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Bonefish (*Albula vulpes*) oocyte lipid class and fatty acid composition related to their development

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Abstract Bonefish (*Albula vulpes*) are a valuable fishery resource of tropical and subtropical ecosystems worldwide. Despite their importance, there is limited information on bonefish life history and ecology. The present study aims to describe, for the first time, oocytes development and their lipid characteristics in wild bonefish during the reproductive season in different tidal flat locations in Grand Bahama Island, Bahamas. Our results have shown that Bonefish follow group-synchronous ovarian development and produce lipid-rich eggs [total lipid (TL) content was >26% of wet mass (WM)]. The major lipid class was a neutral lipid: the wax esters and steryl esters (WE-SE; >48% of TL), suggesting their use to support buoyancy and/or energy storage. Fatty acid (FA) composition of bonefish oocytes was characterized by high levels of monoenes in the neutral lipid fraction (i.e. 16:1 and 18:1n-9) indicating their probable role as energy fuel. However, the most abundant fatty acids in the polar lipids were docosahexaenoic acid (DHA; 22:6 n-3 > 15% of total polar FA), eicosapentaenoic acid (EPA; 20:5 n-3 > 13% of total polar FA) and arachidonic acid (ARA; 20:4 n-6 > 4% of total polar FA) which were selectively conserved among the tidal flat locations, suggesting their importance as essential constituents of cell membranes during the development of bonefish oocytes. Our results bring useful information concerning the reproductive physiology of bonefish and not only serve as a benchmark for determining the nutrient requirements to produce high quality eggs from bonefish captive broodstock, but also will help establish meaningful management practices for this species.

Keywords Bonefish · Oocytes · Lipid · Fatty acid · Wax esters and steryl esters

Abbreviations

ALC	fatty alcohol
ARA	arachidonic acid
BB	Barbary beach
CA	cortical alveolus
CBE	Crabbing Bay east
DHA	docosahexaenoic acid
EFA	essential fatty acids
EPA	eicosapentaenoic acid
FA	fatty acid
FFA	free fatty acids
HC	hydrocarbon

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KET	ketones
LV	late vitellogenic
MUFA	monunsaturated fatty acids
PL	phospholipids
PG	primary growth
PUFA	polyunsaturated fatty acids
SDWC	South Deep Water Caye
SFA	saturated fatty acids
ST	sterols
TAG	triacylglycerol
TL	total lipids
WE-SE	wax ester - steryl ester
WM	wet mass

Introduction

Bonefishes (*Albula spp*) inhabit shallow tropical and subtropical flats worldwide (Alexander 1961). In the Carribbean, the dominant species on the flats that supports the recreational flats fishery is *Albula vulpes*. Hereafter, Bonefish refers to *A. vulpes*. Bonefish are highly prized sport fish, contributing to a recreational flats fishery with an annual economic impact of $465 million in the Florida Keys (USA) (Fedler 2013), $141 million in the Bahamas (Fedler 2010), and $56 million in Belize (Fedler 2014). Despite their economic value and key ecosystem role, there are considerable gaps in the scientific literature about their reproductive physiology and biology, as well as other features in their life history (Danylchuk et al. 2008).

In the Western Atlantic, it is suggested that bonefish spawn between October and May (Mojica et al. 1995; Murchie 2010; Danylchuk et al. 2011). They form large pre-spawning and spawning aggregations 1–3 days prior to the full moon (Johannes and Yeeting 2000; Danylchuk et al. 2011). Then, the fish migrate to spawn near deep-water drop-offs, off coral reef shelves (Danylchuk et al. 2011).

Characterizing the bioenergetics of egg production of bonefish can yield insights into the role of prey composition and abundance in reproduction of wild populations, and can inform the development of feeding strategies for inducing successful reproduction of captive populations of bonefish. Lipids and fatty acids (FAs) are one of the most important maternal components that affect egg quality in fishes that produce lipid-rich eggs (Sargent et al. 1999a, b, 2002). These nutritional components provide energy reserves and structural components of cellular membranes (Copeman et al. 2002; Tocher 2003). In oviparous species, lipids and FAs are transferred from the female to the oocytes during vitellogenesis (Sargent et al. 1997). It is well documented that lipid and FA profiles of developing eggs (oocytes) can reveal the condition of broodstock and have a critical role in successful early development of marine fish (Harel et al. 1994; Brooks et al. 1997; Rainuzzo et al. 1997; Tocher 2003; Mejri et al. 2017).

