph) and later (8–40 dph) larval development stages (Dhert et al., 2001; Aragão et al., 2004). Due to the elevated cost of production of live feed and its potentially variable nutritional quality, significant focus has been allocated to the replacement or reduction of live feed for the culture of marine fish larvae (see review by Conceição et al., 2010), however only with limited success (Cahu and Zambonino-Infante, 2001; Curnow et al., 2006; Conceição et al., 2010).

The enrichment of live feed, generally with microalgae, is a common and necessary practice to boost the quality of the otherwise nutrient deficient feed (Fehér et al., 2013). There is however a high cost associated with on-site microalgae production, therefore commercial production favour a more cost effective, off the shelf option of microalgal pastes (Patil et al., 2005; Spolaore et al., 2006;

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Conceição et al., 2010). These commercially available microalgae include *Nannochloropsis oculata* and *Chlorella vulgaris* and in addition to feed rotifers and/or *Artemia* for fish larvae they are also used to dose the larval tanks to promote "green water". The green water technique not only maintains the nutritional quality of rotifers as a feed, it also provides the fish larvae with a more shaded environment, which results in an improved feed ingestion, survival and growth (Øie et al., 1997; Reitan et al., 1997; Stuart and Drawbridge, 2011).

The culture of barramundi, or Asian sea bass (*Lates calcarifer*), has occurred in Australia for more than 30 years. There has been significant improvement in its culture especially for the juvenile and grow-out stages but bottlenecks still remain for the larval phase. These challenges include mortality at metamorphosis (about 19 dph), vaccuolation of the spine and brain, and high sensitivity to stress (Rimmer et al., 1994). These symptoms have been mainly associated with deficiencies in essential fatty acids (EFA) in particular the long chain highly unsaturated fatty acids (HUFAs). Important HUFAs include docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (ARA). These HUFAs have critical roles in the development of the neural and visual system, structure and function of cell membrane and are linked to growth, survival and stress resistance in marine fish (Rajkumar, 2006; Tocher, 2010). The early larval rearing of barramundi in Australia in most instances relies on two microalgae for the rotifer feeding period: (1) *N. oculata* or (2) *C. vulgaris*. *N. oculata* has no or little amounts of DHA, but tends to be rich in EPA with some moderate amount of ARA (Lubzens et al., 1995; Chini Zittelli et al., 1999). On the other hand, DHA enriched *C. vulgaris* is low in EPA and has no ARA (Matsunari et al., 2013). The aim of this study was to test if the quality and performance of barramundi larvae (growth, stress resistance and development) can be improved by feeding the larvae rotifers enriched with microalgae blends (*C. vulgaris* and *N. oculata*) in comparison to a monospecific diet.

2. Materials and methods

2.1. Algal diets

The four diets tested included *N. oculata* (MBD Energy Ltd.), *C. vulgaris* (SuperFresh V12, Chlorella Industry, Tokyo, Japan), as well as a 50:50 ratio and 75:25 ratio of the two, respectively. The *N. oculata* and *C. vulgaris* purchased ready-made pastes had inter alia different cell densities therefore the microalgae blends and the amount fed to the rotifers were standardized based on dry weights (DW).

2.2. Rotifer culture and enrichment

The inoculum of the L-type rotifer, *Brachionus plicatilis*, was obtained from the Marine and Freshwater Research Facility Unit (MARFU) at James Cook University in Townsville, Australia. The rotifers were maintained in four conical 100 L tanks (water volume 50 L) at a density of 1000 rotifers mL^{-1} in an air conditioned illuminated room (12 L:12 D, from 8:00 am to 8:00 pm). The water was kept at a salinity of 28 g L^{-1} and 28.73 ± 0.01 °C. Individual rotifer tanks were coarse aerated with four open ended 4 mm airlines weighted with a porcelain reel insulator (No 26) to keep the air outlets close to the bottom of the tank. To prevent the influence of the previous rotifer diet (Super fresh V12), the four rotifer cultures were allocated their relative diet in the form of algal paste 48 h prior to the start of the experiment. Rotifers were enriched twice daily 30 min prior to being fed to barramundi larvae at 0800 and 1500 with 0.15 g of microalgae L^{-1} DW. Once enriched, the rotifers were sampled for biochemical analysis. The larval tanks were each dosed with 0.065 g L^{-1} (DW) of each of their respective fresh microalgae paste treatment every morning prior to receiving the rotifers. The microalgae paste was administered to each larval tank using a syringe. The aeration in the larval tanks was sufficient to disperse the microalgae paste throughout the water column. At the time of feeding the rotifer density (from the rotifer and larval tanks) was estimated by counts of 1 mL samples (n = 3) using a Sedgwick rafter counter and an Olympus CX 22 LED microscope. The rotifers were harvested by syphoning the culture over a submerged 55 μm screen to ensure survival. They were then washed with UV filtered seawater and fed to the larvae.

2.3. Broodstock induction and larval rearing

The broodstock used to produce the larvae for this experiment were held at MARFU. The seven broodstock fish, of which three were females, were fed 24 mm pellets of Lansy Breed Maturation (INVE Aquaculture) at 2% body weight per week. To induce a spawn, all seven fish received a single intramuscular injection (0.20 μg L^{-1}) of luteinizing hormone releasing hormone analogue (Aquatic Diagnostic Services International Pty. Ltd.). The fertilised barramundi eggs were transferred to a 500 L fibreglass tank at a density of 2000 eggs L^{-1}. At 1 dph a surface skimmer was positioned in the stock tank in order to facilitate swim bladder inflation. The larvae were stocked at 2 dph at a density of 260 larvae L^{-1} in 12 × 100 L tanks (3 replicates per treatment). This high stocking density was chosen to mimic the commercial practices for intensive barramundi hatchery and to allow for sufficient larvae to be available for biochemical analysis. The larval rearing was conducted in the temperature controlled room described above. The 12 larval tanks, each fitted with a 250 μm stand pipe, were part of a recirculating system which included a biofilter, two 25 μm bag filters, a protein skimmer, a 160 W Ultra Violet light (UV) and a heater-chiller unit with salinity maintained at 32 g L^{-1} and temperature at an average of 31.16 ± 0.01 °C. The larval tanks were each gently aerated using three airstones and the dissolved oxygen was kept at 6 mg L^{-1} in each larval tank using oxygen injection in the system's sump.

Shortly after stocking, the larvae were randomly allocated their treatment and fed at an approximate rate of 15 rotifers mL^{-1}. This density was maintained for both daily feeds (8:00 am and 3:00 pm) until 3 dph and then was increased to 40 rotifers mL^{-1} until 7 dph and to 60 rotifers mL^{-1} from 8 until 13 dph. The flow rate in the larval tanks was increased at the end of the day (8:00 pm) from 0.3 to 1 L min^{-1} to remove the uneaten rotifers from the larval tanks via the internal 250 μm stand pipe and returned to 0.3 L min^{-1} the following morning (7:30 am). This procedure also maintained good water quality in the larval tank with total ammonia and nitrite kept below 0.3 mg L^{-1} (API, Aquarium pharmaceuticals test kits) for the duration of the experiment.

2.4. Sampling and analysis

2.4.1. Biochemical analysis

The composition of endogenous resources is often used as a proxy for nutritional requirements of marine fish larvae (Hamre et al., 2013). In this study, 0.5 g DW samples of eggs, and unfed larvae at 0, 1 and 2 dph (i.e. post-exhaustion of yolk reserve) were taken from the stock tank for biochemical analysis. Biochemical analysis were also conducted on a sample of 0.5 g DW from each algal paste, enriched rotifers (after 48 h of dietary exposure) and each larval tank replicate at 2 dph, after 12 h of exposure to enriched rotifers, and at completion of the experiment, 13 dph, following commercial barramundi hatchery protocols.

