การศึกษาเบื้องต้นของคุณค่าทางอาหารของอาร์ทีเมียที่เสริมด้วยแพลงก์ตอนพืช และผลต่อการสืบพันธุ์ของปลาแมนดารินเขียว (*Synchiropus splendidus* Herre, 1927)

Preliminary Study on the Nutritional Content of *Artemia* fed mixed microalgal Diets and their Effect on the Reproduction of Captive Bred Green Mandarinfish (*Synchiropus splendidus* Herre, 1927)

จารุนันท์ ประทุมยศ* สุพรรณี ลีโทชวลิต ณิษา ศิรนนท์ธนา และ ศิริวรรณ ชูศรี

Jarunan Pratoomyot, Supannee Leethochavalit, Nisa Siranonthana and Siriwan Choosri

สถาบันวิทยาศาสตร์ทางทะเล มหาวิทยาลัยบูรพา

Institute of Marine Science, Burapha University

Received : 8 February 2016

Accepted : 25 June 2016

Published online : 6 July 2016

บทคัดย่อ

อาร์ทีเมียเป็นอาหารมีชีวิตที่ใช้เลี้ยงปลาแมนดารินเขียว *Synchiropus splendidus* ซึ่งเป็นปลาที่มีพฤติกรรมการกินอาหารช้า แต่อย่างไรก็ตามขนาด และคุณค่าทางอาหารที่มีผลต่อการสืบพันธุ์ของปลาแมนดารินเขียว ควรต้องมีการศึกษาทำการทดลองเลี้ยงพ่อแม่พันธุ์ปลาแมนดารินเขียว (F1) เริ่มต้นอายุ 14 เดือนจำนวน 12 คู่ ในตู้กระจกบรรจุน้ำเค็ม 90 ลิตร กินอาหารทดลอง 4 ชนิดวันละ 2ครั้ง 1) อาร์ทีเมียตัวเต็มวัย 3 ตัว/ลิตร/ครั้ง 2) อาร์ทีเมียแรกฟัก 0.5 ตัว/มล/ครั้ง 3) อาร์ทีเมียผสมระหว่างตัวเต็มวัยและอาร์ทีเมียแรกฟัก อัตราส่วน 2 ตัว/ลิตร/ครั้ง: 0.5 ตัว/มล/ครั้ง และ 4) อาร์ทีเมียผสมระหว่างตัวเต็มวัยและอาร์ทีเมียแรกฟัก อัตราส่วน 1 ตัว/ลิตร/ครั้ง: 0.5ตัว/มล/ครั้ง ทำการเลี้ยงอาร์ทีเมียด้วยสไปรูไลนาอบแห้งและเสริมสารอาหารอาร์ทีเมียทุกวันด้วยแพลงก์ตอนพืชผสมกันระหว่าง *Tetraselmis gracilis* และ *Isochrysis galbana* หรือ *T. gracilis* และ *Nanochrolopsis oculata* เป็นเวลา 1 – 3 ชม ผลการทดลองพบว่าอาร์ทีเมียแรกฟักมีโปรตีนและไขมันสูงกว่าอาร์ทีเมียตัวเต็มวัย อาร์ทีเมียตัวเต็มวัยกินแพลงก์ตอนพืชผสม *T. gracilis* และ *N. oculata* มีโปรตีนสูงกว่าอาร์ทีเมียกิน *T. gracilis* และ *I. galbana* แต่อาร์ทีเมียเหล่านี้มีไขมันไม่แตกต่างกัน อาร์ทีเมียทั้งสองขนาดมีกรดไขมันที่จำเป็นขนาดโซ่ยาว eicosapentaenoic acid 3% แต่ไม่มีกรดไขมัน docosahexaenoic acid อาร์ทีเมียตัวเต็มวัยที่กินแพลงก์ตอนพืชผสมมี arachidonic acid 1% แต่ไม่พบกรดไขมันชนิดนี้ในอาร์ทีเมียแรกฟัก ผลการทดลอง 9 เดือนพบว่าปลา *S. splendidus* 1 คู่ที่กินอาร์ทีเมียตัวเต็มวัย 3 ตัว/ลิตร/ครั้ง มีพฤติกรรมการผสมพันธุ์ออกไข่เมื่ออายุ 19 เดือน ปลาออกไข่ตั้งแต่เดือนมิถุนายนถึงกันยายน ไข่ปลามีปริมาณ 48 – 253 ฟอง (ขนาดเฉลี่ย 0.78 ± 0.02 มม) จำนวนไข่ที่ได้รับการผสมพัฒนาและเป็นตัวอ่อนเพิ่มขึ้นตามจำนวนครั้งการออกไข่แสดงว่าการเสริมสารอาหารในอาร์ทีเมียตัวเต็มวัยด้วยแพลงก์ตอนพืชผสมกันและให้ปลาแมนดารินเขียว (F1) อายุ 14 เดือน กินอัตราอย่างน้อย 3 ตัว/ลิตร/ครั้ง จำนวน 2 ครั้งต่อวันเป็นระยะเวลานาน 9 เดือน มีสารอาหารพอเพียงในการเลี้ยงปลา *S. splendidus* ในที่กักขังและปลาสามารถสืบพันธุ์ได้แต่อย่างไรก็ตามวิธีการเสริมสารอาหารในอาร์ทีเมียที่เหมาะสมควรทำการวิจัยต่อไป

