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Differences in egg quality between wild striped trumpeter (*Latris lineata*) and captive striped trumpeter that were fed different diets

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Abstract

This study assessed differences in egg quality between freshly caught striped trumpeter, *Latris lineata*, and captive striped trumpeter that were fed either chopped fish or commercial salmon pellets. Total lipid content of eggs did not differ between the three groups (average of 20% dry weight). The group fed chopped fish produced eggs with the greatest docosahexaenoic acid (DHA)/eicosapentaenoic acid (EPA) ratio (4:1) and, together with eggs collected from the wild group, contained the highest ratio of $(n-3)/(n-6)$ polyunsaturated fatty acids (PUFAs) (9:1 and 8:1, respectively). Ascorbic acid levels in eggs from the pellet-fed group (75 $\mu g\ g^{-1}$ wet weight) were higher than eggs from the wild and chopped-fish groups (48 and 40 $\mu g\ g^{-1}$ wet weight, respectively), but there was no difference in α-tocopherol content (average of 4.4 $\mu g\ g^{-1}$ wet weight). The volume of eggs produced, fertilisation rate and length of the larvae at hatch did not differ, but the pellet-fed group produced eggs of greater diameter, which corresponded to a larger yolk volume, and higher hatching success than the wild group. Each group of broodstock produced viable eggs and yolk-sac larvae, but the relatively low DHA/EPA and $(n-3)/(n-6)$ ratios in the eggs from the pellet-fed fish suggest that this diet may be inferior, when compared to chopped fish, for sustained growth and survival.

Keywords: Striped trumpeter; DHA; EPA; Polyunsaturated fatty acid

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1. Introduction

Teleost eggs can be collected straight from the wild in some situations, but more often they are obtained from either freshly caught wild fish, from captive wild fish or from hatchery-reared fish (Pankhurst, 1998). Regardless of origin, egg quality, or the egg's potential to produce viable fry (Kjørsvik et al., 1989), can be variable. This variability can be attributed to many factors, but two that have been clearly demonstrated, and are independent of the processes that occur post-fertilisation, are the biochemical composition of the eggs and the time to fertilisation after ovulation (Kjørsvik et al., 1989; Bromage, 1995). With captive broodstock, the biochemical composition of the eggs can be altered through changes in the diet (Kjørsvik et al., 1989; Bromage, 1995; Sargent, 1995) and careful monitoring of ovulatory cycles can alleviate problems associated with egg overripening (Norberg et al., 1991; Bromage et al., 1994). As broodstock nutrition has been implicated in egg and larval quality in a number of teleosts (Tandler et al., 1995; Bromage, 1995; Sargent, 1995), it is an area that requires careful consideration, especially when broodstock are being maintained in captivity and fed artificial diets.

In fish, lipids provided by the mother and sequestered by the oocyte during oogenesis provide the embryo and developing larva with energy for metabolism, and the essential material for the formation of cell and tissue membranes (Sargent, 1995; Bell, 1998). Marine fish differ from freshwater fish in that they are unable to convert 18-carbon fatty acids to longer 20- and 22-carbon fatty acids (Sargent, 1995; Takeuchi, 1997). Despite being unable to synthesize these highly unsaturated fatty acids (HUFAs) de novo, marine fish have an absolute requirement for them, particularly the $(n-3)$ series, including docosahexaenoic acid (DHA), 22:6$(n-3)$ and eicosapentaenoic acid (EPA), 20:5$(n-3)$ (Bromage, 1995), and also arachidonic acid (AA), 20:4$(n-6)$ (Sargent, 1995; Sargent et al., 1999). The ratio of DHA/EPA and $(n-3)/(n-6)$ polyunsaturated fatty acids (PUFAs) are commonly used as indices for measuring biochemical composition of marine fish eggs, and deficiencies in these $(n-3)$ PUFAs have been suggested to be one of the most critical hurdles to successful larviculture (Takeuchi, 1997). In addition to the $(n-3)$ HUFAs, marine fish larvae also require ascorbic acid, α-tocopherol and carotenoids, such as astaxanthin, which act as quenchers or scavengers of singlet oxygens and free radicals, effectively preventing reactive damage to other molecules, particularly the PUFAs (Bromage, 1995; Watanabe and Kiron, 1995).

Differences between egg batches often only become apparent well after their collection. Many studies have used physical, chemical or biological parameters as indicators of egg quality. As already stated, egg biochemical composition is one indicator of quality, but it is not easily interpreted. Other indicators of egg quality include fertilisation rate, buoyancy, symmetry of early cell cleavage, appearance of the chorion, the size and shape of the egg, its transparency and distribution of oil drops (Bromage, 1995).