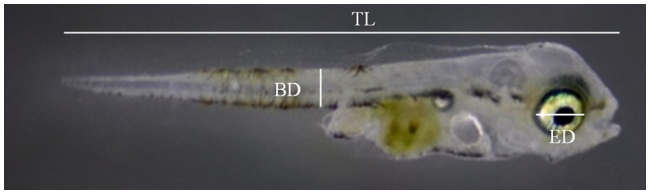

Fig. 1. Morphometric characters measured in the barramundi larvae. Total length (TL), body depth at anus (BD) and eye diameter (ED).

Biochemical analysis was conducted on total lipids and fatty acids composition. Fatty acids were simultaneously extracted and esterified in a direct transesterification method adapted from Carvalho and Malcata (2005) and Cohen et al. (1988) as described in detail in Gosch et al. (2012). The resulting fatty acid methyl esters (FAMEs) were separated and quantified on an Agilent 7890 GC (DB-23 capillary column, 60 m × 0.25 mm id × 0.15 μm) and an Agilent 5975C Electron Ionisation (EI) Turbo Mass Spectrometer (Agilent Technologies Australia Pty. Ltd.).

Total lipids were extracted following a procedure adapted from Folch et al. (1957) by Islam et al. (2013). Once the freeze dried biomass was immersed in a dichloromethane-methanol (2:1) solution the samples were left for 15 min in an ultrasonic cleaner FXP 12D (Unisonic Australia Pty. Ltd.) to be homogenised and improve the lipid extraction process.

2.4.2. Larval morphometrics and stress resistance

Additional samples of 50 larvae per replicate at 4, 6, 10 and 13 dph were taken for morphometric measurements, which included total length, eye diameter and body depth measured to the anal vent (Fig. 1). The sampled larval fish were also assessed for the developmental progress of flexion, which is characterised by the upward bend of the notochord, normally occurring at about 6 dph for barramundi larvae. The different larval measurements were acquired with Image*J* 1.45 software from photos taken by an Olympus SZ-61 dissecting microscope fitted with Olympus DP-26 camera. Stress resistance was determined following an adapted version of the protocol developed by Curnow et al. (2006). At 13 dph, 100 larvae per tank were sampled and exposed to the air in a 250 μm net for 3 min and then returned to a 2 L aerated beaker. Survival was recorded after 7 min by counting the amount of live larvae. The survival rate to the stress test was calculated by dividing the amount of live larvae by the total number of larvae tested (100). The time of air exposure was chosen using preliminary trials (2–9 mins air exposure) on the *N. oculata* fed larvae. As three minutes was enough to kill about half of the *N. oculata* fed larvae and that exposure time was chosen. The overall survival (S), was also determined at the end of the experiment, this was calculated as:

Survival = 100 × (Nf/Ni)where

Nf = Total number of fish left at 13 dph

Ni = Initial number of fish stocked

2.4.3. Statistical analyses

All statistical analyses were carried out using Statistica 7.0 (Statsoft Ltd., 2014). The morphometric data was analysed using a repeated measure ANOVA. Significant differences between the treatment means of the different age barramundi larvae were compared using Tukey's *post hoc* test. Stress resistance, survival, development rate between treatments and fatty acid content of the 2 and 13 DPH larvae were compared using one-way ANOVA followed by Tukey's test. The essential fatty acids were examined with a principal component analysis (PCA). Differences were considered significant at *p*-value of 0.05 or less.

3. Results

3.1. Fatty acid content and lipid endogenous resources of barramundi egg and larvae

Fatty acid composition declined from the egg to larvae at 0, 1 and 2 dph (Table 1); however, the n-3/n-6 fatty acids ratio stayed consistent at 1.8–1.9. ARA, DHA and EPA decreased by 11.4%, 25.6% and 36.6%, respectively from their original (egg) content to the 2 dph larvae. The content of the polyunsaturated fatty acids (PUFA) also decreased substantially over the endogenous feeding period with a loss of 30.8, 31.2, 31.9 and 33.7% for linoleic acid (LA), oleic acid (OLA), docosapentaenoic acid (DPA) and linolenic acid (LNA) respectively. In order of abundance from the time of fertilisation (egg) until 2 dph (larvae) the most abundant fatty acids were DHA followed by OLA and palmitic acid (PAL; Table 1). The total lipid content of the barramundi egg is likely to be erroneous because it is lower than at latter endogenous feeding stages namely 0, 1 and 2 dph. For these later stages the total lipid content decreased steadily from 36.9 to 29.8% in the 0 dph and 2 dph larvae respectively (Table 1).

3.2. Fatty acid content of diets and rotifers

The microalgae blends reflected well the original microalgal pastes but the content of most fatty acids in the rotifers fed the respective diets decreased from the original composition (Table 2). The major fatty acids of the *N. oculata* and 75:25 diets were PAL, EPA and POA (palmitoleic acid). The 50:50 diet had the majority of its fatty acid content represented by PAL, EPA and LA while the fatty acid content of the *C. vulgaris* diet was characterised by LA, DHA and PAL (Table 2). Although the three main fatty acids in the diets remained the same in the enriched rotifer their order of abundance changed. The *N. oculata* fed rotifers were rich in EPA, PAL and POA while for the 75:25 fed rotifers the PAL was the most abundant in front of EPA and POA (Table 2). The 50:50 and *C. vulgaris* fed rotifers were rich in PAL, LA, EPA and LNA, PAL and DHA respectively. C18:0, C18:1n-7, C20:0 and DPA increased from the diets to fed rotifers. The DPA content of the rotifers was inversely proportional to the DPA content of the microalgae pastes (Table 2). The ARA content was higher in the rotifers fed *N. oculata* and *C. vulgaris* with a respective increase of 1.2 and 1.1 mg/g (DW) compared to the original diets and lower for the rotifers fed the blends. The EPA content increased from the algal diet to the rotifer for the *N. oculata* treatment and decreased for the other 3 diets (Table 2). Rotifers fed *N. oculata* were the only diet to induce a higher content of total fatty acid, PUFA, EPA and n-3. The n-3 content decreased in the microalgal paste and likewise in the fed rotifers with the decreased inclusion of *N. oculata* (Table 2). The n-6 content, however, increased for both the microalgal paste and the fed rotifers with the increased inclusion of *C. vulgaris*. The PUFA content of the microalgal pastes was the highest in *C. vulgaris* and decreased in the other diets with the decreasing inclusion of *C. vulgaris*. The PUFA content was similar for the rotifers enriched with *C. vulgaris* and *N. oculata* and for the rotifers enriched with 50:50 and the 75:25 (Table 2). Generally the absolute FA content of the microalgal diets was reduced once it was fed to rotifers and DHA increased with an increasing amount of *C. vulgaris* while EPA and ARA increased with an increasing proportion of dietary *N. oculata* (Table 2). The total lipid content in the microalgal paste was higher in the *N. oculata* paste and decreased with the increased inclusion of *C. vulgaris*, which in comparison had lower lipid content. Once fed to the rotifer, the three diets containing *N. oculata* induced very similar lipid content of the rotifers, approximately 2% above the lipid content of the rotifers fed the *C. vulgaris* diet.

Table 1
Fatty acid content (mg/g DW) and lipid profile (% DW) of the newly released egg and of the 0, 1 and 2 dph barramundi larvae.