คำสำคัญ : อาร์ทีเมีย คุณค่าทางอาหาร การสืบพันธุ์ ปลาแมนดารินเขียว

***Corresponding author: e-mail:** jarunan@buu.ac.th

Abstract

Artemia are used intensively for slow feeding species like the green mandarinfish, *Synchiropus splendidus*, however, the size and nutritional content of *Artemia* on their reproductive performance, requires determination. To investigate this, 12 pairs of 14-month old F1 progeny were maintained in glass aquaria containing 90L of seawater and fed twice daily on either: 1) adult *Artemia* (AA) at 3 individuals (ind.) L^{-1}; 2) newly hatched *Artemia* (NHA) at 0.5 ind.mL^{-1}; 3) mixtures of AA and NHA at a ratio of 2 ind.L^{-1}: 0.5 ind.mL^{-1}; and 4) mixtures of AA and NHA at a ratio of 1 ind.L^{-1}: 0.5 ind. mL^{-1}, respectively. Maintained with dry *Spirulina* sp., *Artemia* were enriched daily with microalgae mixtures, *i.e.*, either *Tetraselmis gracilis* and *Isochrysis galbana* or *T. gracilis* and *Nanochrolopsis oculata*. NHA contained higher protein and lipid levels than AA; the protein levels of AA fed mixed *T. gracilis* and *N. oculata* were high but their lipid levels were similar to that fed *T. gracilis* and *I. galbana*. All *Artemia* contained eicosapentaenoic acid (ca. 3%), but they lacked docosahexaenoic acid, while only AA fed mixed microalgae had ca.1% arachidonic acid. During the 9-month trial, only one pair of mandarinfish, reared on AA at 3 ind.L^{-1}, spawned at 19-months old. The pair subsequently spawned ten times throughout June-September; the number of eggs laid within each batch ranged from 48–253; average diameter of the eggs was 0.78±0.02 mm. Irrespective of the egg number within a batch, the number of fertilized eggs increased in successive batches indicating that application of microalgae-enriched AA at least 3 ind.L^{-1}, when presented to 14-month old green mandarinfish (F1) at least twice daily for 9 months, may provide sufficient nutrients to promote the reproduction of the green mandarinfish reared in captivity, although the enrichment methods require further investigation.

Keywords : *Artemia*, nutritional content, reproduction, green mandarinfish

Introduction

The colourful marine ornamental fish, green mandarinfish, *Synchiropus splendidus* (Herre, 1927), or mandarin dragonet (Family Callionymidae) is among the top twenty species collected from coral reefs (Rhyne *et al*., 2012) and overfishing has placed increasing pressure on wild populations (Sadovy *et al*., 2001). Although the development of a sustainable, captive breeding programme offers a solution to this problem, practices and information relating to the rearing and breeding of adults in captivity is needed. The quality of nutrients in broodstock diets, for example, can influence the reproductive performance of marine aquatic species (Forteath, 1997; Wong & Benzie, 2003; Foster & Vincent, 2004; Lin *et al*., 2007; Fernández-Palacios *et al*., 2011; Migaud *et al*., 2013). The long chain, highly unsaturated n-3 family of fatty acids (FA), *e.g.* eicospentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6 n-3), and the n-6 family of fatty acids, *e.g.* arachidonic acid (AA; C20:4 n-6), are considered essential for the normal growth and reproduction of marine fish species.

In broodstock diets, a deficiency of these essential fatty acids (EFAs) can reduce fecundity and fertilization rates, can result in embryo deformities and can damage larval quality (Izquierdo *et al*., 2001, 2005).

In nature, *S. splendidus* obtains its EFAs from a variety of live feeds, *e.g.* from polychaetes, amphipods, gastropods and various zooplankton species such as harpacticoid copepods (Randall *et al*., 1992; Lieske & Myers, 1994; Sadovy *et al*., 2001). Maintaining a continuous supply of a spectrum of live feeds is, however, in most aquaria, not an easy undertaking in practice. For captive reared species of fish *etc*, *Artemia* has been traditionally used worldwide as a live feed for some of the slower swimming and feeding aquatic species (Wong & Benzie, 2003; Lin *et al*., 2007; Otero-Ferrer, 2012). The rearing of various seahorses, *i.e. Hippocampus* spp., with EFA enriched-*Artemia* has proven successful in getting captive-held stocks to breed and to produce viable, healthy offspring (Forteath, 1997; Chang & Southgate, 2001; Wong & Benzie, 2003; Woods & Valentino, 2003; Woods, 2005; Lin *et al*., 2007; 2008; 2009). Consistent with this, it was hypothesized that EFA enriched-*Artemia* may also serve as an appropriate live feed for the slower moving *S. splendidus* broodstock.

In this preliminary study, adult *Artemia* were maintained with dry *Spirulina* sp., an algae recommended for the culture of *Artemia* (see Zarei, 2013), throughout the trial. Alongside this, mixtures of either *Tetraselmis gracilis* and *Nanochloropsis oculata* or *T. gracilis* and *Isochrysis galbana* were used to enrich the *Artemia* prior to presenting them to the *S. splendidus*. The *T. gracilis* and *N. oculata* represent diets enriched for and containing ARA and EPA (Pratoomyot *et al*., 2005; Jaritkhuan & Chomrung, 2007; Praiboon *et al*., 2012), while the diet with *I. galbana* represents a diet enriched for and containing ARA and DHA (Chomrung *et al*., 2007; Praiboon *et al*., 2012). Consistent with this, the adult *Artemia*, representing large prey, and newly hatched *Artemia*, representing small prey, were assessed as live feeds presented to the F1 progeny from *S. splendidus* broodstock. It was anticipated that the basic information gathered from this trial regarding the utility of *Artemia* as a potential live feed will make a valuable contribution in developing protocols for the culture of captive bred green mandarinfish.

Methods

Source of the *S. splendidus* F1 progeny

Three pairs of wild broodstock originating from the Philippines were obtained from a commercial dealer and then maintained in 150 × 50 × 50 cm (375 L) glass aquaria under a natural photoperiod regime. The broodstock tanks were connected to a >70 m^3main recirculation water system within the research hatchery at the Institute of Marine Science Bangsaen, Burapha University; the water flow through these tanks was 6.0 L min^{-1}. The broodstock were maintained on a mixture of natural zooplankton and *Artemia*. Each evening, a fine plankton net was positioned next to the tank outlet so that eggs released overnight could be collected and then assessed the following morning. After egg hatching, the newly hatched F1 *S. splendidus* were transferred into 20 L glass

aquaria containing 33-34 ppt seawater. During the nursing period, the larval fish were fed rotifers and newly hatched *Artemia*, while live adult *Artemia* were fed to the juvenile fish.