Marine pelagic fish eggs contain a wide variety of lipids. Polar lipids, mainly in the form of phospholipids (PLs) are important compounds of membrane lipids that form the double-layered surface of the cells (Wiegand 1996). Neutral lipids include triacylglycerol (TAG) and wax esters-steryl esters (WE-SE), and provide most of the energy consumed by developing embryos (Sargent et al. 1976; Falk-Petersen et al. 1982; Phleger et al. 1997). In addition to being a reserve energy store, WE provide more buoyancy per unit volume because of their lower densities than TAG (Nevenzel 1970). Some studies have also related embryonic development and hatching success to the content of essential polyunsaturated fatty acids (PUFA) namely docosahexaenoic acid (DHA; 22:6 n-3), eicosapentaenoic acid (EPA; 20:5 n-3) and arachidonic acid (ARA; 20:4 n-6), which are essential building blocks in cell membranes and are contained in storage lipids (March 1993; Marteinsdottir and Begg 2002). Furthermore, EPA and ARA are also precursors of eicosanoids, a group of highly biologically active hormones (Howard and Stanley 1999).

Bonefish show high levels of site fidelity during non-spawning season (Boucek et al. this issue and references therein). Therefore, the diet of benthic invertebrates, crustaceans, mollusks, and fishes (Colton and Alevizon 1983; Crabtree et al. 1998) should reflect variable diet quality among bonefish schools that reside on separate flats. These diet differences should be reflected in the concentrations of highly nutritious lipids and fatty acids in bonefish eggs, which influence the fitness of the future leptocephalus larvae (Fuiman et al. 2015). While there is some knowledge of pre-metamorphic lipid dynamics of the leptocephali of bonefish [i.e., bonefish leptocephali lose 50% of their total lipids during metamorphosis, which provides 80% of the energy required (Padrón et al. 1996)], nothing is known about lipid and FA composition of bonefish eggs.

To gain further insight into reproductive physiology of bonefish, the objective of the present study was to investigate oocyte development and the comparative lipid profile and fatty acid composition of oocytes

collected from female bonefish at three tidal flats adjacent to Grand Bahama Island, Bahamas. We investigated the following questions: (1) What is the reproductive state of bonefish when analyzing the developmental stages of the collected oocytes? (2) What is the total lipid, lipid class and fatty acid composition of bonefish oocytes? (3) Is there evidence of lipid retention or conservation, and which lipid classes and fatty acids are of interest? (4) Is there variation in lipid and fatty acid composition across tidal flat locations? We hypothesize that, based on the known high site fidelity of bonefish, there is a spatial variation in food availability and quality in lipid composition of females' oocytes.

Materials and methods

Experimental fish and sampling

Female bonefish were collected from three shallow (< 1 m) flats habitats, along the southern shore of Grand Bahama Isaland, Bahamas; South Deep Water Cay (SDWC, 26°37' N 77°64' W), Barbary Beach (BB, 26°34'N 78°30'W), and Crabbing Bay East (CBE, 26°39' N 77°58' W) during the full moon of February 2017 (spawning season) (Fig. 1). CB and SDWC were separated by 8 km, with BB located 53 and 60 km from CB and SDWC, respectively. Fish were captured on the flats using a 50 m × 1 m beach seine with 2.5 cm mesh. Captured females were first measured for total length, then a soft-tube catheter (Bard 100% latex-free infant feeding tube, 2.27 mm diameter, 26 cm length, attached to a 3 ml syringe barrel) was inserted through the gonopore into the ovary, and 1–2 ml of oocytes were removed. About two thirds of the oocytes were frozen at −80 °C for biochemical analysis and one third were preserved in 10% neutral buffered formalin for histological analysis. All fish were released alive after sampling.

Histological analysis

Oocytes from bonefish females were fixed in Davidson's solution for 48–72 h before being transferred to 70% ethanol for subsequent histological preparation (Barber 1996; Wilson et al. 2005). Then, oocytes were dehydrated in a series of 70–100% ethanol solutions for a minimum of 1 h each. Samples were then clarified in toluene, and embedded in paraffin wax. Multiple 5–8 μm sections were cut from each embedded sample, stained with hematoxylin and eosin, and then mounted on pre-labeled glass slides for examination. Resultant slides were examined at using a compound microscope with a digital image processing system (cellSens, OLYMPUS, Japan).

Biometric analysis

Oocyte diameters were measured with a high-resolution digital microscope (OLYMPUS, SZX7) that uses the Galilean optical system for brilliant, highly resolved images (1x-5.6x zoom range). The developmental stages observed were identified based on the classification of Crabtree et al. (1997). In total 2835 oocytes from 28 fish were measured, staged and randomly counted, with frequencies of each stage expressed as a relative percentage of the total oocytes for each fish.