Fatty acid	Newly released egg	0 dph	1 dph	2 dph
C14:0	4.1	3.6	2.8	2.5
C15:0	4.2	3.4	2.9	2.4
C16:0 (PAL)	29.8	27.4	23	21.7
C16:1n-9	1.4	1.2	1.1	0.9
C16:1n-7 (POA)	8.9	7.4	6.5	5.5
C16:2n-6	1.6	1.3	1.3	1.3
C16:2n-4	28.2	22.2	21.1	18.4
C16:3n-3	1.5	1.1	1.0	0.8
C18:0	10.2	8.8	8.1	9.2
C18:1n-9 (OLA)	41.7	34.6	31.9	28.7
C18:1n-7	7.4	6.4	6.2	5.6
C18:2n-6 (LA)	17.8	14.8	13.9	12.3
C18:3n-6	11.6	9.5	9.3	8.4
C18:3n-3 (LNA)	8.0	6.3	6.0	5.3
C18:4n-3	1.9	1.7	1.5	1.2
C20:0	2.2	–	2.0	1.7
C20:4n-6 (ARA)	3.5	2.9	3.1	3.1
C20:5n-3 (EPA)	12.0	10.4	8.9	7.6
C23:0	0.6	–	0.7	0.5
C22:5n-6 (DPA)	6.6	5.3	5.1	4.5
C22:6n-3 (DHA)	54.6	47	43.2	40.6
EPA/ARA	3.4	3.6	2.9	2.5
DHA/EPA	4.6	4.5	4.9	5.3
DHA/EPA/ARA	4.6/3.4/1.0	4.5/3.6/1.0	4.9/2.9/1.0	5.3/2.5/1.0
$\sum$ n-3	77.9	66.5	60.5	55.5
$\sum$ n-6	41.0	33.8	32.8	29.6
n-3/n-6	1.9	1.97	1.84	1.88
$\sum$SFA	51.2	43.2	39.5	38.1
MUFA	59.3	49.6	45.7	40.7
PUFA	147.1	122.6	114.4	103.5
Total fatty acid	257.6	215.4	199.6	182.2
Total lipid (% DW)	26.1	36.9	31.7	29.8

Table 2
Fatty acid content (mg/g DW) of the four microalgae diets and enriched rotifers (after 48 h of exposure to their respective diets).

Fatty acid	Microalgal pastes				Enriched rotifers			
	N. oculata	75:25	50:50	*C. vulgaris*	*N. oculata*	75:25	50:50	*C. vulgaris*
C14:0	7.1	5.8	4.0	0.6	5.4	4.1	2.3	0.6
C15:0	0.6	0.5	0.4	0.2	0.5	0.5	0.4	0.3
C16:0 (PAL)	34.9	31.8	26.6	16.2	22.0	21.8	19	14.3
C16:1n-9	0.7	0.8	0.9	1.4	0.7	0.6	0.7	1.1
C16:1n-7 (POA)	23.3	19.7	13.3	0.2	19.7	14.0	7.6	0.6
C16:2n-6	1.2	3.0	5.3	10.4	1.0	1.8	3.1	5.7
C16:2n-4	0.4	0.3	0.3	0.3	0.2	0.2	0.3	0.2
C16:3n-3	0.8	1.6	3.0	5.2	0.4	0.6	0.9	1.5
C18:0	0.7	0.8	1.0	1.4	2.6	3.2	3.0	3.0
C18:1n-9 (OLA)	6.1	5.7	5.0	3.6	4.9	4.3	3.3	2.4
C18:1n-7	0.9	0.7	0.6	–	2.7	2.4	1.5	0.5
C18:2n-6 (LA)	6.5	10.2	15.1	25.6	7.7	10.4	15	22.2
C18:3n-6	0.7	0.6	0.5	–	0.5	0.4	–	–
C18:3n-3 (LNA)	2.4	3.8	5.7	9.7	2.4	3.5	4.6	6.6
C18:4n-3	–	–	–	–	1.2	0.2	–	–
C20:0	0.4	0.4	0.4	0.3	7.1	1.1	1.2	1.1
C20:2n-6	–	–	–	–	–	0.8	1.4	2.5
C20:4n-6 (ARA)	5.7	4.5	3.1	–	6.9	4.4	2.6	1.1
C20:5n-3 (EPA)	30.9	26.9	20.6	7.4	33.5	20.5	12.3	5.3
C23:0	–	–	0.9	1.8	–	0.2	0.8	1.1
C22:5n-6 (DPA)	–	0.7	1.5	3.1	6.5	5.4	4.3	3.9
C22:6n-3 (DHA)	–	3.7	9.0	19.0	–	1.7	6.0	11.5
EPA/ARA	5.4	6.0	6.6	–	4.9	4.7	4.7	4.8
DHA/EPA	–	0.1	0.4	2.6	–	0.1	0.5	2.2
DHA/EPA/ARA	–	0.1/6.0/1.0	0.4/6.6/1.0	–	–	0.1/4.7/1.0	0.5/4.7/1.0	2.2/4.8/1.0
$\sum$ n-3	34.1	35.9	38.3	41.3	37.5	26.5	23.8	24.9
$\sum$ n-6	14.1	19.0	25.5	39.2	22.7	23.4	26.5	35.5
n-3/n-6	2.42	1.89	1.5	1.05	1.65	1.13	0.9	0.7
$\sum$SFA	43.7	39.3	33.3	20.4	37.5	31.0	26.7	20.3
MUFA	31.0	26.9	19.7	5.2	28.1	21.3	13.1	4.5
PUFA	48.6	55.2	64.1	80.7	60.3	50.1	50.5	60.7
Total fatty acid	123.2	121.5	117.2	106.3	125.9	102.3	90.3	85.5
Total lipid (% DW)	28.3	25.7	24.3	17.7	18.4	18.7	18.2	16.2

Table 3
Fatty acid content (mg/g DW) of 2 and 13 dph barramundi larvae fed the four enriched rotifer diets. Mean values (mean ± SD; n = 3) within rows with different superscript letters are significantly different ($P < 0.05$).