Striped trumpeter, *Latris lineata* (Schneider, 1801), inhabit the southern temperate waters of Australia and New Zealand (Last et al., 1983) and are currently being assessed for their aquaculture potential. Broodstock can be sourced from the wild during the natural spawning season, but become stressed (Morehead, 1998) and require exogenous hormone treatment to induce ovulation (Morehead et al., 1998). Recently, wild brood-

stock have been acclimated to captivity and successfully placed onto compressed photothermal regimes to allow out-of-season gamete production (Morehead et al., 2000). However, until now, the quality of the eggs produced had not been compared to those from freshly caught wild fish. Eggs obtained from naturally foraging fish should contain a biochemical composition that is suitable for producing viable offspring (Kjørsvik et al., 1989).

This study assessed differences in egg quality between freshly caught wild fish and captive broodstock fed either chopped fish or commercial pellets. The fatty acid profile and content of ascorbic acid and α-tocopherol in the eggs collected from the different broodstock groups was determined, and egg diameter, occurrence of single oil drops, fertilisation rate, percentage even cell division, hatch rate, and a number of early larval parameters including size of larvae at hatch and survival to mouth-opening were recorded.

2. Materials and methods

2.1. Broodstock groups and treatment

Six spermiating males and 11 females, caught during the spawning season (7–8 Oct. 1997) by drop-lining in waters of ~100 m off the southeast coast of Tasmania (48°32′S,148°02′E), were brought to the marine research laboratories. Females were anaesthetised using 2-phenoxyethanol (0.02%; Sigma) and a sample of ovarian tissue (~0.5 ml) was removed using a biopsy probe and assessed for stage in oocyte development. The following day, seven females undergoing final oocyte maturation (FOM) were implanted intramuscularly with slow-release cholesterol pellets (Lee et al. 1986) containing LHRHa: des-Gly^{10}, [D-Ala^{6}]-LH-RH ethylamide (Sigma) at a dose of 100 μg kg^{-1} body weight. These females were placed with four spermiating males in a 4000-l square tank on flow-through water (~600 l h^{-1}) at ambient temperature (~11.5°C) and simulated ambient photoperiod, and were not fed.

Two groups of mature captivity-acclimated fish were maintained in separate 25 000-l circular tanks on flow-through water (~3600 l h^{-1}) at ambient temperature and simulated ambient photoperiod for between 1 and 3 years (16 females and 8 males in each tank). One group had been fed chopped fish (pilchards and squid) for the 3 months prior to and during the spawning season, while the other had been fed commercial salmon pellets (Pivot, Hobart, Tasmania).

The wild fish were sampled 3 days after the LHRHa implant, as earlier work suggested this was the minimum time for ovulation to occur posttreatment with LHRHa (Morehead et al., 1998). Five of seven females released eggs when gentle abdominal pressure was applied (hand-stripping). Fish from the other two groups had been handled on a number of occasions during the spawning season for the collection of gametes, but all were sampled on 13 October to assess which would be targeted for egg collection the following day. This practice aimed to remove overripe eggs that may have remained in the ovary from the previous ovulation and ensured that freshly ovulated eggs had not been held in the ovary for more than 24 h. Five females from the chopped-fish group

and six from the pellet group underwent ovulation prior to sampling the next day and released eggs when hand-stripped. One batch of eggs from the chopped-fish group did not fertilise, allowing only egg-composition data to be recorded for that batch. Prior to each fish being hand-stripped, it was rinsed in seawater and towel-dried to prevent contamination of the eggs with anaesthetic or seawater. Eggs were collected in glass beakers.

2.2. Egg and larval incubation protocols

Each batch of eggs ($n = 15$) was fertilised with freshly pooled milt from the same four males. Approximately 140 eggs (0.2 ml) from each fish ($n = 15$) were placed into three replicate 70-ml containers and were assessed for egg size, fertilisation, percentage of even cell division, percentage of eggs with a single oil drop, hatching success and larval size at hatch (standard length, body depth at vent, yolk volume and oil drop diameter). Yolk volume (YV) was determined using the formula for a prolate spheroid (Avila and Juario, 1987): $YV = (\pi/6) \times YL \times YD^2$; where YV is the volume of yolk (μl), YL is the yolk length (mm) and YD is the yolk depth (mm). Another sample of approximately 350 eggs (0.5 ml) from each fish was placed into three replicate 200-ml containers for larval size assessments at first-feed and survival. All eggs were incubated at 11°C.

Eggs from the 0.2 ml samples that were undergoing cell division (2–32 cell stage) were examined for fertilisation and even cell division. A 90% water exchange using 1 μm filtered seawater maintained at 11°C was conducted after fertilisation had been determined, and on days 3, 5 and 7 post-fertilisation. Nonviable eggs (sinking) were counted and removed from the containers after each water exchange. Samples of larvae ($n = 5$) were removed for morphometrics when 50–80% of the eggs in each container had hatched. Final hatch proportions were assessed ~ 32 h after the first eggs had hatched, to allow time for all viable eggs to hatch.

