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Dietary fatty acid enrichment increases egg size and quality of yellow seahorse *Hippocampus kuda*

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ABSTRACT

Seahorses populations in the wild have been declining and to restore them a better knowledge of seahorse reproduction is required. This study examines the effect of dietary quality on seahorse fecundity and egg quality. Two different diets were tested with *Hippocampus kuda* females: frozen mysis (control) and frozen mysis enriched with a liposome spray containing essential fatty acids. Diets were given to females (two groups of five) over a seven week period. After this period, males (fed the control diet) and females were paired and the eggs dropped by the females were collected. Fatty acid profile were analysed and eggs were counted and measured. Results showed that females fed on enriched mysis had larger eggs and that these had a higher content of total polyunsaturated fatty acids. The size of the egg was especially affected in the first spawn, where egg size for females fed the enriched diet was significantly higher than the egg size from control females. This effect was reduced in the following spawning where no significant differences were found. Egg size is an important quality descriptor as seahorse juveniles originating from smaller eggs and/or eggs of poor quality will have less chances of overcoming adverse conditions in the wild and consequently have lower survival and growth rates. This study shows that enriching frozen mysis with polyunsaturated fatty acids increases egg size and egg quality of *H. kuda*.

1. Introduction

Seahorses are in great demand by the Chinese traditional medicine trade, and are attractive species for aquaria (Lourie et al., 1999) with the result that at least seven millions of seahorses are being traded globally each year (Evanson et al., 2011). Seahorse overexploitation, together with habitat degradation or loss, has led to a rapid decline of the natural stocks and some seahorse species have been included in the International Union for the Conservation of Nature red list as vulnerable species (Job et al., 2002). A better understanding of their reproductive biology and the development of effective breeding programmes is crucial for the creation of conservation strategies needed for the recovery of wild seahorse populations (Planas et al., 2010). For this reason, interest in seahorse aquaculture has been increasing (Payne and Rippingale, 2000), but the high mortality observed during the first weeks of life and the lack of knowledge of seahorse reproduction are still problems that need to be overcome (Woods, 2003).

The yellow seahorse, *Hippocampus kuda*, is a highly valued tropical Indo-Pacific seahorse species, which is widely distributed along the tropical Indo-Pacific region, from the Indian subcontinent in the west to the Pacific islands in the east (Lourie et al., 1999). It is generally found in shallow inshore habitats such as mangroves, seagrass beds and estuaries (Job et al., 2002). *H. kuda* generally reaches gonadal maturation at about 7 to 10 months of age (Lin et al., 2007), although younger males, 3–4 months old, have been observed to become pregnant and release viable

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offspring (Dzyuba et al., 2006). In order to achieve a successful spawning, it is necessary to provide a large amount of food, frozen mysis and/or live food, up to 15% of their body weight per day (Lin et al., 2007). This will not only improve survival and growth rates but will especially improve ovarian development of female seahorses (Forteeth, 1996; Chang and Southgate, 2001; Wong and Benzie, 2003). The quantity and quality of food is thought to play an important role in both female fecundity and number of spawns (Lin et al., 2007). Female seahorses are fractional spawners, releasing part of a post-vitellogenic clutch at each mating (Vincent, 1990). The ovaries are always active with oogenesis, vitellogenesis and atresia but egg hydration seems to occur after courtship begins and some hours prior to egg transfer (Boisseau, 1967). Once a female deposits her yolk-rich eggs into the male's brood pouch, fertilisation occurs (Van Look et al., 2007). Then embryos are implanted, the brooding tissues undergo cell differentiation and the epithelial structures enclose the embryos (Laksanawimol et al., 2006). During incubation, most energy required for embryonic development is thought to be derived from the mother (Boisseau, 1967) and the main role of the male is to protect the embryos and provide the gaseous exchange through a capillary network. Nevertheless it has been suggested there might be some nutrient transfer from the male to his offspring (Linton and Soloff, 1964; Foster and Vincent, 2004).Fatty acid composition of eggs is of major importance because lipid reserves are the main substrate for energy production during embryo development, but they are also essential structural components of biomembranes and precursors of eicosanoids (Sargent et al., 2002). The nutritional composition of broodstock diets, before and during the spawning period has been shown to affect egg chemical composition and quality (Fernandez-Palacios et al., 1995). This happens because most lipids stored in female's ovaries originate from the diet and/or endogenous storage (Almansa et al., 1999). For this reason, egg chemical composition is often used to evaluate egg quality as its fatty acid content must satisfy nutritional needs of the embryos (Bell et al., 1997). There is little information on egg composition and the effects of diet on the quality of seahorse eggs and more studies are required for the improvement of broodstock management.