Fatty acid	2 dph				13 dph			
	N. oculata	75:25	50:50	*C. vulgaris*	*N. oculata*	75:25	50:50	*C. vulgaris*
C14:0	1.4 ± 0.0^{a}	1.5 ± 0.1^{a}	1.4 ± 0.0^{a}	1.2 ± 0.1^{b}	1.9 ± 0.1^{a}	1.2 ± 0.3^{b}	0.8 ± 0.1^{c}	0.3 ± 0.0^{d}
C15:0	1.3 ± 0.0^{a}	1.1 ± 0.1^{a}	1.1 ± 0.0^{a}	1.1 ± 0.1^{a}	0.5 ± 0.0^{a}	0.4 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}
C16:0 (PAL)	17.7 ± 0.5^{a}	17.9 ± 0.6^{a}	16.9 ± 0.7^{b}	16.7 ± 0.6^{b}	21.1 ± 0.5^{a}	19.2 ± 1.2^{b}	17.6 ± 1^{c}	15.8 ± 0.8^{d}
C16:1n-9	0.5 ± 0.1^{a}	0.6 ± 0.0^{a}	0.5 ± 0.0^{a}	0.6 ± 0.0^{a}	0.5 ± 0.1^{a}	0.5 ± 0.1^{a}	0.5 ± 0.1^{b}	0.7 ± 0.1^{c}
C16:1n-7 (POA)	3.8 ± 0.2^{a}	3.6 ± 0.3^{a}	3.5 ± 0.3^{a}	2.7 ± 0.1^{b}	7.3 ± 0.2^{a}	4.8 ± 0.7^{b}	3.1 ± 0.3^{c}	0.5 ± 0.0^{d}
C16:2n-6	0.8 ± 0.0^{a}	0.8 ± 0.3^{a}	0.4 ± 0.0^{b}	0.7 ± 0.0^{a}	0.3 ± 0.0^{a}	0.5 ± 0.1^{b}	0.7 ± 0.1^{c}	1.4 ± 0.2^{d}
C16:2n-4	8.7 ± 0.8^{a}	8.3 ± 0.7^{a}	8.0 ± 0.2^{a}	7.8 ± 0.5^{a}	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}	0.6 ± 0.0^{a}
C16:3n-3	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.4 ± 0.1^{b}	0.4 ± 0.0^{b}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{b}
C18:0	8.9 ± 0.2^{a}	8.7 ± 0.1^{a}	8.2 ± 0.3^{b}	8.5 ± 0.2^{b}	7.9 ± 0.3^{a}	8.7 ± 0.2^{b}	8.5 ± 0.4^{c}	9.3 ± 0.3^{d}
C18:1n-9 (OLA)	17.7 ± 0.5^{a}	17.1 ± 0.4^{a}	16.3 ± 0.5^{b}	16.4 ± 0.6^{b}	5.7 ± 0.2^{a}	5.1 ± 0.2^{b}	4.5 ± 0.4^{c}	4.0 ± 0.1^{d}
C18:1n-7	3.9 ± 0.1^{a}	3.9 ± 0.1^{a}	3.7 ± 0.1^{b}	3.5 ± 0.1^{b}	3.6 ± 0.1^{a}	2.9 ± 0.2^{b}	2.2 ± 0.2^{c}	0.8 ± 0.0^{d}
C18:2n-6 (LOA)	7.6 ± 0.2^{a}	8.6 ± 0.2^{a}	7.5 ± 0.4^{a}	9.1 ± 0.6^{b}	4.1 ± 0.0^{a}	5.5 ± 0.5^{b}	7.5 ± 0.7^{c}	12.4 ± 1.0^{d}
C18:3n-6	4.3 ± 0.3^{a}	4.0 ± 0.3^{b}	4.0 ± 0.2^{b}	3.9 ± 0.3^{b}	0.5 ± 0.0^{a}	0.5 ± 0.1^{a}	0.5 ± 0.0^{a}	0.6 ± 0.0^{b}
C18:3n-3 (αLNA)	2.9 ± 0.2^{a}	3.0 ± 0.0^{b}	2.7 ± 0.1^{a}	3.1 ± 0.2^{b}	0.8 ± 0.0^{a}	1.1 ± 0.1^{b}	1.5 ± 0.2^{c}	2.3 ± 0.3^{d}
C18:4n-3	0.6 ± 0.0^{a}	0.6 ± 0.0^{a}	0.6 ± 0.0^{a}	0.6 ± 0.0^{a}	–	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{b}
C20:0	1.2 ± 0.0^{a}	1.2 ± 0.0^{a}	1.1 ± 0.0^{a}	1.2 ± 0.1^{a}	0.9 ± 0.1^{a}	0.7 ± 0.1^{a}	0.6 ± 0.1^{b}	0.7 ± 0^{b}
C20:2n-6	–	–	–	–	–	0.8 ± 0.1^{a}	1.1 ± 0.1^{b}	2.0 ± 0.1^{c}
C20:4n-6 (ARA)	3.0 ± 0.1^{a}	2.9 ± 0.2^{a}	2.8 ± 0.1^{a}	2.4 ± 0.1^{b}	7.1 ± 0.1^{a}	5.2 ± 0.1^{b}	3.8 ± 0.1^{c}	0.8 ± 0^{d}
C20:5n-3 (EPA)	6.0 ± 0.2^{a}	6.1 ± 0.6^{a}	5.8 ± 0.5^{a}	4.8 ± 0.2^{b}	15.1 ± 0.0^{a}	9.3 ± 0.9^{b}	6.8 ± 0.6^{c}	3.9 ± 0.3^{d}
C23:0	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.2 ± 0.0^{a}	0.4 ± 0.0^{a}	–	0.3 ± 0.1^{a}	0.4 ± 0.1^{a}	0.6 ± 0.1^{b}
C22:5n-6 (DPA)	3.3 ± 0.1^{a}	3.2 ± 0.3^{a}	3.1 ± 0.1^{a}	3.1 ± 0.2^{a}	13.1 ± 0.6^{a}	10 ± 0.4^{b}	7.0 ± 0.6^{c}	4.7 ± 0.4^{d}
C22:6n-3 (DHA)	28.4 ± 1.0^{a}	28 ± 0.7^{a}	26.6 ± 0.7^{b}	28.5 ± 1.3^{a}	1.1 ± 0.1^{a}	4.2 ± 0.2^{b}	8.9 ± 0.3^{c}	15.6 ± 0.8^{d}
EPA/ARA	1.9 ± 0.0^{a}	2.1 ± 0.0^{a}	2.0 ± 0.0^{a}	1.9 ± 0.0^{a}	2.1 ± 0.0^{a}	1.7 ± 0.1^{b}	1.7 ± 0.0^{b}	5.0 ± 0.0^{c}
DHA/EPA	4.7 ± 0.0^{a}	4.6 ± 0.0^{a}	4.6 ± 0.2^{a}	5.9 ± 0.2^{b}	–	1.3 ± 0.0^{a}	0.4 ± 0.0^{b}	3.9 ± 0.0^{c}
DHA/EPA/ARA	–	4.6/2.1/1.0	4.6/2.0/1.0	5.9/1.9/1.0	–	1.3/1.7/1.0	0.4/1.7/1.0	3.9/5.0/1.0
$\sum$ n-3	38.3 ± 1.2^{a}	38.2 ± 0.4^{a}	36.1 ± 1.1^{b}	37.3 ± 1.7^{a}	17.3 ± 0.1^{a}	15.1 ± 0.9^{b}	17.6 ± 1.2^{a}	22.2 ± 1.4^{c}
$\sum$ n-6	19.0 ± 0.7^{a}	19.3 ± 0.8^{a}	17.7 ± 0.5^{b}	19.2 ± 0.9^{a}	25.1 ± 0.7^{a}	22.4 ± 1.2^{b}	20.5 ± 1.5^{c}	21.9 ± 1.7^{b}
n-3/n-6	2.0 ± 0.4^{a}	2.0 ± 0.2^{a}	2.0 ± 0.3^{a}	1.94 ± 0.6^{a}	0.7 ± 0.2^{a}	0.7 ± 0.4^{a}	0.9 ± 0.6^{b}	1.0 ± 0.6^{c}
$\sum$SFA	30.7 ± 0.8^{a}	30.7 ± 0.8^{a}	28.9 ± 1.0^{b}	29 ± 1.0^{b}	32.2 ± 0.8^{a}	30.5 ± 1.5^{b}	28.3 ± 1.5^{c}	27.1 ± 1.2^{d}
MUFA	25.9 ± 0.9^{a}	25.1 ± 0.4^{a}	24 ± 0.7^{b}	23.2 ± 0.8^{b}	17.1 ± 0.4^{a}	13.2 ± 1.1^{b}	10.3 ± 0.8^{c}	6.0 ± 0.3^{d}
PUFA	66.0 ± 2.6^{a}	65.8 ± 1.1^{a}	61.9 ± 1.8^{b}	64.2 ± 3.1^{a}	42.9 ± 0.7^{a}	38 ± 2.2^{b}	38.6 ± 2.7^{b}	44.6 ± 3.1^{c}
Total fatty acid	122.6 ± 4.2^{a}	121.6 ± 1.8^{a}	114.8 ± 3.4^{b}	116.5 ± 4.8^{c}	92.1 ± 1.7^{a}	81.7 ± 4.7^{b}	77.2 ± 5.0^{c}	77.7 ± 4.5^{c}
Total lipid (% DW)	29.7 ± 0.3^{a}	29.5 ± 0.2^{a}	29.9 ± 0.3^{a}	29.6 ± 0.3^{a}	17.7 ± 0.2^{a}	16.8 ± 0.4^{b}	16.3 ± 0.3^{b}	17.7 ± 0.2^{a}

3.3. Fatty acid content of barramundi larvae fed enriched rotifer diets

The fatty acid content between treatments differed significantly more in the 13 dph larvae than in the 2 dph larvae (Table 3). However, the total fatty acid content of the 2 dph larvae was the lowest when fed the *C. vulgaris* diet ($P < 0.05$) and highest when fed with *N. oculata* and the 75:25 diet ($P < 0.05$). The EPA and ARA content in the 2 dph larvae was significantly lower when fed the *C. vulgaris* diet than when fed with the other three diets ($P < 0.05$). The DHA content of the 2 dph larvae was lowest ($P < 0.05$) when fed the 50:50 diet; however, DHA levels did not differ significantly ($P > 0.05$) when they were fed the monospecific diets (Table 3). The fatty acid contents of the larvae at 13 dph reflected the one of its diet with the larvae fed the *C. vulgaris* diet being significantly richer in DHA ($P < 0.05$). The DHA content of the 13 dph larvae decreased with the reduced dietary inclusion of *C. vulgaris* (Table 3). The same decreasing trend with decreasing inclusion occurred with the EPA and ARA content in the 13 dph larvae fed the *N. oculata* diet ($P < 0.05$; Table 3). C18:1n-7, DPA, MUFA, SFA and total fatty acids of the 13 dph larvae also decreased with the decreasing dietary inclusion of *N. oculata* in the rotifer diet (Table 3). The content of LA, C20:2n-6 and n-3 in the 13 dph larvae increased following the increase in dietary inclusion of *C. vulgaris* (Table 3). The fatty acid content of all four treatments in the 13 dph was dominated by PAL but its content decreased with the decreasing inclusion of dietary *N. oculata*. The following two most abundant fatty acids were EPA and DPA for the 13 dph larvae fed the *N. oculata* and the 75:25 fed 13 dph larvae (Table 3). The second most abundant fatty acid was DHA for both the 50:50 and the *C. vulgaris* fed 13 dph larvae which was followed by C18:0 and LA for the larvae fed the former and later diet respectively.