Eggs from the 0.5-ml samples were treated the same as those in the 0.2-ml containers until hatch. Chorions and unhatched eggs were removed from the containers after hatching and larval mortalities were recorded on days 2, 4, 6 and 8 posthatch (ph). A single water exchange (40%) was conducted on day 4 ph. Larval survival was recorded every two days until day 8 ph, which coincided with mouth-opening and the completion of yolk-sac absorption. A sample of 5 larvae in total from each group was sampled on day 9 ph (first-feed).

Images of eggs and larvae were recorded for later analysis using a Sony video camera connected to a Nikon dissecting microscope attached to an IBM computer running Scion ImagePC.

2.3. Egg composition — sample preparations

2.3.1. Fatty acids

Eggs were rinsed in 0.5 M ammonium formate and two 5-ml samples from each fish were placed into glass test tubes and stored at −30°C. Chopped fish (50/50 w/w whole pilchards and squid, homogenised presampling) and salmon pellets were analysed

in triplicate. Lipids were extracted using the method of Bligh and Dyer (1959). Lipid extracts were stored at −20°C under nitrogen prior to analysis. The amount of lipid was determined gravimetrically using a Metler AE 163 digital balance. An aliquot of each lipid extract was analysed for lipid class. Separation of the lipid classes was performed using the following solvent system; hexane:diethyl ether:acetic acid, 600:170:1. The lipid classes were quantified using an Iatroscan Mk-5. An aliquot of each lipid was also transesterified with a methanolic hydrochloric acid solution to form fatty acid methyl esters (FAME). Samples were stored under nitrogen at −20°C until analysis. The FAME samples were analysed with a Varian 3410 gas chromatograph equipped with an FID and autosampler injector. The column used for the analysis was a polar 70% cyanopropyl siloxane (BP-X70) fused-silica capillary column (50 m × 0.32 mm i.d.) (SGE, Australia). Samples were injected at 45°C and after 1 min the oven temperature was raised to 120°C at 30°C/min and then to 245°C at 3°C/min. The final temperature was maintained for 20 min. Hydrogen was used as the carrier gas. The detector temperature was 260°C. Peak areas were quantified with DAPA software on an IBM-compatible computer. Egg dry weights were determined in duplicate. A complete fatty acid profile was determined for each batch of eggs, but only fatty acids present at levels of 1% or greater are presented. Fatty acids present at levels below 1% comprised 6.1% of the total fatty acid content.

2.3.2. Ascorbic acid (vitamin C)

Two 3-ml samples of eggs from each fish were transferred to cryovials, then stored in liquid nitrogen for 2 weeks prior to analysis. Samples were then thawed at 4°C, and transferred to 10-ml plastic tubes together with 4.0 ml of 3% metaphosphoric acid + 8% acetic acid, and 20 μl of EGTA–glutathione solution (0.24 M ethyleneglycol-bis-(β-aminoethyl ether)-*N,N,N′,N′*,-tetraacetic acid and 0.20 M glutathione; pH 6.5), at 4°C (Hapette and Poulet, 1990). Samples were sonicated at 150 W with a Labsonic 1510 sonicator (B. Braun, Melsungen, W. Germany) for 20 s, then left overnight at 4°C to complete extraction. Volumes of extracts were made up to 9.0 ml with water, then 2.7-ml aliquots were transferred to clean test tubes. Samples were then neutralised with a sodium acetate buffer (4.5 M, pH 6.2) and the extracted ascorbic acid converted to a fluorescent derivative, 3-(1,2 dihydroxyethyl) furo [3,4-*b*]-quinoxaline-1-one by the method of Speek et al. (1984). HPLC analysis of samples and standards was carried out with a Varian Model 5000 LC by injecting 90 μl of the derivatised extracts (containing up to 2 μg of AA) onto a C18 Novapak (Waters; 3.9 × 150 mm) column. The column was eluted isocratically with 0.08 M potassium dihydrogen phosphate:methanol, pH 7.8 (8:2 v/v) at a flow rate of 0.8 ml min^{-1}. The ascorbic acid derivative was detected with a Varian Fluorichrom set for an excitation maximum of 355 nm and an emission maximum of 425 nm; the peak area was quantified with Star Integration software (Varian).