Lipid and fatty acid analysis

Lipids from a pool of eggs from individual females were extracted according to procedures developed by Folch et al. (1957) and modified by Parrish (1999). The relative proportions of the different lipid classes; ketones (KET), triacylglycerols (TAG), wax esters-steryl esters (WE-SE), free fatty acids (FFA), hydrocarbon (HC), fatty alcohol (ALC), sterols (ST), and phospholipids (PLs) were determined using an Iatroscan Mark-VI analyzer (Iatron Laboratories Inc., Tokyo, Japan) and were developed in a four-solvent system (Parrish 1987, 1999). In addition, lipid extracts were separated into neutral and polar fractions using silica gel column (30 × 5 mm i.d., packed with Kieselgel 60, 70–230 mesh; Merck, Darmstadt, Germany) hydrated with 6% water, and eluted with 10 mL of chloroform:methanol (98:2 *v*/v) for neutral lipids followed by 20 mL of methanol for polar lipids (Marty et al. 1992). The neutral lipid fraction was further eluted on an activated silica gel with 3 mL of hexane and diethyl ether to eliminate free sterols. All fatty acid methyl esters (FAME) were prepared as described by Lepage and Roy (1984) and analysed in MSMS scan mode (ionic range: 60–650 m/z) on a Polaris Q ion trap coupled to a Trace GC (Thermo Finnigan, Mississauga, ON, CA) equipped with a Valcobond VB-5 capillary column (VICI Valco Instruments Co. Inc., Broakville, ON, CA). FAME were identified by comparison of retention times with known standards (37 component FAME Mix, PUFA-3, and menhaden oil; Supelco Bellefonte, PA, USA) and quantified with nonadecanoic

Fig. 1 Map showing sampling sites at Grand Bahama Island, Bahamas

acid (19:0) as internal standard. Chromatograms were analyzed using the Xcalibur 1.3 integration software (Thermo Scientific, Mississauga, ON, CA).

Statistical analysis

Variation in oocyte diameter and total lipids by site were tested with one-way analysis of variance (ANOVA) after assumption verification of homoscedasticity and normality with Levene and Shapiro-Wilk tests, respectively. These analyses were performed with the JMP 13 package (SAS Institute Inc., Cary, NC). Permutational analysis of variance (PERMANOVA with 9999 permutations), including a posteriori pair-wise comparisons, was performed on lipid classes and fatty acid profiles and sums of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and PUFA from polar and neutral lipid fractions. Each PERMANOVA was tested with one factor: sites (SDWC, BB, and CBE). Assumptions of multivariate homoscedasticity were verified with a PERMDISP test, and data were transformed (arcsine square root) when necessary. To analyze the similarity in fatty acid profiles among different sites, SIMPER analyses were run using a Bray-Curtis similarity matrix with PRIMER 7 (v. 7.1.12) and PERMANOVA+ (v.1.0.2).

Results

Oocyte stage characteristics

A total of 28 wild female bonefish were sampled; South Deep Water Cay (SDWC, $n = 13$), Barbary Beach (BB, $n = 6$), and Crabbing Bay East (CBE, $n = 9$). Females ranged from 397 to 564 mm total length, without significant differences among sites $t\,(27) = 76.06$, $p = 0.18$. Three reproductive stages were documented in all ovaries (Fig. 2). Primary growth (PG) oocytes and cortical alveolus (CA) stages were present in all ovaries at a percentage of 12 ± 7.5 (mean ± SD) and $15 \pm 4.4\%$ of total oocytes, respectively. Late vitellogenic oocytes (LV) were present in greatest number in all females ($73 \pm 9.6\%$ of total oocytes) (Fig. 2). The diameter of oocytes at LV stage did not vary between the sites, with an average size of 601 ± 63 μm.

Lipids and lipid classes

Total lipids accounted for 264.78 ± 72.05 mg g^{-1} of the egg wet mass (WM) and did not differ significantly among the sites ($F_{(2,37)} = 2.67$, $p = 0.08$). The major lipid classes in bonefish eggs were wax esters-steryl esters and phospholipids, accounting