The lipid content of the fed 2 dph larvae did not differ significantly ($P > 0.05$) between treatments and was comparable to the unfed 2 dph larvae (Tables 1 and 3). The 13 dph larvae fed the two microalgal blends had similar lipid ($P > 0.05$) content but significantly lower ($P < 0.05$) than the monospecific diets fed larvae (Table 3).

The PCA indicated that the fatty acid composition of the 13 dph larvae varied according to their diet (Fig. 2). The PC1 axis, which accounted for 95.11% of the variance, was well represented by DHA. EPA and ARA were better represented on the PC2 axis which only accounted for 4.73% of the variance. EPA and ARA had little influence over the variation between treatments (Fig. 2). The 2 dph larvae were clumped together with little difference among treatments and more DHA than any of the 13 dph larvae (Fig. 2). The influential groups of fatty acids in the variance observed between treatments were PUFA, n-3 and then MUFA. A clear distinction existed between treatments for EFA at 13 dph however much less pronounced than with the fatty acid groups (Fig. 2).

3.4. Larval morphometrics and survival

3.4.1. Total length

No difference between treatments was found for total length at 4 and 6 dph ($P > 0.05$) but at 10 dph, the larvae fed with the *C. vulgaris* diet were significantly shorter than the larvae fed with the *N. oculata* ($P < 0.05$) and 75:25 diet (Fig. 3, $P < 0.05$). Larvae fed the 50:50 diet were significantly longer at 10 and 13 dph ($P < 0.05$) compared to those fed the other 3 diets. The larvae fed with the *N.*

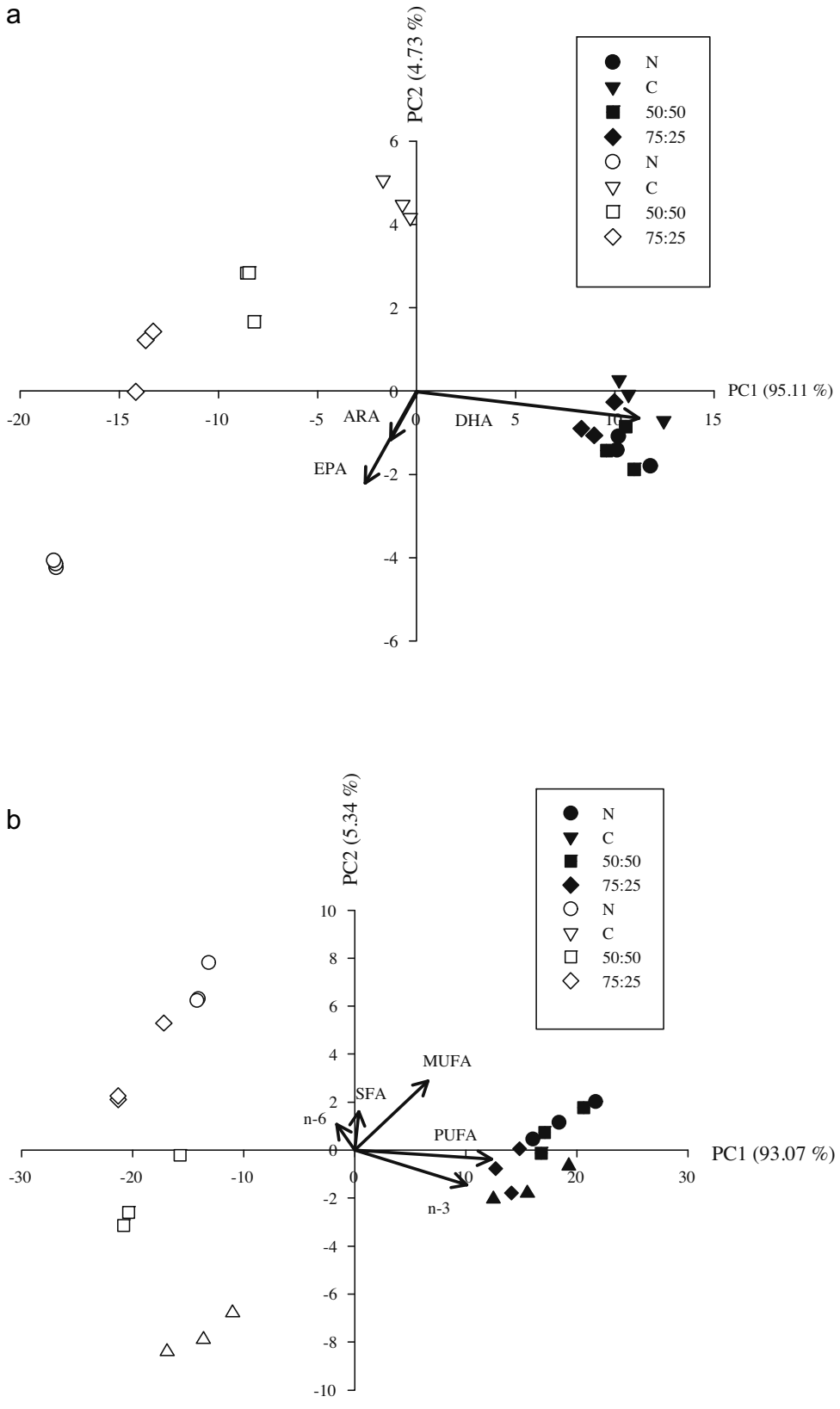

Fig. 2. Principle Component Analysis of the three EFA (DHA, EPA and ARA) and fatty acid groups (n-3, n-6, SFA, MUFA and PUFA) from the four treatments at 2 dph (black filled patterns) and 13 dph (empty patterns) where: N: *N. oculata*; C: *C. vulgaris*; 50:50 and 75:25 = proportional blends of *N. oculata* and *C. vulgaris* respectively.

oculata and *C. vulgaris* diets did not differ in length ($P > 0.05$) at 13 dph, but the larvae fed the *N. oculata* diet were significantly shorter ($P < 0.05$) than the ones fed the 75:25 diet (Fig. 3).