2.3.3. α-Tocopherol (vitamin E)

The saponification and subsequent extraction of lipid extracts for α-tocopherol was based on the method of Phronen et al. (1984). Lipid extracts, containing 1 to 5 mg lipid

(prepared during fatty acid extraction procedure) were transferred to 5-ml minivials and evaporated to dryness under a stream of nitrogen. Ascorbic acid (20 mg), distilled water (0.4 ml) and 96% ethanol (1 ml) were added, and vials contents were mixed and then left at room temperature for 10 min. KOH (0.2 ml of 50%) was added and the vials were then purged with nitrogen before sealing the lids. Vials were incubated in a shaking water bath at 25°C overnight. Vitamin E was then extracted from the saponified extract

Table 1
Composition of chopped-fish (pilchards and squid) and salmon pellets fed to captive broodstock of striped trumpeter

Diet composition (±SD)	Chopped fish	Salmon pellets
Diet composition		
Water content (%)	76.4	11.0
Total lipid (% dry weight)	16.7	26.6
Lipid class (% of total)		
Polar lipids	41.1 (4.0)	14.6 (1.1)
Sterols	4.3 (0.5)	1.0 (0.1)
Triglycerides	48.3 (3.7)	81.4 (1.1)
Wax esters/sterol esters	0.5 (0.0)	0.4 (0.0)
Other	5.8 (0.4)	2.7 (0.1)
Major fatty acids (% of total)		
Saturated		
14:0	6.0 (0.2)	6.2 (0.2)
16:0	22.5 (0.3)	18.6 (0.3)
18:0	5.3 (0.1)	4.5 (0.1)
Sum	33.9 (0.1)	29.2 (0.4)
Monounsaturated		
16:1 $n-9$	0.5 (0.1)	0.4 (0.1)
16:1 $n-7$	4.3 (0.1)	5.4 (0.1)
18:1 $n-9$	5.4 (0.2)	11.6 (0.4)
18:1 $n-7$	3.0 (0.2)	3.3 (0.4)
20:1 $n-9$	1.0 (0.0)	4.0 (0.1)
Sum	14.1 (0.1)	24.6 (0.3)
Polyunsaturated		
18:2 $n-6$	2.3 (0.0)	3.0 (0.1)
20:4 $n-6$	1.5 (0.1)	1.0 (0.0)
20:4 $n-3$	0.0 (0.0)	0.0 (0.0)
20:5 $n-3$ (EPA)	11.7 (0.3)	8.6 (0.3)
22:5 $n-3$	1.5 (0.1)	1.6 (0.3)
22:6 $n-3$ (DHA)	22.3 (0.7)	12.2 (0.3)
Sum	39.3 (0.7)	26.5 (0.5)
Sum other	12.7	19.7
Sum total	100	100
DHA/EPA ratio	1.9:1	1.4:1
$(n-3)/(n-6)$ ratio	9.3:1	5.6:1
Ascorbic acid (ppm)	7.5	150[a]

[a]Manufacturers standard.

with hexane (3 × 2 ml). The hexane extracts were combined, washed with water (3 × 2 ml), then dried under a stream of nitrogen at 50°C. One milliliter of a hexane–ethanol mixture (1:1) was added and the samples dried down again, before finally redissolving in 0.5 ml of ethanol. Aliquots (60 μl) were then chromatographed on a C18 column (SGE, Australia; 4.6 × 250 mm) with a mobile phase of 7% dichloromethane in methanol at a flow rate of 1 ml min^{-1}. The vitamin was measured by UV detection at 294 nm.

2.4. Statistics

Percentage data were arcsin $\sqrt{x}$ transformed prior to statistical analysis. Analysis of variance established if statistical differences ($P < 0.05$) occurred between the parameters examined and a Tukey–Kramer (all pairs comparison) test was conducted when differences were found. Data met the requirements of normality (Shapiro–Wilk) and homogeneity (O'Brien), and were assessed using the JMP statistical package.

Table 2
Broodstock, egg and larval parameters from wild broodstock ($n = 5$) and captive broodstock of striped trumpeter fed either chopped fish ($n = 4$) or pellets ($n = 5$)