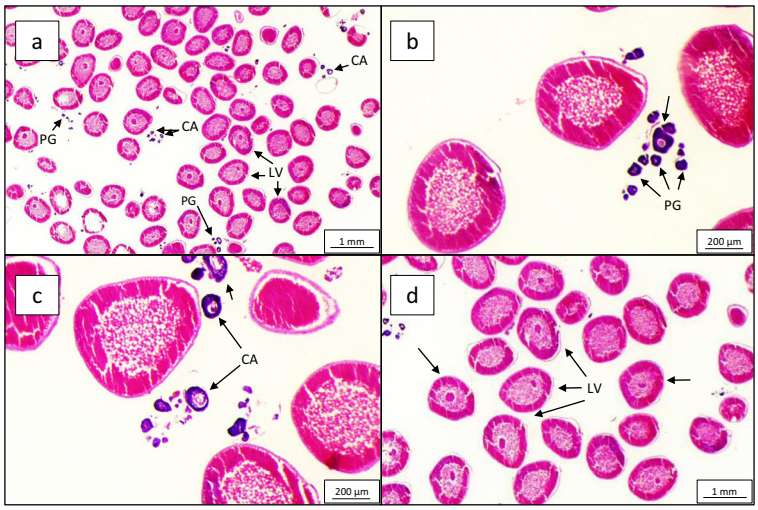

Fig. 2 Micrographs of oocytes from bonefish (*Albula vulpes*) females sampled during the reproductive season (February 2017) in three different tidal flat locations (South Deep Water Cay [SDWC], $n = 13$, Crabbing Bay East [CBE], $n = 9$, and Barbary Beach [BB], $n = 6$) in Grand Bahama Island, Bahamas. **a** wild individual showing oocytes at primary growth (PG), cortical alveolus (CA), and late vitellogenic (LV) stages. **b** primary growth oocytes in the ovary of a wild specimen captured on 13 February 2017. **c** cortical alveolus oocytes in the ovary of a wild specimen captured on 13 February 2017. **d** late vitellogenic oocytes from a wild fish caught on 14 February 2017

for more than 48 and 27% of total lipids, respectively (Table 1). Three of the lipid classes varied significantly among collection sites (*Pseudo-F* $_{(2,\ 37)} = 13.63$, $p = 0.0001$). SIMPER analysis showed that triacylglycerol percentages explained up to 24% of the differences observed between the SDWC, CBE, and BB sites. The percentages of TAG were significantly higher at SDWC (22% of total lipids) compared to BB and CBE, where it was 0.7 and 3.4% of total lipids, respectively.

Fatty acids

Neutral lipid fraction of fatty acids

The fatty acid composition varied according to the flat sites (*Pseudo-F* $_{(2,\ 37)} = 7.63$, $p = 0.0001$) (Table 2). Oocytes sampled from the three flat locations had different fatty acid composition. Overall, in all sites, monounsaturated fatty acids made up the larger fraction (>35% of total neutral fatty acids) compared to

Table 1 Lipid class composition (mean ± S.D., expressed as percentage of the total lipid composition) in bonefish (*Albula vulpes*) oocytes at three tidal flat locations (south Deep Water Cay [SDWC], $n = 13$, Crabbing Bay East [CBE], $n = 9$, and Barbary Beach [BB], $n = 6$) in Grand Bahama Island, Bahamas

Lipid classes	SDWC	CBE	BB
HC	0.50 ± 0.04	0.96 ± 0.47	1.97 ± 1.26
WE-SE	48.27 ± 1.12	52.81 ± 7.22	55.92 ± 1.59
KET	0.21 ± 0.11	0.18 ± 0.12	0.31 ± 0.38
TAG	22.35 ± 0.04^{a}	3.43 ± 0.28^{b}	0.66 ± 0.33^{b}
FFA	0.43 ± 0.00^{b}	2.23 ± 1.40^{a}	0.00 ± 0.05^{b}
ALC	0.00 ± 0.00	0.01 ± 0.06	0.05 ± 0.02
ST	1.27 ± 3.46^{b}	9.39 ± 4.59^{a}	7.95 ± 5.45^{a}
PL	26.96 ± 3.30	31.00 ± 4.43	33.14 ± 2.06
Total lipids ($mg.g^{-1}$)	274.34 ± 74.57	215.84 ± 53.13	295.73 ± 48.28

HC, hydrocarbons; WE-SE, wax esters-steryl esters; KET, ketones; TAG, triacylglycerols; FFA, free fatty acids; ALC, fatty alcohols; ST, sterols; PL, phospholipids. Different letters indicate significant differences among sites

Table 2 Fatty acid composition (mean ± SD, expressed as percentage of total neutral and polar lipids detected) in bonefish (*Albula vulpes*) oocytes sampled at three tidal flat locations (South Deep Water Cay [SDWC], n = 13, Crabbing Bay East [CBE], n = 9, and Barbary Beach [BB], n = 6) in Grand Bahama Island, Bahamas