3.4.2. Body depth

No differences were observed for the body depth between the treatments at 4 and 6 dph ($P > 0.05$), but the body depth of the larvae

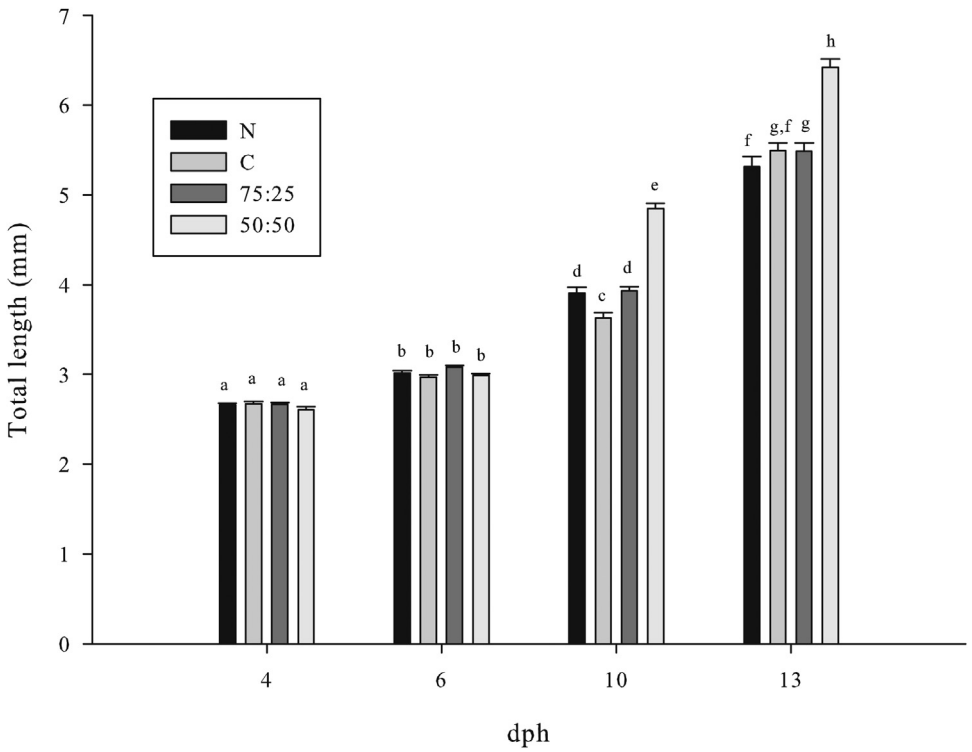

Fig. 3. Total length of the 4, 6, 10 and 13 dph barramundi larvae fed one of four diets where: N*: N.* oculata; C: *C. vulgaris*; 50:50 and 75:25 = proportional blends of *N. oculata* and *C. vulgaris* respectively (mean ± SD; n = 150). Different letters denote significant differences ($P < 0.05$).

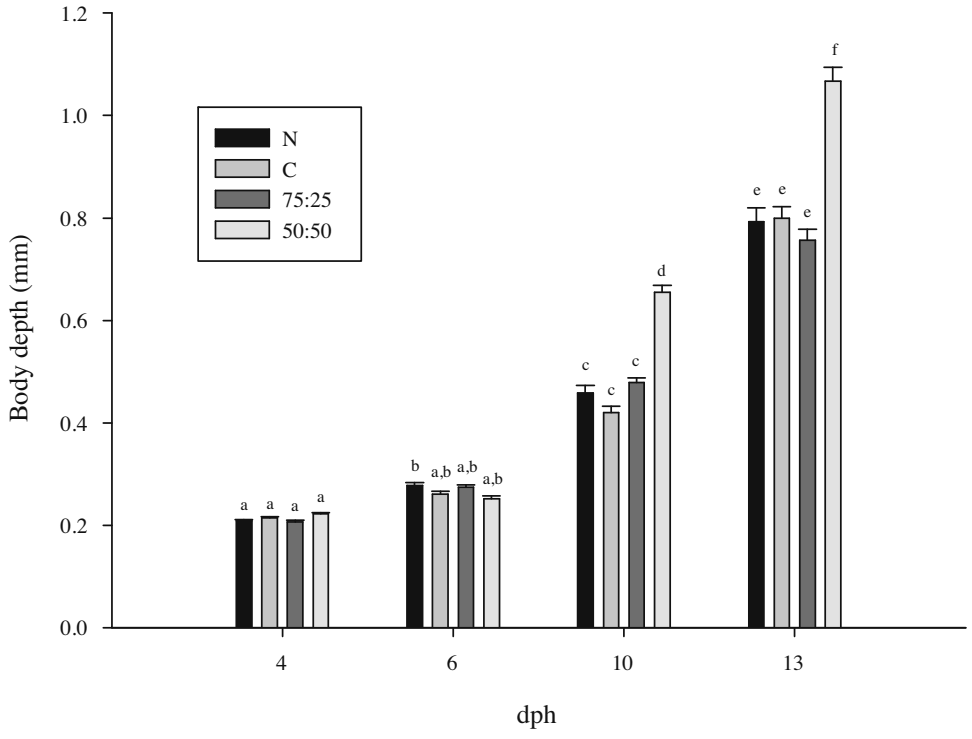

Fig. 4. Body depth of the 4, 6, 10 and 13 dph barramundi larvae fed the four diets where: N: *N. oculata*; C: *C.* vulgaris; 50:50 and 75:25 = proportional blends of *N. oculata* and *C. vulgaris* respectively (mean ± SD; n = 150). Different letters denote significant differences ($P < 0.05$).

fed the *N. oculata* diet was significantly larger ($P < 0.05$) from 4 to 6 dph. There was however no difference ($P > 0.05$) between the 6 dph larvae fed the other 3 diets (Fig. 4). The main difference between diets appeared at 10 dph and 13 dph with the larvae fed the 50:50 diet significantly outperforming ($P < 0.05$) all other diets (Fig. 4).

3.4.3. Eye diameter

The eye diameter of the larvae remained constant (0.25–0.28 mm) at 4 and 6 dph. At 10 dph, the eye diameter of the larvae fed the *C. vulgaris* diet did not differ significantly from the larvae fed the *N. oculata* ($P > 0.05$) and 75:25 ($P > 0.05$)

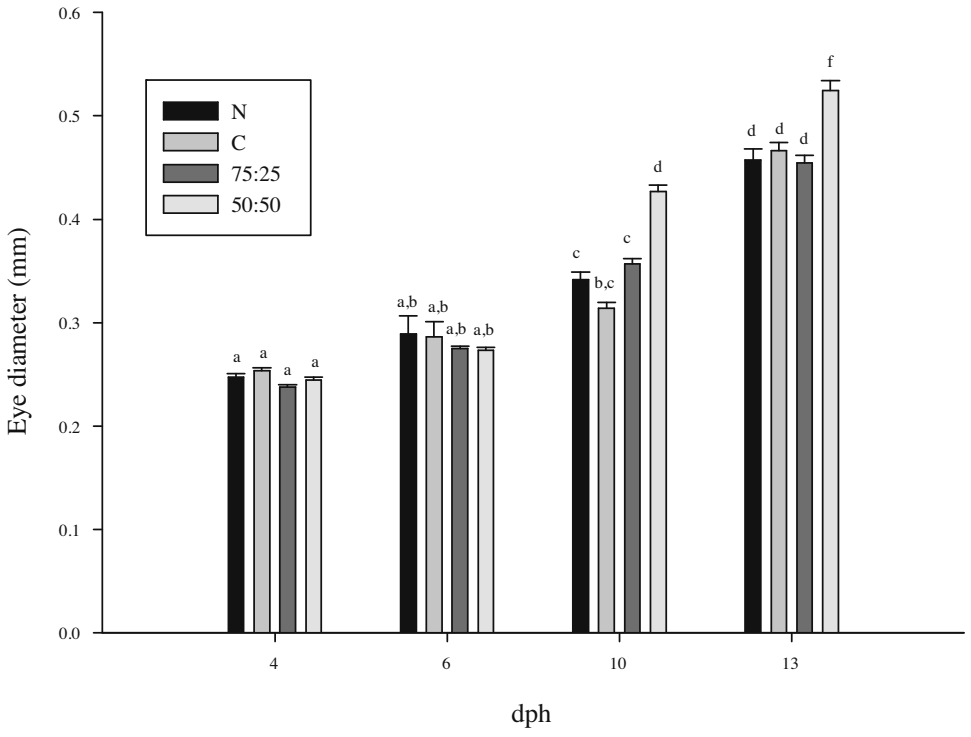

Fig. 5. Eye diameter of the 4, 6, 10 and 13 dph larvae fed the different diets where: N: *N. oculata*; C: *C. vulgaris*; 50:50 and 75:25 = proportional blends of *N. oculata* and *C. vulgaris* respectively (mean ± SD; n = 150). Different letters denote significant differences ($P < 0.05$).