Sources of *Artemia*

Adult *Artemia* (AA) were obtained from a local dealer three times each week and were maintained in a 100 L fibre glass tank containing 33-34 ppt seawater. Each batch of *Artemia* were fed a daily diet of ca. 1 g of dry *Spirulina* sp. and live *T. gracilis* at a density of 1.4×10^{3} - 2.0×10^{4} cells mL^{-1}. Prior to each feed presented to the *S. splendidus* broodstock (F1), the adult *Artemia* were enriched with mixtures of *T. gracilis* (3.5×10^{5} – 5.0×10^{5} cell mL^{-1}) and *I. galbana* (2.3×10^{6} - 2.4×10^{6} cell mL^{-1}) or with *T. gracilis* (3.5×10^{5} – 5.0×10^{5}cell mL^{-1}) and *N. oculata* (1.6×10^{6} – 3.6×10^{6}cell mL^{-1}) for a period of 1-3 h. *Artemia* cysts were bought from a local shrimp feed supplier and kept at -20°C until required. *Artemia* were hatched on a daily basis in 32 ppt seawater and the newly hatched *Artemia* (NHA) were used within 24 h. The NHA were enriched with mixtures of microalgae using the same methods used for the adult *Artemia*.

Experimental aquaria

The 45 × 120 × 50 cm experimental aquaria, which contained 270 L of 33-34 ppt seawater, were sub-divided into a rearing area, which measured 45 × 40 × 50 cm (90 L), and an egg collecting zone, which measured 45 × 80 × 50cm (180 L); both zones were aerated by air stones. Sand and artificial rocks were added to the rearing zone of each tank; 1L of plastic biomedia was used within a biological filter positioned in the egg collecting zone.

Experimental protocol

Twelve pairs of 14 month-old *S. splendidus* with a mean weight of 1.52 ± 0.22 g (females) to1.81 ± 0.33 g (males) and length of 3.61 ± 0.26 cm (females) to 3.82 ± 0.22 cm (males) were randomly assigned to the experimental aquaria. Three pairs of *S. splendidus* were fed enriched adult *Artemia* (AA) at 3 individuals (ind.) L^{-1} twice per day, a further three pairs were fed newly hatched *Artemia* (NHA) at 0.5 ind. mL^{-1} twice per day, while the remaining six pairs were fed a combination of enriched AA and NHA at ratios of 2 ind. AA L^{-1}: 0.5 ind. NHA mL^{-1} (3 pairs of fish) and 1 ind. AA L^{-1}: 0.5 ind. NHA mL^{-1} (3 pairs of fish). The trial ran for 9 months. Every evening, a 15 cm long ×15 cm diameter PVC pipe sealed with 50 µm mesh was positioned in the egg collecting zone of each aquarium so that any eggs released overnight could be collected and enumerated the following morning.

Throughout the trial, dead or uneaten *Artemia* from the bottom of the aquaria were siphoned daily and the sea water was then refilled to the previous level. Every two months, algae growing on the sides of the tanks were manually scrapped down and siphoned out prior to a 50% sea water change. Consistent with this, water quality was maintained within the following parameters: pH 8.2-8.7, temperature 25.5-27.7°C, salinity 32-33 ppt, alkalinity 93-140 mg^{-l}, ammonia-nitrogen < 0.08mg^{-l}, and nitrite-nitrogen 0.02-0.07mg^{-l}.

Proximal analysis

The proximate compositions of the NHA and the microalgae-enriched adult *Artemia* were determined following standard methods (AOAC, 2000) and the instrumentation protocols. In brief, the samples were placed in a freeze drier to obtain their dry weight. The crude protein was analysed using Kjeldahl analysis on a Tecator Kjeltec TM 2300 analyser (Foss, Warrington, UK), while the crude lipid content was extracted by petroleum ether (40-60^{o}C boiling point) using a Tecator Soxtec System 2050 Auto Extraction Apparatus (Foss, Warrington, UK). The total ash content of each species was determined by burning samples in a muffle furnace at 550^{o}C for 16 h. The nitrogen free extract (NFE) and fibre content of each sample was subsequently determined by subtracting the crude protein, crude lipid and ash values from 100%.

Total lipid extraction and fatty acid analysis

Total lipids were extracted from each *Artemia* sample following the methods detailed by Folch *et al.* (1957). Each 1 g dry sample was homogenised in 20 volumes of ice-cold chloroform:methanol (2:1, by vol.) containing 0.1 % butylated hydroxytoluene (BHT) and then left for 1 h. The liquid layer was transferred to a separating funnel and the residues were once again extracted using the same conditions detailed above and then by removing the liquid phase into the separating funnel. To separate the non-lipid phase, 0.88% (w/w) KCl (ca. 25% of the total volume) was added to the funnel, then agitated, and then left until the solution separated into two layers. Thereafter, the top phase was removed and the lower fraction was filtered through anhydrous sodium sulphate before evaporating the collected fraction (Folch *et al*., 1957). The sample was then placed in a desiccator for 1 h before it was weighed and then dissolved in chloroform:methanol (2:1, by vol.) containing 0.01% BHT to give a final concentration of 10 mg mL^{-1}. The samples were subsequently stored in brown vials at -40°C until required. Fatty acid methyl esters (FAME) were subsequently prepared from the total lipid samples by subjecting each to acid-catalysed transesterification by adding 1% sulphuric acid and then incubating them at 50^{o}C for 16 h (Christie, 1993) and quantifying them by gas-liquid chromatography (Agilent Technologies GC 7820A, USA). The individual FAMEs within each sample were identified by their comparison to known standards (Supelco 37-Component FAME Mix, Supelco, USA). The FAMEs were split injected (ratio) through a wall-coated capillary column (HP-Innowax column, 30 m × 0.25 mm id, 0.25µm film thickness, Agilent J & W, USA) and then detected by a flame ionisation detector (FID) at 250°C. Helium gas was used as the carrier at a constant flow rate of 1.1 mL min^{-1}. The temperature program used was an initial 150°C for 0.5 min, increasing to 170°C at a rate of 5°C min^{-1}, hold at 170°C for 10 min, after that increasing to 190°C at a rate of 3°C min^{-1}, and then hold at 190°C for 28 min. Temperatures at the injection and detection ports were 230°C and 250°C respectively.