Fatty acid	Neutral lipid fraction			Polar lipid fraction		
	SDWC	CBE	BB	SDWC	CBE	BB
14:0	3.38 ± 0.95	4.55 ± 0.61	4.96 ± 0.13	1.17 ± 0.25	1.36 ± 0.22	1.10 ± 0.20
15:0	0.80 ± 0.11	1.13 ± 0.10	1.49 ± 0.23	0.74 ± 0.15	0.91 ± 0.16	1.03 ± 0.35
16:0	12.67 ± 2.58	14.41 ± 1.69	14.71 ± 1.03	26.36 ± 2.12	26.07 ± 0.81	23.42 ± 2.00
17:0	1.30 ± 0.26	1.47 ± 0.17	2.47 ± 0.29	1.63 ± 0.34	1.66 ± 0.13	2.26 ± 0.15
18:0	6.09 ± 0.65	6.29 ± 0.91	9.37 ± 2.06	13.48 ± 1.54	14.57 ± 1.37	14.69 ± 0.50
21:0	0.12 ± 0.03	0.13 ± 0.02	0.11 ± 0.00	0.74 ± 0.12	0.68 ± 0.10	0.59 ± 0.06
23:0	0.15 ± 0.05	0.22 ± 0.05	0.09 ± 0.08	0.35 ± 0.12	0.42 ± 0.04	0.19 ± 0.08
24:0	0.18 ± 0.07	0.21 ± 0.05	0.21 ± 0.05	0.64 ± 0.30	0.68 ± 0.16	0.38 ± 0.10
∑SFA$^{\alpha}$	25.53 ± 5.04	29.17 ± 4.24	34.76 ± 4.90	47.53 ± 6.28	50.00 ± 3.77	51.63 ± 12.52
14:1	0.13 ± 0.04	0.19 ± 0.06	0.11 ± 0.02	1.21 ± 0.69	1.51 ± 0.56	0.35 ± 0.05
16:1	16.19 ± 3.85^{a}	17.28 ± 2.37^{a}	7.52 ± 5.51^{b}	3.48 ± 0.81	3.36 ± 0.62	3.57 ± 1.48
17:1	1.59 ± 0.23	1.67 ± 0.33	3.06 ± 0.29	1.24 ± 0.79	1.37 ± 0.13	2.17 ± 1.40
18:1 n-9	23.44 ± 1.71^{a}	25.09 ± 2.52^{a}	20.87 ± 1.14^{b}	3.99 ± 0.80	4.95 ± 0.76	4.52 ± 0.34
20:1 n-9	1.54 ± 0.34	2.08 ± 0.26	3.51 ± 0.00	0.45 ± 0.18	0.48 ± 0.10	0.47 ± 0.17
22:1 n-9	0.89 ± 0.64	0.94 ± 0.54	0.30 ± 0.14	0.79 ± 0.45	0.72 ± 0.28	1.05 ± 0.58
∑MUFA$^{\beta}$	43.89 ± 6.92	47.46 ± 6.26	35.51 ± 18.26	11.91 ± 4.15	13.42 ± 2.62	12.80 ± 4.74
20:2	0.78 ± 0.29	0.58 ± 0.05	0.97 ± 0.71	0.74 ± 0.22	0.70 ± 0.14	0.64 ± 0.15
18:2 n-6	2.41 ± 0.46	2.46 ± 0.44	2.84 ± 0.30	1.56 ± 0.38	1.90 ± 0.12	1.17 ± 0.21
18:3 n-6	0.35 ± 0.14	0.31 ± 0.04	0.10 ± 0.01	0.35 ± 0.15	0.35 ± 0.14	0.36 ± 0.08
18:3 n-3	0.81 ± 0.22	0.69 ± 0.19	1.01 ± 0.11	0.49 ± 0.17	0.49 ± 0.10	0.32 ± 0.16
20:3 n-3	7.22 ± 2.31	4.69 ± 0.63	6.02 ± 0.40	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:3 n-6	1.13 ± 0.26	1.15 ± 0.19	1.16 ± 0.13	1.51 ± 0.40	1.96 ± 0.28	1.34 ± 0.10
18:4 n-3	0.54 ± 0.12	0.61 ± 0.11	1.03 ± 0.32	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:4 n-6 (ARA)	4.83 ± 2.11	3.65 ± 0.68	4.70 ± 0.68	4.40 ± 1.08	4.10 ± 0.69	3.80 ± 1.44
20:5 n-3 (EPA)	8.11 ± 2.62	5.19 ± 0.58	6.96 ± 0.50	15.21 ± 2.06	13.09 ± 1.62	12.24 ± 3.13
22:6 n-3 (DHA)	4.13 ± 1.23	3.67 ± 0.68	4.31 ± 0.34	15.65 ± 1.82	15.31 ± 1.26	15.06 ± 6.09
∑PUFA$^{\delta}$	30.40 ± 9.93	23.16 ± 3.72	29.52 ± 4.05	39.92 ± 6.29	37.90 ± 4.36	35.19 ± 11.70
n-3	17.52 ± 2.82	13.31 ± 1.88	17.07 ± 2.27	20.54 ± 6.71	19.89 ± 6.56	19.19 ± 6.50
n-6	8.11 ± 3.51	5.19 ± 2.13	6.96 ± 3.01	15.21 ± 7.05	13.09 ± 5.89	12.24 ± 5.66

Different letters indicate significant differences among sites

α: includes 11:0, 12:0, 13:0, 20:0, whose combined percentages are ≤0.2% of total fatty acids

β: includes 15:1, 24:1 n-9, whose combined percentages are ≤0.2% of total fatty acids

δ: includes 22:2, whose combined percentages are ≤0.5% of total fatty acids

saturated fatty acids and polyunsaturated fatty acids (>25 and > 23% of total neutral FAs, respectively). The fatty acid profiles of bonefish oocytes sampled at SDWC and CBE flats were characterized by high levels of oleic FA (18:1 n-9) and FA (16:1) compared to oocytes sampled at BB flat site, as shown by n-MDS analysis (Fig. 3 and Table 2). The two fatty acids explained up to 47% of the differences among sites as determined by SIMPER analysis.