Table 4
Survival of barramundi larvae fed one of four enriched rotifer diets at 13 dph. Mean values (± SE; n = 3) with different superscript letters are significantly different ($P < 0.05$).

	N. oculata	75:25	50:50	*C. vulgaris*
Survival rate (%)	66.7 ± 2.6^{a}	54.0 ± 2.6^{a}	62.3 ± 2.0^{a}	61.7 ± 4.0^{a}

diet and from all treatments at 6 dph ($P > 0.05$). At 10 dph the larvae fed the 50:50 diet had significantly ($P < 0.05$) larger eyes (0.52 mm ± 0.009) than the larvae fed the other three diets, but of similar size ($P > 0.05$) compared to the same treatments at 13 dph (Fig. 5).

3.4.4. Flexion

The proportion of larvae that underwent flexion at 6 dph did not differ between treatments ($P > 0.05$). At 10 dph the larvae fed the *N. oculata* diet had the lowest proportion ($P < 0.05$) of flexed larvae but it was not significantly different ($P > 0.05$) from the larvae fed the *C. vulgaris* diet (Fig. 6). The larvae fed the 50:50 diet had the highest proportion of flexed larvae at 10 dph ($P < 0.05$) followed by the larvae fed the 75:25 diet, which were not different from the *C. vulgaris* treatment ($P > 0.05$). No difference ($P > 0.05$) was observed in the percentage of flexed larvae at 13 dph as more than 90% in each diet had completed the metamorphosis (Fig. 6).

3.4.5. Stress resistance and survival

The 13 dph larvae fed the 50:50 diet had the highest survival ($P < 0.05$) after exposure to the stress stimulus (Fig. 7). No difference ($P > 0.05$) was observed between the larvae fed the *C. vulgaris* and 75:25 diets while the larvae fed the *N. oculata* diet had the lowest survival ($P < 0.05$) after the stress test (Fig. 7). Overall survival (S) did not differ significantly ($P > 0.05$) among the four treatments at 13 dph (ranged from 54.0–66.7%; Table 4).

4. Discussion

Blending microalgae species to obtain a balanced fatty acid diet is a common practice and often essential for the successful larval culture of bivalve and crustacean species (Epifanio, 1979; Gallardo et al., 1995; Borowitzka, 1997; Brown et al., 1997; Al-Abdul-Elah et al., 2001; Becker and Richmond, 2004) but it is not often used for the larval culture of fish. This study demonstrated that an equiproportional blend of *N. oculata* and DHA enriched *C. vulgaris* improved growth, development and stress resistance of barramundi larvae compared to the 75:25 blend of the two or when fed as monospecific diets. Generally, the performance of the barramundi larvae did not differ when fed either the other microalgae mix (75:25) or the two monospecific diets. However, the *N. oculata* diet induced the lowest survival rate for the stress test and similarly to the *C. vulgaris* diet fed larvae, it induced slower larval development when compared to the mixed diets.

4.1. Endogenous resources of barramundi egg and larvae

Conducting dose response based experiments to establish the nutritional requirements of marine fish larvae is challenging, due to their small size, undeveloped digestive tract, poor acceptance of artificial diets, live feed's metabolism and the challenges associated with maintaining constant abiotic environmental factors in larval tanks. These limitations necessitate the endogenous resources of marine fish to be used as a proxy for indicating nutritional requirements of marine fish larvae. In this study, endogenous EFA in barramundi steadily decreased from the time of fertilisation until the start of exogenous feeding at 2 dph with ARA being relatively conserved compared to EPA and DHA. The biochemical strategy of conserving EFA at the expense of other fatty acids has been reported for multiple species (Fraser et al., 1988; Heming and Buddington, 1988; Izquierdo, 1996). The retention of ARA at a content of 3.1 mg/g (DW) in the 2 dph barramundi larvae suggests that

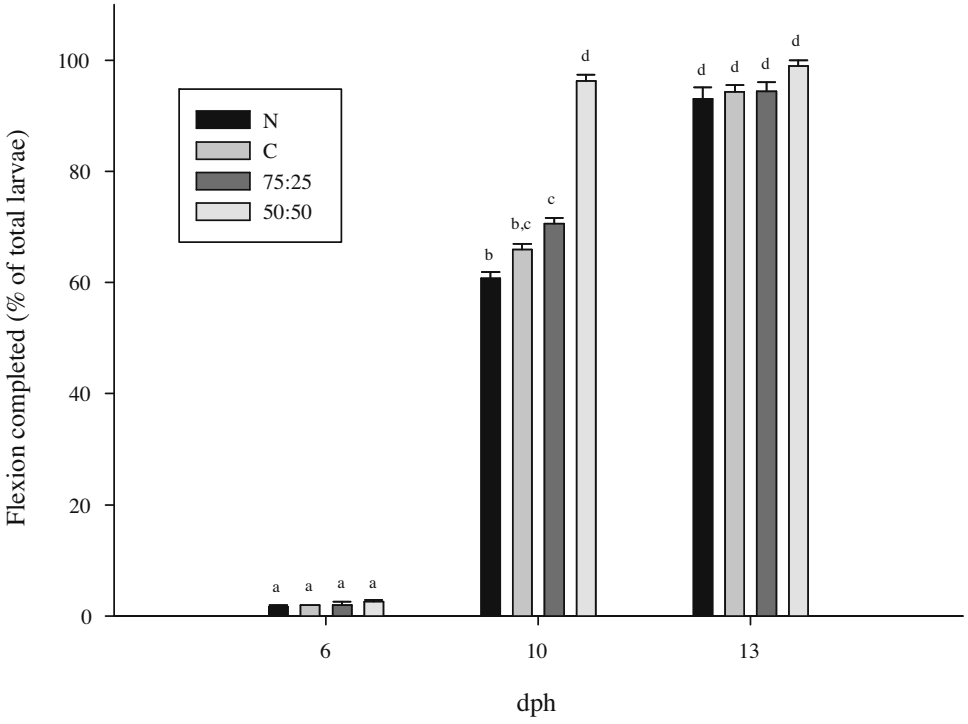

Fig. 6. Percentage of larvae which completed flexion at 6, 10 and 13 dph for the four diets where: N: *N. oculata*; C: *C. vulgaris*; 50:50 and 75:25 = proportional blends of *N. oculata* and *C. vulgaris* respectively (mean ± SD; n = 150). Different letters denote significant differences ($P < 0.05$).

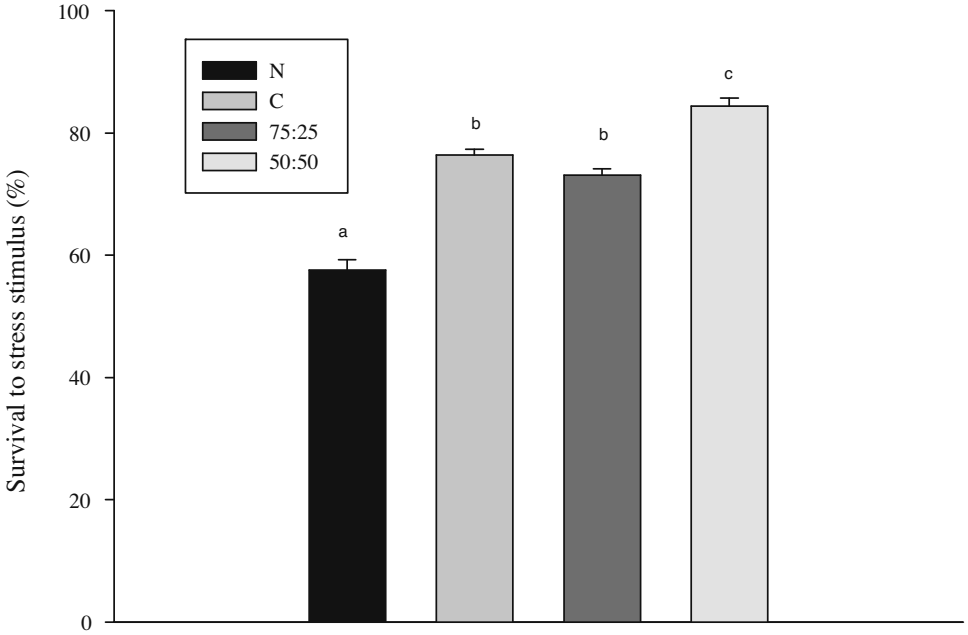

Fig. 7. Percentage of 13 dph larvae fed the four diets which survived the stress stimulus where: N: *N. oculata*; C: *C. vulgaris*; 50:50 and 75:25 = proportional blends of *N. oculata* and *C. vulgaris* respectively (mean ± SD; n = 300). Different letters denote significant differences ($P < 0.05$).