Broodstock, eggs and larvae	Wild	Captive (chopped fish)	Captive (pellets)	Significant difference ($P = 0.05$)
Broodstock (± SD)				
Broodstock weight (kg)	3.0 (1.1)	4.0 (1.5)	3.0 (0.5)	NS
Egg volumes produced (ml)	11 (58)	11 (47)	21 (88)	NS
Relative fecundity (ml kg^{-1})	3 (12)	3 (19)	6 (26)	NS
Eggs (± SE)				
Egg diameter (μm)	125 (6)^{b}	128 (20)^{ab}	131 (7)^{a}	*
Single oil drop (%)	98.0 (0.5)	99.0 (0.1)	96.0 (2.5)	NS
Fertilisation rate (%)	75.0 (6.0)	73.0 (5.7)	82.0 (2.7)	NS
Even cell division (%)	97.0 (1.3)	99.0 (0.8)	96.0 (2.5)	NS
Hatch rate (%)	38.0 (4.3)^{b}	55.0 (8.6)^{ab}	64.0 (5.1)^{a}	*
Larvae (± SE)				
Length at hatch (μm)	357 (38)	369 (49)	369 (21)	NS
Depth at vent at hatch (μm)	224.0 (2.5)	227.0 (1.8)	224.0 (1.9)	NS
Yolk volume at hatch (μl)	0.4 (0.03)^{b}	0.5 (0.05)^{b}	0.8 (0.06)^{a}	*
Oil drop diameter at hatch (μm)	28 (2)^{a}	27 (2)^{b}	29 (4)^{a}	*
Survival to day 2 ph (%)	6 (8)	7 (7)	7 (8)	NS
Survival to day 4 ph (%)	3 (9)	2 (9)	2 (9)	NS
Survival to day 6 ph (%)	1 (9)	1 (6)	1 (7)	NS
Survival to day 8 ph (%)	(5)	1 (4)	1 (7)	NS
Length at day 9, first-feed (μm)	474 (149)	469 (89)	472 (244)	NS
Depth at vent at first-feed (μm)	25 (10)	258 (12)	26 (12)	NS

(*) Denotes significant differences. Superscripts denote where differences occur.

3. Results

3.1. Diet composition:

Samples of chopped fish (whole pilchards and squid, 50/50 w/w, homogenised presampling) and commercial salmon pellets were analysed in triplicate as described above and their composition is presented in Table 1.

Table 3
Composition of eggs from wild broodstock ($n=5$) and captive broodstock of striped trumpeter fed either chopped fish ($n=5$) or pellets ($n=5$)

Egg composition (±SD)	Wild	Captive (chopped fish)	Captive (pellets)	Significant differences ($P=0.05$)
Egg composition				
Wet weight (mg)	1.04 (0.02)	1.02 (0.01)	1.03 (0.01)	NS
Water content (%)	91.1 (0.2)	91.8 (1.0)	90.9 (0.1)	NS
Total lipid (% dry weight)	19.7 (0.7)	21.6 (1.6)	18.9 (0.5)	NS
Lipid class (% of total)				
Polar lipids	59.6 (4.0)	59.5 (4.9)	62.8 (3.7)	NS
Sterols	1.9 (0.5)	2.9 (0.9)	2.3 (0.4)	NS
Triglycerides	32.5 (4.1)	31.5 (5.5)	29.8 (3.9)	NS
Wax esters/sterol esters	6.0 (0.8)	6.2 (0.9)	5.1 (1.0)	NS
Major fatty acids (% of total)				
Saturated				
14:0	2.9 (0.5)	2.5 (0.3)	2.9 (0.2)	NS
16:0	17.8 (0.3)^{b}	19.6 (0.3)^{a}	19.5 (0.3)^{a}	*
18:0	2.9 (0.1)^{b}	3.6 (0.1)^{a}	3.6 (0.1)^{a}	*
Sum	23.7 (0.4)^{b}	25.6 (0.9)^{a}	26.0 (0.5)^{a}	*
Monounsaturated				
16:1 $n-9$	1.4 (0.1)	1.1 (0.1)	1.1 (0.0)	NS
16:1 $n-7$	3.8 (0.2)^{b}	4.1 (0.2)^{b}	4.8 (0.1)^{a}	*
18:1 $n-9$	18.6 (0.8)	17.4 (0.8)	18.1 (0.3)	NS
18:1 $n-7$	3.9 (0.2)	3.6 (0.0)	3.7 (0.0)	NS
20:1 $n-9$	4.5 (1.6)^{a}	0.9 (0.2)^{b}	1.1 (0.1)^{b}	*
Sum	32.2 (1.2)^{a}	27.1 (2.0)^{b}	28.9 (0.6)^{b}	*
Polyunsaturated				
18:2 $n-6$	1.1 (0.0)^{c}	1.9 (0.1)^{b}	3.8 (0.2)^{a}	*
20:4 $n-6$	2.4 (0.3)	2.0 (0.1)	2.0 (0.0)	NS
20:4 $n-3$	1.3 (0.2)^{a}	0.7 (0.0)^{b}	0.8 (0.0)^{b}	*
20:5 $n-3$ (EPA)	6.8 (0.1)^{b}	7.4 (0.3)^{b}	9.3 (0.2)^{a}	*
22:5 $n-3$	3.8 (0.2)	3.8 (0.2)	3.8 (0.1)	NS
22:6 $n-3$ (DHA)	20.5 (0.3)^{b}	26.5 (0.6)^{a}	20.4 (0.5)^{b}	*
Sum	36.0 (0.3)^{c}	42.2 (2.0)^{a}	40.0 (0.8)^{b}	*
Sum other	8.1	5.1	5.1	
Sum total	100	100	100	

(*) Denotes significant difference. Superscripts denote where differences occur.