Polar lipid fraction of fatty acids

We observed no effect of sites on fatty acid composition in polar lipids (*Pseudo-F* $_{(2,\ 17)} = 1.35$, $p = 0.2091$).

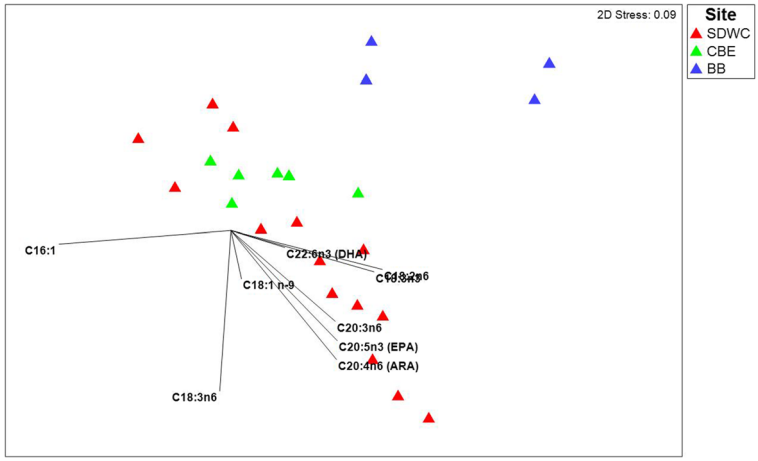

Fig. 3 Non-metric multi-dimensional scaling of the Bray-Curtis similarity matrix based on the relative abundance of neutral fatty acid profiles associated with oocytes sampled from females at three flats sites: South Deep Water Caye (SDWC), Crabbing Bay east (CBE), and Barbary beach (BB). The arrows represent the fatty acid responsible for most of the variation

Percentages of different FAs at the three different sites examined are presented in Table 2. Here, saturated fatty acid and polyunsaturated fatty acid percentages made up the larger fraction (>47 and > 35% of total polar FAs, respectively; Fig. 4 and Table 2) compared to monounsaturated fatty acids (>12% of total polar FAs). The highest essential FA concentrations were DHA (22:6 n-3) followed by EPA (20:5 n-3) and ARA (20:4 n-6; Table 2).

Discussion

Reproductive behavior

Female bonefish exhibited three stages (clutches) of oocytes: the dominant clutch is late vitellogenic stage, a second clutch is likely 'arrested' in the cortical alveoli (yolk vesicle) stage, and a third clutch of non-yolky oocytes in the primary growth phase. This pattern allows for multiple, distinct ovulatory events that typically follow seasonal, lunar, or diurnal cycles (Parenti and Grier 2004), which suggests that bonefish follow a group-synchronous ovarian development. Group synchronous fish have two or more distinct populations of oocytes present at the same time and ovulate once in a season, or undergo multiple ovulations over a few days or weeks within the spawning season (Parenti and Grier 2004). This is the most common ovarian type among teleost fishes (Asturiano et al. 2002; Murua and Saborido-Rey 2003) and has been documented in white mullet (*Mugil curema*) (Solomon and Ramnarine 2007), European sea bass (*Dicentrarchus labrax* L.) (Carrillo et al. 1989; Mayer et al. 1990) and tucunare (*Cichla kelberi*) (Normando et al. 2009). Recent studies have shown that females of these species can have up to four ovulations during the natural reproductive period (Asturiano et al. 2000). Thus, one bonefish female

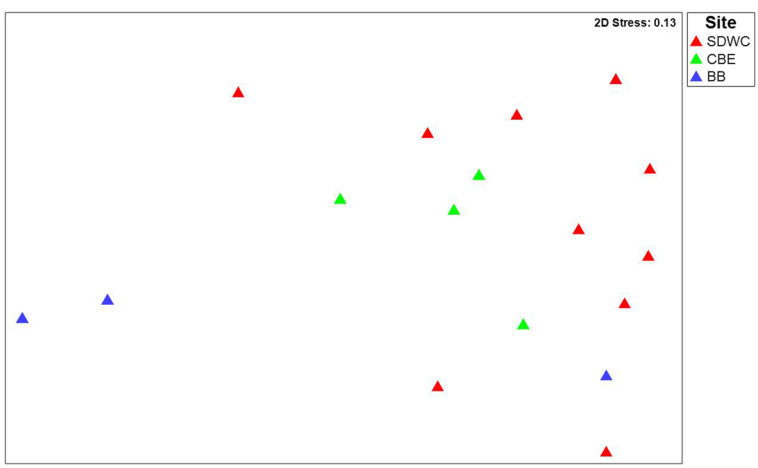

Fig. 4 Non-metric multi-dimensional scaling of the Bray-Curtis similarity matrix based on the relative abundance of polar fatty acid profiles associated with oocytes sampled from females at three flats sites: South Deep Water Caye (SDWC), Crabbing Bay east (CBE), and Barbary beach (BB)

may ovulate more than once during the extended (November through April) reproductive season.