Reproduction determination

Reproduction was determined from the mean age of the fish at each spawning, the number of spawning events, egg quantity and quality, and, the hatch rate (*i.e.* no. of pro-larvae obtained). Egg diameter and larval development was determined microscopically under an Olympus SZ30 stereo microscope at magnifications of ×1-×4. Egg quality was calculated using the following:

% of fertile eggs = no. of clear floating eggs ×100/ total no. of eggs from each spawning

% of unfertile eggs = no. of opaque sinking eggs ×100/ total no. of eggs from each spawning

Statistical analysis

All data are presented as the mean ± st. dev. Differences between samples were determined using a one-way analysis of variance (ANOVA) and Duncan's *post-hoc* test using SPSS 22 for Windows. Statistical significance was set at $p<0.05$.

Results and discussion

As filter feeders, the nutritional composition of adult *Artemia* and consequentially their offspring is influenced by the adult's diet (Lavens *et al*., 1989). In this study, the NHA were found to contain ca. 65% protein and 14% lipid while the adult *Artemia* contained 48 - 52% protein and 7 - 8% lipid (Table 1). The NHA have higher protein and lipid as this is derived from the maternal source deposited in eggs while the adult *Artemia* have already depleted the maternal source and rely on protein and lipid obtained from its diet. The Great Salt Lake nauplii and juveniles from super-intensive culture systems have levels of between 41.6 - 47.2% to 49.7 - 62.5% protein and lipid levels that range from 20.8 - 23.1% and 9.4 - 19.5% (Dhont & Lavens, 1996).

From the current study, the adult *Artemia* fed a mixture of *T. gracilis* and *N. oculata* had over 50% protein, while the *Artemia* fed the other mixtures contained almost 50% protein. The farmed *Artemia* and the *Artemia* fed the mixed microalgae had lipid contents of around 7 - 8% which were significantly ($p<0.05$) different from those fed freshwater *Spirulina*, which were determined to contain only 3% lipid (Table 1). The nutritional composition of the *Artemia* used in this study were in the same range as various *Artemia* meals, which typically have 40 - 60% protein and 8 - 20% lipid (Zarei, 2013). *Spirulina* is classified into the class Cyanophyceae, and cyanophytes including *Spirulina* do not contain high levels of fat (De Oliveira *et al*., 1999; Uslu *et al*., 2011). Cultured in a mineral medium under continuous light at temperature of between 20 - 40°C, *S. maxima* and *S. platensis* contain 5.97 - 7.3% and 6.32 - 7.24% lipid respectively (De Oliveira *et al*., 1999). As mentioned above, the nutrients content of adult *Artemia* is dependent on their diets, therefore, the low level of lipid found in the adult *Artemia* fed on freshwater *Spirulina* is due to low level of lipid in the freshwater *Spirulina*.

Table 1 Proximate composition of newly hatched *Artemia,* adult *Artemia* obtained from farms, *Artemia* fed *Spirulina* sp. and also *Artemia* fed a mixture of either *Tetraselmis gracilis* and *Nanochoropsis oculata* or a mixture of *T. gracilis* and *Isochrysis galbana*.

Proximate composition	NH *Artemia*	Farmed *Artemia*	Adult *Artemia* sp. fed on a variety of phytoplankton		
			Spirulina sp.	*T. gracilis*+*N. oculata*	*T. gracilis*+*I. galbana*
Protein	65.2 ± 0.2^{a}	47.7 ± 0.2^{cd}	48.2 ± 0.6^{c}	52.5 ± 0.1^{b}	47.2 ± 0.1^{d}
Lipid	14.1 ± 0.2^{a}	6.6 ± 0.2^{c}	3.4 ± 0.4^{d}	7.9 ± 0.1^{b}	7.9 ± 0.1^{b}
Ash	10.7 ± 0.2^{e}	20.6 ± 0.3^{a}	11.3 ± 0.4^{d}	13.1 ± 0.3^{c}	18.5 ± 0.2^{b}
NFE + fibre	11.0 ± 0.1^{d}	25.1 ± 0.3^{c}	37.0 ± 0.9^{a}	26.4 ± 0.2^{b}	26.4 ± 0.2^{b}

NFE= nitrogen free extract

The fatty acid (FA) composition of the NHA, the farmed adults and the adult *Artemia* that were enriched by feeding them mixtures of microalgae are shown in Table 2. The NHA contained comparatively higher quantities of the essential n-3 polyunsaturated fatty acid (PUFA) and PUFAs than monounsaturated fatty acids (MUFAs) and saturated FAs. In this study, the farmed adult *Artemia* and the *Artemia* fed on a mixture of *T. gracilis* and *N. oculata* contained 3% n-3 highly unsaturated FA (n-3 HUFA); much of which was represented by eicosapentaenoic acid (EPA, C20:5n-3). High levels of EPA were seen in the farmed *Artemia* and this is most likely because they are reared on a mixed diet consisting of microalgae, dry algae, protozoa, bacteria and various yeasts (Dhont & Lavens, 1996), while the *Artemia* fed the mixture of *T. gracilis* and *N. oculata* derive this EFA directly from both species of microalgae (Pratoomyot *et al*., 2005; Jaritkhuan & Chomrung, 2007; Praiboon *et al*., 2012). The *Artemia* fed the mixture of *T. gracilis* and *I. galbana* was found to contain lower quantities of EPA, a consequence of *I. galbana* containing little amounts or are lacking in this EFA (Chomrung *et al*., 2007), and so the EPA measured in this diet is derived principally from *T. gracilis.*

Table 2 The fatty acid compositions (% of total fatty acid) determined in samples of newly hatched *Artemia*, farmed adult *Artemia* sp. and in farmed *Artemia* sp. fed on two different mixtures of microalgae.