3.2. Broodstock, eggs and larvae

Broodstock weights, the volume of eggs produced and the relative fecundity of the broodstock did not differ significantly between groups (Table 2). However, the diameter of the eggs collected from the pellet-fed group was significantly larger than that from the wild group. There was no significant difference in terms of percent single oil drop, fertilisation rate or percent even cell division between the three groups, but hatch rates were significantly higher in the pellet group when compared to eggs from the wild group.

Posthatching, there was no significant difference in the length or depth at vent of the larvae from the three groups (Table 2). However, larvae from the pellet group had a significantly larger yolk volume than larvae from both the wild and chopped-fish groups. Oil drop diameters at hatch in larvae from both the wild and pellet groups were significantly larger than in larvae from the chopped-fish group. However, there was no difference in larval survival to day 2, 4, 6 or 8 ph, or length and depth at vent of larvae at first feed (day 9 ph) between the three groups. Yolk reserves were depleted and only a small quantity of lipid remained by the time the mouth was beginning to open (day 8).

3.3. Egg composition

Wet weight, water content and total lipid content of the eggs collected from the three broodstock groups did not differ significantly (Table 3). Lipid class comprised approxi-

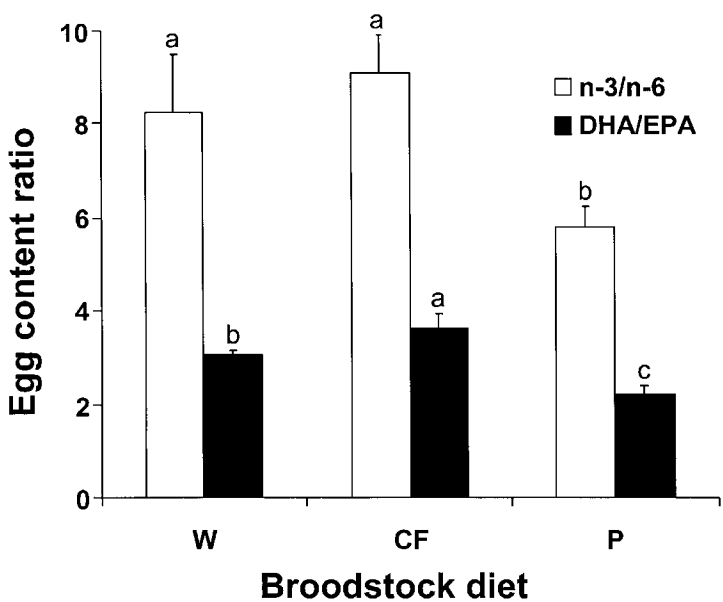

Fig. 1. Ratios of $n-3/n-6$ PUFA and DHA/EPA in striped trumpeter eggs collected from wild fish (W) and captive fish fed either chopped fish (CF) or commercial pellets (P). Mean ± SD. For each ratio, values with different letters are significantly different (Tukey–Kramer, $P = 0.05$).

mately 61% polar lipids, 2 % sterols, 31% triglycerides and 6% wax esters/sterol esters, with no significant difference between the groups (Table 3). Eggs from both the chopped fish and pellet group had significantly higher levels of saturated fatty acids compared to eggs from the wild group. Conversely, levels of monounsaturated fatty acids were significantly higher in eggs from the wild group than the other two. Eggs from each of the groups differed significantly in their level of total PUFA, with the highest level obtained from the chopped-fish group, followed by the pellet group and then the wild group. The level of EPA, 20:5($n-3$) was highest in the pellet group, while the level of DHA, 22:6($n-3$) was highest in eggs from the chopped-fish group.

The ratio of DHA to EPA differed significantly between each broodstock group (Fig. 1), with the greatest ratio in eggs from the chopped-fish group (3.6:1), followed by the wild group (3.0:1) and then the pellet-fed group (2.2:1). The ($n-3$)/($n-6$) PUFA ratio also differed significantly, with eggs from both the wild and chopped-fish group (8.2:1 and 9.1:1, respectively) having a higher ratio than eggs from the pellet fed group (5.8:1).

Levels of ascorbic acid in eggs from the pellet group (75 μg g^{-1} wet weight) were significantly higher than levels in the wild (48 μg g^{-1} wet weight) and chopped-fish (40 μg g^{-1} wet weight) group, but there was no significant difference in α-tocopherol levels; with levels of 4.8, 3.4 and 4.9 μg g^{-1} wet weight for the wild, chopped-fish and pellet group, respectively (Fig. 2).