The purpose of this study was to evaluate the quality of the eggs dropped by the females after being fed lipid-enriched and non-enriched mysis. Egg quality was analysed in terms of fatty acid content, size of the egg and egg viability.

2. Materials and methods

2.1. Husbandry and experimental set-up

This study was carried out at the Sea life London Aquarium during 2011 and 2012. 11 months old *H. kuda* (acquired from Simply seahorses, Norwich, UK) were kept in ten 120 L glass tanks (30 cm × 50 cm × 75 cm) working in a closed system. Before the individual males and females were put together to form couples, temperature was 22.7 ± 0.3 °C; it was slightly increased (24.2 ± 0.3 °C) once the couples were put together. Salinity was 33.5 ± 0.4 ppt, oxygen had a saturation of 99 ± 2% and water flow was kept at 1.2 L/min. Photoperiod was 12 L:12D.

At the beginning of the experimental trial, fish were not measured in order to avoid too much handling. Three months later, at the end of the trial females had an average weight of 5.4 ± 0.8 g and average length of 10.6 ± 1.1 cm in the enrichment treatment and 4.7 ± 0.4 g and 10.0 ± 0.7 cm in the non-enriched treatment. Males had an average weight of 7.5 ± 1.8 g and an average length of 11.1 ± 0.8 cm in the enriched group and 7.2 ± 2.0 g and 11.2 ± 1.2 cm in the non-enriched group.

2.2. Experimental design

During the first seven weeks of the experimental period, males and females were kept separately, in different tanks. A group of five females was divided between two tanks and was fed a control diet consisting of mysis (Gamma slice, Tropical Marine Centre, UK). A second group of five females, also divided between two tanks, was fed mysis enriched with a liposome mixture containing a supplement of fatty acids (New Era®, UK). Both groups had three meals of frozen mysis and one meal of adult live *Artemia* daily. The adult *Artemia* were not liposome-enriched. The male partners were all fed the control diet, non-enriched mysis, three times per day and a meal of live adult *Artemia*. Although the diet was given ad libitum, a daily average of 7.3 ± 2.9 g of mysis (11.3% body weight) was given to the enriched group (total for males and females) and an average of 6.1 ± 1.9 g of mysis (10.2% body weight) to the non-enriched group. Seven weeks after the experiment started, males and females were randomly paired to form couples. Each couple was then kept in a separate tank. From this point on, all fish were fed non-enriched mysis. Unfertilised egg clutches found in the tank were freshly collected (males were often seen with the eggs hanging in the pouch which after fell), rinsed in distilled water, measured using a stereo microscope and egg viability was determined (eggs were considered unviable if they were opaque or were empty). After being measured the eggs were stored in liquid nitrogen until the content in fatty acid was analysed. As seahorse eggs are ovoid and therefore possess large longitudinal and small transverse diameters, the egg sizes presented in this study represent the diameters measured along the longer axis.

2.3. Fatty acid analysis

Fatty acid compositions were measured essentially as described by Burdge et al. (2000) with some modifications. Briefly, samples (approximately 100 mg) were ground to powder under liquid nitrogen and total lipids were extracted with chloroform and methanol (Folch et al., 1957). Total lipid extracts were dried under nitrogen, dissolved in toluene and transesterified by heating in the presence of methanol containing 2% (v/v) sulphuric acid (Burdge et al., 2000). The resulting fatty acid methyl esters (FAMEs) were recovered by repeated extraction with hexane, dried under nitrogen and dissolved in hexane (120 μl). FAMEs were resolved on a BPX-70 fused silica capillary column (30 m × 250 μm × 0.25 μm) (SGE Europe Ltd.) using a

Table 1
Fatty acid composition (%) of female eggs of *Hippocampus kuda* fed enriched mysis (ENR) and non-enriched mysis (NE) in three different spawns (S1, S2 and S3).