Lipids and lipid classes

Bonefish have lipid-rich eggs containing more than 26% of their wet mass in lipids. Fish eggs can be classified into two energetic categories according to their lipid characteristics (Mourente and Vázquez 1996); eggs with high (> 15% of egg WM) or low (<15% of egg WM) lipid content. The high lipid content in eggs of some fish species as compared to others may arise from different energy needs of the fertilized eggs (i.e., duration of embryogenesis and length of endogenous feeding) (Mourente and Vázquez 1996). The relatively high lipid concentration in bonefish eggs thus suggests high energetic requirements for embryonic and pre-feeding larval development.

Lipids within bonefish eggs from all sites were mainly composed of WE-SE (> 48% of total lipids) and PLs. The WE is the dominant lipid class of eggs and gonads from many marine fish species, including European sea bass (Navas et al. 1997), mullet (Spener and Sand 1970), striped bass (*Morone saxatilis*) (Eldridge et al. 1983), and golden perch (Anderson et al. 1990). Wax esters have three possible functions when present in marine organisms; as a reserve energy store, as a buoyancy agent, and as a structural element (Nevenzel 1970). There are indications that WE have slightly lower densities than TAG, and thus provide more buoyancy per unit volume (Nevenzel 1970). Possibly, the WE-SE, in contrast to TAG, are not subject to hormone-controlled mobilization during periods of energy demand, thus stabilizing the buoyancy of the organism against short term fluctuations (i.e., buoyancy control is divorced from energy demand) (Bogevik 2011). The high concentration of WE-SE in bonefish oocytes suggests that the eggs of this species could be positively buoyant, which would assist eggs spawned at depth to rise to the surface. During the reproductive season, bonefish from Western Atlantic move from their typical shallow water flats to deep waters overlying coral reef edges to spawn (Danylchuk et al. 2008; Danylchuk et al. 2011). It has been shown some fish species rich in WE-SE (eggs and muscles) undertake daily vertical migrations of 300 m or more; during such movements pressure changes of 30 atm are experienced, with WE-SE lipids thought to play an important role as constituents in osmotic regulation and buoyancy control (Bogevik 2011). For instance, eggs of *Merluccius paradoxus*, *M. hubbsi*, and *M. capensis* are positively buoyant because of their high amount of WE-SE (>17% of total lipids) (Kayama and Hirata 1986; Olivar and Fortuno 1991; Sundby et al. 2001).

Bonefish mark-recapture data (Boucek et al., this issue and references therein) reveal high site fidelity of this species. Our results showed significant differences in lipid classes (TAG, FFA, and ST) among oocytes from three different shallow water sites within 8 to 66 km from one another. TAG was higher in oocytes from SDWC site (22% of total lipids) than BB and CBE sites (< 4% of total lipids). The benefits of greater TAG content in bonefish oocytes from SDWC site are likely to be advantageous for the future larvae due to the potential role of TAG as energy reserves. Hydrolysis of TAG is significantly faster than WE-SE, from 1 to 2 orders of magnitude to four- fivefold dependent on species (Bogevik 2011). Indeed, Padrón et al. (1996) have shown that TAG was the principal lipid class broken down during metamorphosis in bonefish leptocephali. It has been observed that decreases in TAG over the spawning season in oocytes of the striped trumpeter (*Latris lineata*) was indicative of decrease in egg quality as maternal resources diminish over time (Bransden et al. 2007; Bachan et al. 2012). Thus, females from SDWC site may have access to better feeding resources comparing to the two other sites (BB and CBE), and increased portion of TAG in eggs may, therefore, provide larvae from SDWC with a potential survival advantage during the transition to exogenous feeding.

The results on the other lipids are less conclusive. The variability of FFA is probably too low with levels of 0% in BB to 2.2% in CBE to have a biological effect. High FFA is an indicator of lipid degradation, otherwise it is undoubtedly a metabolic intermediate (Parrish 1999), but as no difference in oocyte development was observed between sites, there are probably not related to biological activity. Sterol is an essential component in animal membranes, with multiple effects on their physical properties including membrane fluidity, phase behavior, thickness, and permeability (Crockett 1998). Levels in ST were the lowest in SDWC site (1.2% comparaed to over 8% in other sites). Clearly, the oocytes from SDWC seem less thick or less fluid. However, this needs to be further investigated to see if there are differences in water salinity and/or temperature between sites that contribute to these differences. The absence of site effect on WE-SE composition in

bonefish oocytes indicates that the provisioning of this lipid may be tightly regulated in bonefish, however this remains unknown and warrants further investigation.