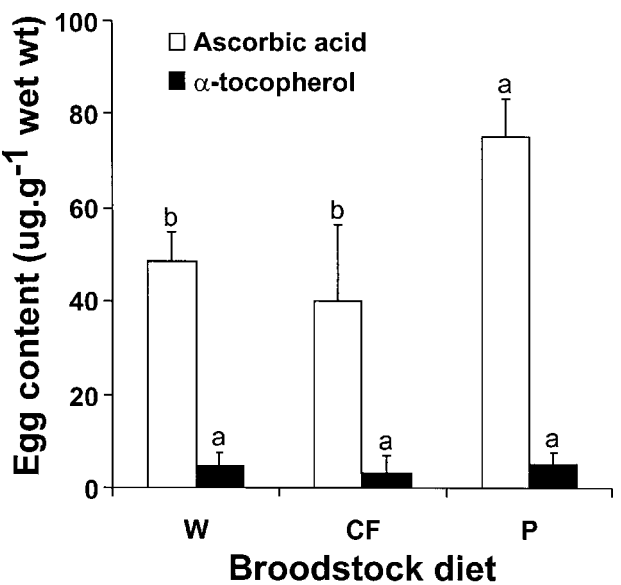

Fig. 2. Ascorbic acid and α-tocopherol levels (μg/g wet weight) in striped trumpeter eggs collected from wild fish (W) and captive fish fed either chopped fish (CF) or commercial pellets (P). Mean ± SD. For each vitamin, values with different letters are significantly different (Tukey–Kramer, $P = 0.05$).

4. Discussion

All fish used in this trial were of similar body weight and there was no difference in their relative fecundity. Differences in fatty acid profile, ascorbic acid content, egg size, hatch rate and volume of endogenous reserves in the yolk-sac larvae were detected between the three broodstock groups. These results suggest that broodstock diet affects egg biochemical composition and certain egg and larval parameters.

Total lipid content of the eggs did not differ between the broodstock groups and comprised on average 20% of the egg dry weight. Eggs from the wild group contained a lower overall percentage of saturated fatty acids, as a result of their significantly lower levels of 16:0 and 18:0, when compared to either of the acclimated broodstock groups. The level of monounsaturated fatty acids was highest in the wild group due mainly to a fourfold increase in the percentage of 20:1($n-9$). In the Northern Hemisphere, this monoenoic fatty acid indicates the presence in the diet of lipids from fish feeding on calanoid copepods, such as herring and capelin (Silversand et al., 1996). It is a characteristic feature of farmed fish fed diets rich in oils derived from northern Atlantic fisheries (Sargent, 1995). This contrasts with our study which found high levels of 20:1($n-9$) in the wild group, but not the chopped-fish or pellet group, despite concentrations of 20:1($n-9$) in the pellet diet being fourfold higher than the chopped-fish diet. Furthermore, total saturated and monounsaturated fatty acid profiles in eggs from the wild group were quite different to those of the chopped-fish and pellet group. Striped trumpeter are forage feeders and their diet includes pilchards, squid, sprats, pipefish, seahorses and decapods (Nichols et al., 1994); however, the reason for these significant differences in saturates and monoenes remains unclear.

The DHA content, ratio of DHA/EPA and ratio of ($n-3$)/($n-6$) PUFAs are commonly associated with marine finfish egg quality (Izquierdo, 1996; Takeuchi, 1997), with high DHA content and high DHA/EPA and ($n-3$)/($n-6$) PUFAs ratios considered desirable. Eggs from marine fish usually have a DHA/EPA ratio of around 2:1 and an ($n-3$)/($n-6$) PUFA ratio between 5:1 and 10:1 (Sargent, 1995). Differences in lipid composition between wild and captive fish have been recorded in gilthead sea bream (*Sparaus aurata*), (Mourente et al., 1989), striped bass, (*Morone saxatilis*) (Harrel and Woods, 1995), turbot (*Scophthalmus maximus*) (Silversand et al., 1996) and sea bass (*Dicentrarchus labrax*) (Bell et al., 1997). These differences have been attributed to broodstock diet. The current study found DHA and the ratio of DHA/EPA to be significantly higher in eggs from the chopped-fish group and this reflects the twofold increase of DHA in the chopped-fish diet when compared to pellets. DHA levels between the wild and pellet group did not differ, reflecting the substantial contribution of fish oils to the commercial pellets. The ($n-3$)/($n-6$) PUFA ratios ranged from 6:1 in the pellet group to 9:1 in the chopped-fish group and mirrored those found in the diet (6:1 and 9:1, pellet and chopped-fish group, respectively), while ($n-3$)/($n-6$) PUFA ratios in eggs from the wild group were 8:1. The lower ($n-3$)/($n-6$) PUFA ratio in eggs from the pellet group was primarily a result of increased levels of linoleic acid (LA), 18:2($n-6$). LA is scarce in the marine environment and increased LA in egg lipids is typical of fish given a formulated diet containing vegetable products (Silversand et al., 1996).