Fatty acids	Newly hatched *Artemia*	Farmed *Artemia*	Adult *Artemia* fed on microalgae		
			Spirulina sp.	*T. gracilis* + *N. oculata*	*T. gracilis*+ *I. galbana*
C14:0	0.62 ± 0.04 ^{d}	1.34 ± 0.06 ^{b}	0.83 ± 0.10 ^{b}	1.34 ± 0.07 ^{b}	2.08 ± 0.03 ^{a}
C16:0	10.05 ± 0.39 ^{c}	14.14 ± 0.20 ^{b}	14.39 ± 0.70 ^{b}	15.0 ± 0.23 ^{b}	16.36 ± 0.63 ^{a}
C18:0	4.92 ± 0.18 ^{c}	5.48 ± 0.03 ^{cb}	7.82 ± 0.64 ^{a}	6.25 ± 0.80 ^{b}	6.18 ± 0.23 ^{b}
C16:1n7	1.87 ± 0.07 ^{d}	0.15 ± 0.02 ^{e}	2.40 ± 0.14 ^{c}	4.59 ± 0.31 ^{a}	3.92 ± 0.12 ^{b}
C18:1n9	22.08 ± 0.51 ^{b}	22.65 ± 0.15 ^{b}	21.55 ± 0.78 ^{b}	25.40 ± 0.71 ^{a}	22.52 ± 0.73 ^{b}
C20:1n9	0.70 ± 0.13 ^{a}	0.55 ± 0.06 ^{b}	nd	nd	nd
C18:2n6	4.25 ± 0.22 ^{e}	12.46 ± 0.15 ^{a}	9.79 ± 0.73 ^{c}	7.62 ± 0.62 ^{d}	11.00 ± 0.20 ^{b}
C18:3n6	0.36 ± 0.02 ^{a}	0.22 ± 0.02 ^{b}	2.38 ± 0.20 ^{a}	0.37 ± 0.07 ^{a}	0.32 ± 0.06 ^{a}
C18:3n3	26.83 ± 0.89 ^{a}	8.32 ± 0.08 ^{c}	11.25 ± 0.48 ^{b}	7.87 ± 0.68 ^{c}	8.39 ± 0.27 ^{c}
C20:4n6	nd	1.76 ± 0.04 ^{a}	nd	1.13 ± 0.17 ^{b}	1.11 ± 0.05 ^{b}
C20:5n3	1.34 ± 0.05 ^{c}	3.23 ± 0.03 ^{a}	1.41 ± 0.11 ^{c}	3.16 ± 0.49 ^{a}	1.97 ± 0.15 ^{b}
C22:6n3	nd	nd	nd	nd	nd
Others	26.96 ± 2.17	29.70 ± 0.40	28.16 ± 2.74	27.27 ± 2.09	26.16 ± 1.22
SFA	15.36 ± 0.58 ^{c}	19.62 ± 0.23 ^{b}	22.22 ± 1.34 ^{a}	21.25 ± 1.00 ^{a}	22.54 ± 0.81 ^{a}
MUFA	25.36 ± 0.52 ^{c}	23.35 ± 0.13 ^{c}	23.96 ± 0.92 ^{c}	29.99 ± 1.00 ^{a}	26.44 ± 0.84 ^{b}
PUFA	32.79 ± 1.13 ^{a}	25.99 ± 0.28 ^{b}	24.83 ± 1.03 ^{bc}	20.16 ± 1.92 ^{d}	22.78 ± 0.63 ^{c}
n-3PUFA	28.18 ± 0.90 ^{a}	11.55 ± 0.09 ^{bc}	12.66 ± 0.52 ^{b}	11.03 ± 1.16 ^{c}	10.36 ± 0.42 ^{c}
n-3HUFA	1.34 ± 0.05 ^{c}	3.23 ± 0.03 ^{a}	1.41 ± 0.11 ^{c}	3.16 ± 0.49 ^{a}	1.97 ± 0.15 ^{b}

Data represent the mean ± st. dev., n=3. The C20:0, C22:0 and C22:1n9 content of the samples was less than 0.4% and are not shown. nd = not detected.

In the present study, a single pair of *S. splendidus* fed on marine microalgae-enriched *Artemia* given as 3 ind. L^{-1} twice per day began spawning after five months on this dietary regime and when the breeding pair of fish were aged 19 months old (female 3.0 g, 5.39 cm; male 3.7 g, 5.54 cm). The *S. splendidus* pair subsequently spawned 10 times over the period June through to September (Figure 1). Determining the age at which wild dragonets mature is difficult as this depends on the species and the sex of the fish (Chang, 1951; Gibson & Ezzi, 1979). It has been reported that *S. splendidus* mature once they exceed 30 mm in length (Sadovy *et al*., 2001)