FA	ENR			NE		
	S1	S2	S3	S1	S2	S3
14:0	1.53 ± 0.27	1.62 ± 0.16	1.51 ± 0.16	1.28 ± 0.09	1.60 ± 0.13	1.56 ± 0.16
16:0	18.89 ± 0.95	17.91 ± 2.03	20.00 ± 1.27	20.80 ± 3.58	18.15 ± 1.14	18.65 ± 1.76
18:0	4.85 ± 0.44	4.39 ± 0.44	4.74 ± 0.36	5.51 ± 0.74	4.69 ± 0.46	4.85 ± 0.64
20:0	0.26 ± 0.04a	0.24 ± 0.02[a]	0.25 ± 0.02a	0.33 ± 0.06b	0.26 ± 0.04a	0.25 ± 0.03a
22:0	0.15 ± 0.07	0.16 ± 0.04	0.24 ± 0.14	0.21 ± 0.05	0.22 ± 0.04	0.16 ± 0.06
24:0	0.78 ± 0.07ab	0.68 ± 0.13a	0.71 ± 0.05a	0.88 ± 0.08b	0.67 ± 0.07a	0.72 ± 0.07a
Total SFA	26.48 ± 0.70	25.28 ± 1.93	27.44 ± 1.39	29.02 ± 4.20	25.59 ± 1.56	26.19 ± 2.31
14:1	0.48 ± 0.08	0.51 ± 0.09	0.46 ± 0.12	0.39 ± 0.20	0.59 ± 0.14	0.67 ± 0.25
15:1	0.22 ± 0.03	0.22 ± 0.04	0.26 ± 0.09	0.19 ± 0.08	0.26 ± 0.06	0.28 ± 0.04
16:1n-7	4.50 ± 0.85	4.78 ± 0.70	4.66 ± 0.68	3.93 ± 0.47	5.18 ± 0.32	5.12 ± 0.50
18:1n-9	11.38 ± 0.73	11.34 ± 1.06	11.43 ± 1.31	10.52 ± 1.44	12.50 ± 1.36	13.13 ± 1.60
18:1n-7	4.79 ± 0.36	4.71 ± 0.47	4.85 ± 0.41	4.30 ± 0.72	5.11 ± 0.63	5.28 ± 0.18
20:1n-9	0.48 ± 0.04	0.45 ± 0.06	0.60 ± 0.22	0.46 ± 0.02	0.44 ± 0.04	0.46 ± 0.03
Total MUFA	21.85 ± 1.12ab	22.00 ± 1.51ab	22.24 ± 1.95ab	19.78 ± 2.50b	24.08 ± 2.09a	24.95 ± 2.50a
18:2n-6	4.03 ± 0.64	4.25 ± 0.66	3.98 ± 0.97	3.30 ± 1.53	4.88 ± 1.03	5.52 ± 1.10
18:3n-6	0.61 ± 0.07	0.59 ± 0.18	0.43 ± 0.39	0.51 ± 0.47	0.77 ± 0.19	0.85 ± 0.10
18:3n-3	1.64 ± 0.15	2.65 ± 1.65	1.62 ± 0.17	1.52 ± 0.26	1.86 ± 0.22	1.75 ± 0.35
18:4n-3	1.47 ± 0.37	1.73 ± 0.43	1.64 ± 0.04	1.11 ± 0.24	1.71 ± 0.31	1.45 ± 0.11
20:3n-6	0.19 ± 0.02	0.20 ± 0.05	0.24 ± 0.09	0.51 ± 0.58	0.19 ± 0.02	0.23 ± 0.04
20:4n-6	1.86 ± 0.36	1.59 ± 0.31	1.57 ± 0.18	1.94 ± 0.45	1.78 ± 0.21	1.83 ± 0.05
20:4n-3	0.55 ± 0.10	0.65 ± 0.14	0.62 ± 0.08	0.51 ± 0.06	0.61 ± 0.11	0.55 ± 0.06
20:5n-3	12.11 ± 0.79	13.81 ± 1.64	12.04 ± 0.62	12.69 ± 0.77	12.73 ± 1.40	11.97 ± 0.28
22:5n-3	1.70 ± 0.59	2.51 ± 1.79	2.14 ± 0.27	1.55 ± 0.51	1.47 ± 0.57	1.56 ± 0.44
22:6n-3	21.92 ± 2.81	21.42 ± 2.86	22.38 ± 1.32	23.97 ± 3.01	20.70 ± 1.56	19.26 ± 2.19
Total PUFA	48.16 ± 1.62ab	49.40 ± 2.42a	46.64 ± 1.04ab	47.60 ± 1.59ab	46.70 ± 2.12ab	44.95 ± 1.04b
Sn-3	41.46 ± 1.72ab	42.77 ± 3.04a	40.43 ± 2.14ab	41.35 ± 2.30ab	39.08 ± 3.34ab	36.53 ± 1.96b
Sn-6	6.70 ± 1.00	6.62 ± 1.09	6.22 ± 1.29	6.25 ± 2.71	7.62 ± 1.36	8.42 ± 1.26
Sn-3/Sn-6	6.32 ± 1.14	6.63 ± 1.41	6.73 ± 1.64	7.81 ± 3.73	5.35 ± 1.65	4.42 ± 0.81
DHA/EPA	1.81 ± 0.19	1.58 ± 0.33	1.86 ± 0.04	1.90 ± 0.34	1.64 ± 0.18	1.61 ± 0.21
EPA/AA	6.79 ± 1.78	9.12 ± 2.82	7.76 ± 1.35	6.78 ± 1.31	7.28 ± 1.67	6.54 ± 0.14