Recently, more attention has been given to the role of AA in embryonic and larval development (Bell et al., 1997; Sargent et al., 1999), but no differences in this PUFA were detected between the broodstock groups. AA is more commonly known for its role as a prostaglandin precursor involved in spawning behaviour and ovulation in fish (Rosenblum et al., 1995). Despite differences in lipid composition between the broodstock groups, no group produced eggs with a biochemical composition that would be considered unsuitable for embryonic and larval development. As stated by Sargent (1995), if broodstock feeds are produced primarily from marine fish or commercial fish oils, it is unlikely that broodstock lipid nutrition will be the cause of poor egg quality.

Ascorbic acid content was highest in eggs collected from the pellet group (75 $\mu g\ g^{-1}$ wet weight) and, similar to studies with cod (*Gadus morhua*), (Mangor-Jensen et al., 1994), this suggests the direct uptake of this vitamin from the ascorbic acid-enriched feed. However, α-tocopherol did not differ between the broodstock groups with average levels of 4.4 $\mu g\ g^{-1}$ wet weight. Both vitamins act as antioxidants and are important in larval development (Bromage, 1995; Watanabe and Kiron, 1995). Severe deficiencies in these vitamins may reduce egg quality, but when compared to eggs from the wild group, it is unlikely that these vitamins would be limiting in the captive broodstock diets. Merchie et al. (1997) demonstrated that a transfer of high concentrations of ascorbic acid to larvae can improve their stress resistance, which may be beneficial during intensive larval production where major stresses are often encountered.

Despite the larger egg size, yolk volume and lipid diameters of the pellet-fed group, larval length and depth at hatch and survival to mouth-opening did not differ between the groups. Egg size as an indicator of quality has been a source of controversy. Bromage et al. (1992) suggest that, under good hatchery conditions, differential egg size in rainbow trout, *Oncorhynchus mykiss*, is not a primary determinant of egg and fry quality. As a rule, larger eggs produce larger larvae, but this does not necessarily give any permanent or long-term advantages as far as growth and survival of larvae are concerned (Kjørsvik et al., 1989). This study found larger eggs from the pellet group to result in larger yolk and lipid reserves, but not larger larvae per se. It remains unclear why eggs from the pellet group were larger, when egg weights between the three groups did not differ. Egg weight was determined using eggs stripped from the ovary and stored on dry ice, while egg diameter was determined from eggs that had been fertilised. It is suggested that the larger egg diameter may have resulted from increased water uptake during water hardening. Another consideration is that weight is not proportional to volume and that the larger eggs may have contained a greater amount of less dense material than the smaller eggs. It is also unclear why hatching proportions from the pellet group were almost twofold higher than those from the wild group. However, this may relate to the use of LHRHa to induce ovulation in the wild group, as oocytes can become arrested in development for a period between capture and stimulation via exogenous hormones. Foscarini (1988) found the use of hormones in red sea bream, *Pagrus auratus*, to be implicated with loss of egg viability, and this may have also occurred in the current study.

Egg overripening has also been reported as a major egg quality determinant in teleosts (Kjørsvik et al., 1989; Bromage, 1995). In our study, the wild fish were hand-stripped within 3 days of treatment with LHRHa, which is reported as the

minimum time for ovulation posttreatment to occur (Morehead et al., 1998), and all of the captive fish were hand-stripped on the day prior to eggs being collected, to ensure aged eggs that may have remained in the ovary from the previous ovulation were removed and that newly ovulated eggs had not been held within the ovary for more than 24 h. Bromage et al. (1992) reported up to 10–20% of rainbow trout eggs can be retained in the ovary after stripping, but these are usually removed with a second stripping. Differences in egg biochemical composition were not obviously correlated with the egg- and larval-quality parameters measured and, despite no obvious deficiencies in egg composition being noted in this study, it is suggested that egg composition may provide a more accurate reflection of the egg's potential, once fertilised, to produce viable offspring.

To obtain a reliable supply of eggs for larviculture throughout the year it is necessary to use captive broodstock. Broodstock fed the chopped fish produced eggs with higher levels of DHA, and desirable DHA/EPA and $(n-3)/(n-6)$ ratios, while the broodstock fed pellets produced eggs with a relatively high LA and ascorbic acid content. Each diet resulted in the production of viable eggs and yolk-sac larvae, but the lower DHA/EPA and $(n-3)/(n-6)$ ratios in eggs from the pellet-fed fish, and in the pellets themselves, suggests that this diet may be inferior for sustained larval growth and survival.

In summary, this study found egg biochemical composition and select egg and yolk-sac larval parameters to differ between the three broodstock groups, and that eggs from captive striped trumpeter fed chopped fish provide a suitable alternative to eggs from freshly caught fish.

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