it could be used as an initial guideline regarding the dietary ARA inclusion for the start of exogenous feeding of barramundi larvae. The ratio of DHA/EPA increased over the endogenous feeding period of barramundi, a consequence of DHA being conserved relative to EPA which is also seen in cod larvae, *Gadus morua* (Fraser et al., 1988) and Atlantic halibut, *Hippoglossus hippoglossus* (Rønnestad et al., 1995). As for other marine fish species, DHA, OLA and PAL were the three major fatty acid acids retained in the endogenous resources of barramundi, from egg until 2 dph (Falk-Petersen et al., 1989; Anderson et al., 1990; Ako et al., 1991; Vázquez et al., 1994). Although compared to EFA, non-essential monounsaturated and saturated fatty acid such as OLA and PAL respectively, have not received significant attention for the enrichment and quality of live feed offered to marine fish larvae, they nonetheless have been reported to be important source of energy for the developing fish larvae and to spare the utilisation of EFA for energy (Meeren et al., 1993; Izquierdo, 1996).

4.2. Growth and development of barramundi larvae

The barramundi larvae fed the 50:50 diet outperformed the larvae fed the other three diets on the criteria of growth development. The higher DHA content of the 50:50 diet may have induced comparatively better performance over the 75:25 and *N. oculata* diets. In comparison, the *C. vulgaris* diet might have underperformed due to its lower EPA, ARA, saturated and monounsaturated fatty acid content. The importance of DHA over other EFA for the promotion of growth and survival has been established for various marine fish larvae such as yellowtail, *Seriola lalandi* (Furuita et al., 1996), striped jack, *Pseudocaranx dentex* (Takeuchi et al., 1996) and dolphin fish *Coryphaena hippus* (Ostrowski and Divakaran, 1990). DHA has been shown in multiple species to be important for visual and neural development, which in turn may affect the behaviour and prey capture abilities of developing marine fish larvae (Bell and Dick, 1993; Castell et al., 1994; Masuda et al., 1995; Ishizaki et al., 2001; Uauy et al., 2001). The results of this study demonstrated that the second highest level of DHA, the 50:50 treatment, outperformed those fed the higher DHA *C. vulgaris* treatment, which suggests that the DHA content of 50:50 diet might have already fulfilled the nutritional requirements of the barramundi larvae for that fatty acid. Similarly, Rimmer et al. (1994) did not find high dietary requirement of DHA for barramundi larvae but EPA was essential to prevent mortality syndromes associated with metamorphosis. Other marine fish larvae such as the Senegalese sole, *Solea senegalensis* (Villalta et al., 2005), Japanese flounder, *Paralichthys olivaceus* (Izquierdo et al., 1992) and plaice, *Pleuronectes platessa* (Dickey-Collas and Geffen, 1992) do not require dietary DHA if sufficient EPA is present. This has been attributed to the benthic fauna, on which these flat fish feed following metamorphosis, and its high EPA and ARA content compared to DHA rich pelagic zooplankton the pre-metamorphosed fish fed on (Villalta et al., 2005). Similarly, metamorphosed barramundi migrate from a salt water to a fresh water habitat and then feed on insect larvae and small invertebrates; food sources rich in EPA and ARA but poor in DHA (Russell and Garrett, 1985; Barlow et al., 1993; Villalta et al., 2005). Although barramundi larvae are predominantly found in a salt water environments, the spawning period of barramundi in northern tropical Australia coincides with the monsoon season (Staunton-Smith et al., 2004). Monsoonal events can potentially change the zooplankton fauna in estuaries from DHA rich zooplankton such as copepods and replace it with freshwater zooplankton such as cladocerans, which in comparison are poor in DHA but rich in EPA and ARA (Kainz et al., 2004; Chew and Chong, 2011; Makhutova et al., 2014). In an experiment conducted by Barlow et al. (1993), the barramundi larvae actually showed a preference for cladocerans over copepods. Actually, Rimmer et al. (1994) did not find any DHA in pond reared juvenile barramundi and hypothesised that the initial DHA requirements might have been met by the endogenous resources of the larvae. The fluctuating environment of the natural habitat of barramundi larvae and the migration from a salt to a fresh water environment may have led to intrinsic physiological and biochemical pathways to evolve conserving DHA.

4.3. Stress resistance of barramundi larvae

Growth is one of the main factors of interest in aquaculture but resistance to stress is equally important in an environment where fish are exposed to different stressors such as changes in water quality and handling. Blended microalgae feeds improved barramundi larvae capacity to cope with exposure to a stress event. ARA has been identified as the fatty acid responsible for stress resistance in multiple fish species (Koven et al., 2001; Bell and Sargent, 2003; Carrier et al., 2011). Although, Ishizaki et al. (1998) reported that high dietary inclusion of ARA caused an increase of mortality and inhibited growth in the larvae of yellow tail flounder. In this experiment, the larvae fed the *N. oculata* diet, high in ARA compared to the other three diets, had the lowest survival rate past the stress test. This result could be due to excess ARA supplied in the *N. oculata* diet or one or more nutrients (i.e. DHA) in that diet, which did not fulfil the minimal nutritional requirements of the barramundi larvae. A potential confounding factor on the dietary effect of ARA is its capacity to modify the bacterial flora of the rotifer and larval rearing tanks, which in turn, depending on the type of bacteria (detrimental versus beneficial), may influence growth and survival of fish larvae (Skjermo and Vadstein, 1999; Seychelles et al., 2013). This study only focused on blending microalgae for rotifer enrichment and its effect on barramundi larvae growth and development; however, other potentially important factors such as bacterial composition of the rotifer and larval tanks may need to be investigated in the future to further understand the early larval period of this species.

5. Conclusion

Although EFA has been recognised for some time as critically important nutrients for aquaculture species, their clear role in biochemical pathways leading to optimal growth in marine fish species remains largely unknown (Glencross, 2009). This study highlights the importance of considering blends of microalgae species over the monospecific enrichment of rotifers fed to barramundi larvae which is the current standard protocol; this has a clear potential application to commercial barramundi hatcheries. HUFA's were the main fatty acid influencing the observed differences between the treatments and, as for other marine fish species, other fatty acid groups (e.g. PUFA, SFA and MUFA) also had decisive roles in the parameters assessed. However, quantifying the requirements for DHA, EPA and ARA, was beyond the scope of this study and further research is needed to better understand the specific role of individual fatty acid and their interaction affecting the growth and development of the fast developing barramundi larvae. The microalgae diets used in this study have not only different fatty acid profiles but also contain amino acids, vitamins, pigments, protein, lipid and carbohydrate content. It is important to consider that this study only focused on the fatty acids in the diet of barramundi larvae and that other nutrients will likely also have an important role in influencing larval growth and development. Once a detailed understanding of the dietary requirements for EFA is achieved, live feeds can then be enriched with optimised blends of microalgae species, including the use of other species not considered in this study. Targeted dietary nutrient specifications of live feeds will improve growth and survival of barramundi larvae reducing the production bottlenecks currently experienced in commercial hatchery environments.

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