which is smaller than the captive bred F1 progeny used in the present study, however, the age and size of fish may not be correlated especially when comparing wild and captive bred individuals. The number of eggs produced ranged from 48 to 253 eggs per batch, with an average 151.40 ± 70.91 eggs per batch; the eggs were 0.78 ± 0.02 mm in diameter; these figures agree with egg data gathered from individuals spawning in the wild, *i.e.*12 - 205 eggs; 0.7 - 0.8 mm in diameter (Sadovy *et al*., 2001). Green mandarinfish are partial spawners, releasing small batches of eggs on a regular basis. Eggs within the ovary develop at different rates, with small numbers of mature eggs only being released. This mode of egg release is different to total spawners where there is a synchronized development and release of mature eggs in a single batch (Miranda *et al.,* 1999). From Figure 1, it can be seen that there was gradual improvement in the number/proportion of fertilized eggs produced with each sequential spawning event irrespective of the number of eggs produced in a batch. It has been suggested from previous studies that the number of eggs produced in a batch and the proportion that are fertilized is influenced by nutrition (Carcupino, 2002; Foster & Vincent, 2004; Callan *et al*., 2014), and also by the age and quality of the broodstock, and, by egg and sperm quality (Wong & Benzie, 2003; Dzyuba *et al*., 2006; Fernández-Palacios, 2011; Callan *et al*., 2014).

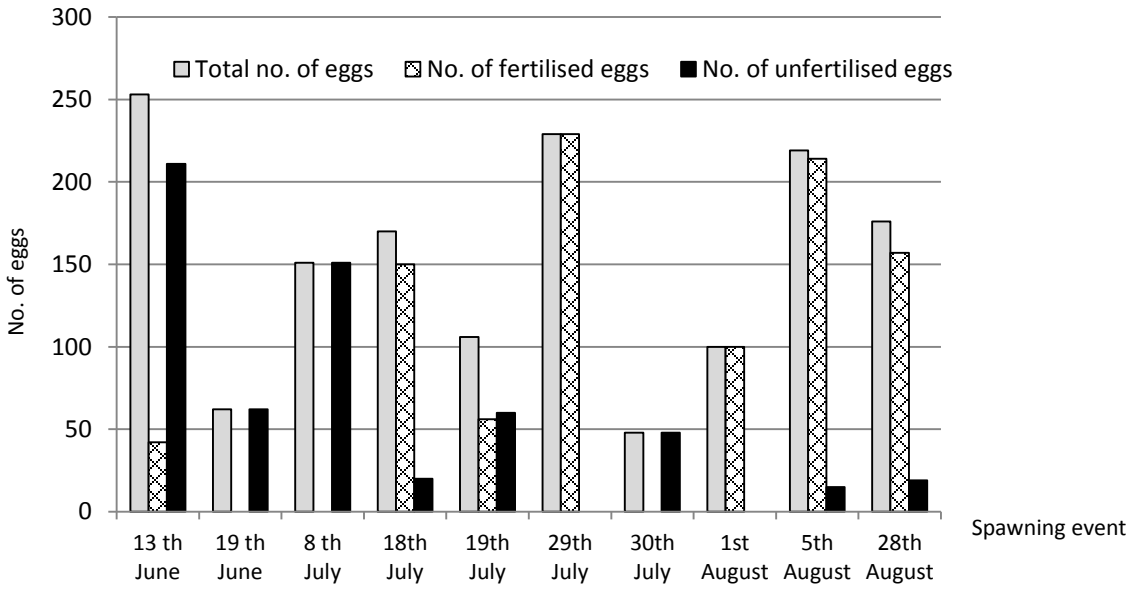

Figure 1 The total number and the quality of the eggs of *Synchiropus splendidus* obtained from ten separate spawning events that were reared on adult *Artemia* (3 ind. L^{-1}) twice per day that began after approximately five months on this feeding regime. The spawning data is from a single pair of fish aged 19 months old.

In studies looking at the nutrition of broodstock fish, apart from amino acids, vitamins and carotenoids, lipids are also commonly studied. Lipids not only provide energy for growth but also are a source of EFA required for the formation of cell membranes in marine organisms (Sargent *et al*., 2002). ARA and DHA are essential for the early development of the eggs and larvae from marine species *e.g.* European sea bass, *Dicentrachus labrax* (L.), (see Bruce *et al.,* 1999; Sorbera *et al.,* 2001). EPA, for example, is particularly important for the fertility of gilthead seabream, *Sparus aurata* L., broodstock (Fernández-Palacios *et al.,* 1995). Thus qualities of egg and larvae are dependent on nutrient delivery from the female (Izquierdo *et al.,* 2000, 2001) and from the broodstock diet (Izquierdo *et al.,* 2001). EFA requirements in fish are species dependent; Mediterranean fish species require between 0.64 and 2.2 % n-3 highly unsaturated fatty acid (n-3HUFA) in their broodstock diets (Izquierdo *et al.,* 2001). Optimum EFA levels are found around 0.6-2.3% for DHA, 0.7 - 2.3% for EPA and up to 1% for ARA (Izquierdo *et al.,* 2001). Specifically, the inclusion of 0.6% ARA dry weight (DW) in the broodstock diet was found to improve the egg quality of Japanese flounder, *Paralichthys olivaceus* (Temminck et Schlegel, 1846) (see Furuita *et al.,* 2003). Atlantic halibut, *Hippoglossus hippoglossus* (L.), fed on a diet containing 1.8% ARA of the total fatty acid content resulted in significantly higher fertilization and hatching rates when compared to a group of fish fed 0.4% ARA (Mazorra *et al.,*2003). Gilthead seabream broodstock require a minimum dietary DHA level of about 0.6% DW and EPA at 1.2% DW (Fernández-Palacios *et al.,* 1995). It is also important to consider the ratio between the polar lipids ARA, EPA and DHA and the competition between them, as an excess of one will displace the others, to the potential detriment of egg quality (Izquierdo *et al.,* 2000; 2001; Sargent *et al.,* 2002). In the present study, the enrichment of *Artemia* with a mixture of microalgae gave1% ARA and 2-3% EPA and the *S. splendidus* spawned. The results suggest that these levels may be appropriate for *S. splendidus* to spawn when they are ca. 19 months old. The absence of DHA in their diet, however, suggests that alternative methods of dietary enrichment must be sought to allow for its incorporation into diets given to captive reared *S. splendidus*.