Fatty acids

Fatty acids mobilized by female fish during gonadogenesis are transferred via serum vitellogenin to developing eggs in the ovary. Thus, the essential fatty acids, vital for early survival and development of newly hatched larvae, are determined by the lipids derived directly from the dietary input of the female during the period preceding gonadogenesis (Kjørsvik et al. 1990; Sargent et al. 1995). In our study, neutral FAs varied among the three sites, where we observed higher percentages of MUFA (i.e, 18:1 n-9 and 16:1) at SDWC site. Given the high site fidelity of bonefish, this suggests that a possible spatial variability in food availability and/or quality that could affect the egg quality and the future larval survival. For instance, levels of essential fatty acids in gilthead sea bream and red drum eggs were found to be closely tied to recent diet (Harel et al. 1994; Fuiman and Faulk 2013), suggesting that some fishes may undergo spawning migrations to incorporate nutrients into their eggs that are specifically available at the spawning site (Fuiman and Faulk 2013). Bonefish may fall into this category where they allocate nutrients available in their tidal flat locations to the eggs a few hours or days before spawning.

SFA and PUFA are important components of cell membrane lipids and in the build-up of oocytes during vitellogenesis (McKenzie et al. 1998; Sargent et al. 2002). Our results showed that both the sums of SFA and PUFA were particularly high in the polar lipid fraction of bonefish oocytes accounting for more than 47 and 35% of total fatty acids, respectively, which fall within the published range for early-metamorphosing bonefish leptocephali (Padrón et al. 1996). Indeed, all classes of fatty acids (SFA, MUFA, and PUFA) were utilized and contributed to energy production during metamorphosis with a selective conservation of DHA (Padrón et al. 1996). Clearly, the higher levels of DHA present in bonefish oocytes in our study would allow more DHA to be incorporated into vitally important neural membranes, thereby enhancing physiological function and survivability.

We found that two other essential fatty acids dominate bonefish eggs: EPA and ARA. These EFAs have been identified as being important to a variety of functions in various marine fish species, including survival (Bessonart et al. 1999; Arendt et al. 2005), growth (Shields et al. 1999; Wacker and Von Elert 2001; Bell and Sargent 2003; Copeman and Laurel 2010), sensory and nervous system function (Shields et al. 1999), stress tolerance (Koven et al. 2001; Montero et al. 2003) and ecological performance (Ishizaki et al. 2001; Fuiman and Ojanguren 2011). Arachidonic acid, which accounted for >4% of total FAs in both lipid fractions, is known as the major eicosanoid precursor in fish cells, including prostaglandins, thromboxanes and leukotrienes, among others (Bell et al. 1994; Van Der Kraak and Biddiscombe 1999). These metabolites are important in the control of ovulation and are probably involved in embryonic development of the immune system, hatching and early larval performance (Mustafa and Srivastava 1989; Wade and Van Der Kraak 1993).

Conclusions

Although many studies have examined with changes in lipid class and fatty acid composition during early development in teleost fishes, no previous data are available for developing eggs from fishes in the subdivision Elopomorpha, which includes bonefishes, tarpons, and true eels, a group that has have unique larvae called leptocephali. This study indicates that bonefish likely follow a group-synchronous ovarian development and produce lipid-rich eggs. The eggs are particularly rich in wax esters-steryl esters, which suggest that this lipid class is used for buoyancy and/or energy reserves. Levels of neutral lipids (TAG and MUFA) varied based on the tidal flat locations. Thus, the lower TAG percentages in bonefish oocytes from BB and CBE sites may be indicative of either poorer feeding conditions experienced by bonefish females on the individual flats, or the fish were at a different developmental stage relative to spawning (i.e., the SDWC fish may have been getting ready to spawn in the next lunar phase, whereas the BB and CBE fish would spawn in a future lunar phase). More comprehensive sampling over large spatial and temporal scales is needed to better address these differences. Moreover, the results demonstrate the importance of EFAs (DHA, EPA, and ARA) that were selectively conserved among the sites, suggesting their importance during the ontogeny of bonefish. Finally, analyses of lipids and fatty acids in wild-caught oocytes are a useful tool for the assessment of egg quality and larval viability

of bonefish. These findings highlight that tidal flat location, habitat quality, prey availability, and similar factors could be important determinants for nutrients incorporated into the eggs, which in turn is crucial for egg and larval development and survival. Thus, this should be further investigated and must be considered in the management of this important fisheries resource.

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Compliance with ethical standards

Ethical approval The experimental protocol received approval from the Florida Atlantic University's Institutional Animal Care and use Committee (IACUC, protocol A16–34).

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