Conclusions

Green mandarinfish are difficult to maintain in aquaria as they typically feed on a broad spectrum of zooplankton and as such, maintaining a continuous and varied supply of live feeds for their culture is a limiting factor to their broader commercial aquaculture. In the current trial, a pair of F1 progeny from captive bred *S. splendidus* broodstock, were fed adult *Artemia* enriched with microalgae for a period of 9 months during which the pair spawned ten times from June to September. The trial findings highlight that fish maintained on this diet are able to breed and suggests that adult *Artemia* enriched with various species of phytoplankton may serve as an alternative live feed for *S. splendidus* broodstock cultured in captivity.

Ethics statement

These experimental procedures were reviewed by and conducted under the approval of Burapha University's internal ethical review board (ethics project certificate ID#14/2555).

Acknowledgements

This work was financially supported by a research grant from the National Research Council of Thailand (Grant no. 149/2557) administered through Burapha University.

References

AOAC (2000). *Official Methods of Analysis* 17th ed. Association of Official Analytical Chemists International, Gaithersburg, Maryland.

Bruce, M., Oyen, F., Bell, J., Asturiano, J. F., Farndale, B., & Carillo, M. (1999). Development of broodstock diets for the European sea bass (*Dicentrarchus labrax*) with special emphasis on the importance of n-3 and n-6 highly unsaturated fatty acids to reproductive performance. *Aquaculture, 177*, 85–97.

Callan, C.K., Laidley, C.W., Kling, L.J., Breen, N.E., & Rhyne, A.L. (2014). The effects of dietary HUFA level on flame angelfish (*Centropyge loriculus*) spawning, egg quality and early larval characteristics. *Aquaculture Research, 45*, 1176–1186.

Carcupino, M., Baldacci, A., Mazzini, M., & Franzoi, P. (2002). Functional significance of the male brood pouch in the reproductive strategies of pipefishes and seahorses: a morphological and ultrastructural comparative study on three anatomically different pouches. *Journal of Fish Biology, 61*, 1465–1480.

Chang, H.W. (1951). Age and growth of *Callionymus lyra*L. *Journal of the Marine Biological Association of the United Kingdom, 30*, 281-296.

Chang, M., & Southgate, P.C. (2001). Effects of varying dietary fatty acid composition on growth and survival of seahorse, *Hippocampus* sp., juveniles. *Aquarium Sciences & Conservation, 3*, 205–214.

Chomrung, A., Pratoomyot, J., Srivilas, P., & Muthuwon, V. (2007). Effects of nitrogen concentrations on biochemical composition of cultured microalgae *Isochrysis galbana* growth and survival rate of anemonefish (*Amphiprion ephippium*). *Journal of Scientific Research Chulalongkorn University,* Section T (6) (special issue 1), 339-347.

Christie, W.W. (1993). Preparation of derivatives of fatty acids for chromatographic analysis. In W.W. Christie, (Ed.). *Advances in Lipid Methodology* Two (pp. 69-111). The Oily Press, U.K.

De Oliveira, M.A.C.L., Monteiro, M.P.C., Robbs, P.G., & Leite, S.G.F. (1999). Growth and chemical composition of *Spirulina maxima* and *Spirulina platensis* biomass at different temperatures. *Aquaculture International*, *7* 261–275.

Dhont, J., & Lavens, P. (1996). Tank production and use of on grown *Artemia*. In: P. Lavens, & P. Sorgeloos, (Eds.). Manual in the production and use of live food for aquaculture. *FAO Fisheries Technical Paper no 361*, 137-163.

Dzyuba, B., Van Look, K.J.W., Cliffe, A., Koldewey, H., & Holt, W.V. (2006). Effect of parental age and associated size on fecundity, growth and survival in the yellow seahorse, *Hippocampus kuda*. *Journal of Experimental Biology*, *209*, 3055–3061.

Fernández-Palacios, H., Izquierdo, M. S., Robaina, L., Valencia, A., Salhi, M., & Vergara, J. (1995). Effect of n-3 HUFA level in broodstock diets on egg quality of gilthead seabream (*Sparus aurata* L.). *Aquaculture*, *132*, 325-337.

Fernández-Palacios, H., Norberg, G., Izquierdo, L. M., & Hamre, K. (2011). Broodstock diets on eggs and larvae. In J. Holt. (Ed.). *Larval Fish Nutrition* (pp.153–183). Wiley-Blackwell, John Wiley and Sons, London, UK.

Folch, J., Lees, M. & Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, *226*, 497-509.

Forteath, N. (1997). The large bellied seahorse, *Hippocampus abdominalis*, a candidate for aquaculture. *Austasia Aquaculture, 11,* 52–54.

Foster, S.J., & Vincent, A.C.J. (2004). Life history and ecology of seahorses: implications for conservation and management. *Journal of Fish Biology*, *65*, 1–61.

Furuita, H., Yamamoto, T., Shima, T., Suzuki, N., & Takeuchi, T. (2003). Effect of arachidonic acid levels in broodstock diet on larval and egg quality of Japanese flounder *Paralichthys olivaceus*. *Aquaculture*, *220*, 725–735.

Gibson, R.N., & Ezzi, I.A. (1979). Aspects of the biology of the spotted dragonet *Callionymus maculates* Rafinesque-Schmaltz from the west coast of Scotland. *Journal of Fish Biology*, *15*, 555-569.

Izquierdo, M. (2005). Essential fatty acid requirements in Mediteranean fish species. In: D. Montero, B. Basurco, I. Nengas, M. Alexis, M. Uzquierdo (Eds.), *Mediterranean Fish Nutrition* (pp.91-101).Zaragoza:CHHEAM.

Izquierdo, M.S., Fernandez-Palacios, H., & Tacon, A.G.J. (2001). Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture*, *197*, 25-42.

Izquierdo, M.S., Socorro, J., Arantzamendi, L., & Hernandez-Xruz, C.M. (2000). Recent advances in lipid nutrition in fish larvae. *Fish Physiology and Biochemistry*, *22*, 97-107.

Jaritkhuan, S. & Chomrung, A. (2007). Effect of photoperiods on protein lipid and fatty acid content of *Nannochloropsis oculata*. *Journal of Scientific Research Chulalongkorn University,* Section T (6) (special issue 1), 331-338.

Lavens, P., Leger, P., & Sorgeloos, P. (1989). Manipulation of the fatty acid profile in *Artemia* offspring produced in intensive culture systems. In: N. De Pauw, E. Jaspers, H. Ackefors, N. Wilkins (Eds.). *Aquaculture - A Biotechnology in Progress* (pp 731-739). European Aquaculture Society, Bredene, Belgium.

Lieske, E., & Myers, R. (1994). *Collins Pocket Guide*. *Coral Reef Fishes*. Indo-Pacific & Caribbean including the Red Sea. Harper Collins Publishers.

Lin, Q., Gao, Y., Sheng, J., Chen, Q., Zhang, B., & Lu, J. (2007). The effects of food and the sum of effective temperature on the embryonic development of the seahorse, *Hippocampus kuda* Bleeker. *Aquaculture, 262* (12), 481–492.

Lin, Q., Lin, J., & Zhang, D. (2008). Breeding and juvenile culture of the lined seahorse, *Hippocampus erectus* Perry, 1810. *Aquaculture*, *277*, 287–292.

Lin, Q., Lin, J., Zhang, D., & Wang, Y. (2009). Weaning of juvenile seahorses *Hippocampus erectus* Perry, 1810 from live to frozen food. *Aquaculture*, *292*, 224–229.

Mazorra, C., Bruce, M., Bell, J.G., Davie, A., Alorend, E. & Jordan, N. (2003). Dietary lipid enhancement of broodstock reproductive performance and egg and larval quality in Atlantic halibut (*Hippoglossus hippoglossus). Aquaculture*, *227*, 21–33.

Migaud, H., Bell, G., Cabrita, E., McAndrew, B., Davie, A., Bobe, J., Herráez, M.P., & Carrillo, M. (2013). Gamete quality and broodstock management in temperate fish*. Reviews in Aquaculture*, *5 (Suppl. 1)*, S194–S223.

Miranda, A.C.L., Bazzoli, N., Rizzo, E., & Sato, Y. (1999). Ovarian follicular atresia in two teleost species: a histological and ultrastructural study. *Tissue & Cell, 31,* 480-488.

Otero-Ferrer, F., Molina, L., Socorro, J., Fernández-Palacios, H., & Izquierdo, M. (2012). Effect of different live prey on spawning quality of short-snouted seahorse, *Hippocampus hippocampus* (Linnaeus, 1758*). Journal of the World Aquaculture Society*, *43*, 174-186.

Praiboon, J., Kaewsuralikit, C., Tankitjanukit, S., & Jitchum, P. (2012). Fatty acid composition of five species of marine phytoplankton. In *The first Asian Marine Biology Symposium, Phuket. Program and Abstracts.* (p.96). Department of Marine Science, Faculty of Fisheries, Kasetsart University.

Pratoomyot, J., Srivilas, P., & Noiraksar, T. (2005). Fatty acids composition of 10 microalgal species. *Songklanakarin Journal of Science & Technology*, *27* (6), 1179-1187.

Randall, J.E., Allen, G.R., & Steene, R.C. (1990). *Fishes of the Great Barrier Reef and Coral Sea*. University of Hawaii Press, Honolulu, Hawaii.

Rhyne, A.L., & Tlusty, M.F. (2012). Trends in the marine aquatic trade: the influence of global economics and technology. *Aquaculture, Aquarium, Conservation & Legislation*, *5,* 99–102.

Sadovy, Y., Mitcheson, G., & Rasotto, M.B. (2001). Early development of the mandarinfish, *Synchiropus spendidus* (Callionymidae), with notes on its fishery and potential for aquaculture*. Aquarium Sciences &Conservation, 3*, 253-363.

Sargent, J.R., Tocher, D.R., & Bell, J.G. (2002). The lipids. In J.E. Halver, & R.W. Hardy (Eds). *Fish Nutrition*, 3rd. (pp. 181-257). Academic Press, San Diego, California.

Sorbera, L.A., Asturiano, J.F., Carrillo, M., & Zanuy, S. (2001). Effects of polyunsaturated fatty acids and prostaglandins on oocyte maturation in a marine teleost, the European sea bass (*Dicentrachus labrax*). *Biology of Reproduction, 64*, 382-389.

Uslu, L., Isik, O., Koç, K., & Göksan, T. (2011). The effects of nitrogen deficiencies on the lipid and protein contents of *Spirulina platensis*. *African Journal of Biotechnology*, 10 (3), 386-389.

Wong, J.M., & Benzie, J.A.H. (2003). The effects of temperature, *Artemia* enrichment, stocking density and light on the growth of juvenile seahorses, *Hippocampus whitei* (Bleeker, 1855), from Australia. *Aquaculture*, *228*, 107–121.

Woods, C.M.C. (2005). Growth of cultured seahorses (*Hippocampus abdominalis*) in relation to feed ratio. *Aquaculture International*, *13*, 305–314.

Woods, C.M.C., & Valentino, F. (2003). Frozen mysids as an alternative to live *Artemia* in culturing seahorses *Hippocampus abdominalis*. *Aquaculture Research*, *34*, 757–763.

Zarei, A. (2013). Chapter 20: *Artemia*, a new source of animal protein ingredient in poultry nutrition. In: M.D. Matovic (Ed.) Biomass now - sustainable growth and use. InTech, Croatia. pp. 503-510.