SFA, saturated fatty acid; MUFA, mono-unsaturated fatty acid and PUFA, poly-unsaturated fatty acids. S, sum; DHA, docohexaenoic acid; EPA, eicosapentaenoic acid; ARA, arachidonic acid. Different letters represent significant differences for each fatty acid for a $p < 0.05$).

stepwise temperature gradient on a HP6890 gas chromatograph equipped with flame ionisation detection (Burdge et al., 2000). FAMEs were identified by their retention times relative to commercial standards (Sigma) and quantified by integration of the area under the peak using Chemstation software (Agilent).

2.4. Data analysis

Differences between egg sizes from the two treatments and their three spawnings were determined using two separate analyses of variance (ANOVAs) with Statistica© (v10; Statsoft UK). Initially a repeated measures ANOVA was used to compare treatment effects across the three different spawning (i.e. Treatment × Spawning interaction). Once it was shown that the treatment effect was only apparent in the first spawning, a "customised" nested ANOVA was used to verify the result and to avoid pseudoreplication. In this ANOVA eggs were "nested" within the females, and females (replicates) were "nested" within treatments.

The overall effect of treatments on the number of eggs produced was examined using the Mann–Whitney *U*-test. Significant differences in egg viability were also calculated using the non-parametric Kruskal–Wallis. To determine the significant differences in the fatty acid profiles a Kruskal–Wallis non-parametric ANOVA was used for 22:0, 15:1, 18:1n-7, 18:3n-6, 18:3n-3, 18:4n-3, 20:3n-6 and 22:5n-3. For the remaining fatty acids a two-way ANOVA was used (for 20:0 and 20:4n-3 an outlier had to be removed to fulfil the parametric ANOVA assumptions). Post hoc analysis was undertaken using Newman–Keuls test.

3. Results

3.1. Behaviour

Seahorses are sedentary animals as they spend most of their time attaching themselves to a plant. In this study, when the fish were fed they generally showed immediate interest but usually tried to reach the feed, stretching themselves, and avoiding letting go of the holdfast. Sometimes, but not often, they swam in the water column to catch the feed. This happened more commonly when they were fed the adult *Artemia*. Their most interesting behaviour happened during courtship. Courtship consisted of several complex movements which involved the male persistently following the female, and grabbing her by the tail or trunk. While doing this the male opened and closed its pouch, contracting its abdomen and moving forward and backwards with his trunk. This way the male showed the female he had an empty pouch ready to receive her eggs. Courtship lasted two or three days and ended either with a pregnant

male or with eggs dropped in the tank. Once pregnant the males became very inactive and reduced their uptake of feed. In this study there was only one pregnant male which produced offspring and the pregnancy lasted 12 days. Most pregnant males aborted or absorbed their eggs at some point during pregnancy.

3.2. Periodicity of spawning

During this study 24 clutches of eggs were collected (12 from each treatment). Females fed the control diet dropped a total of 571 eggs and females fed the enriched diet dropped a total of 744 eggs. Females dropped the eggs either when trying to transfer the eggs to the males or without any apparent relationship with the male. On the first spawning, all females from both treatments dropped eggs. On the second spawning, all females fed the enriched diet and 80% of the females fed the non-enriched diet dropped eggs. On the third spawning only 50% of females dropped eggs.

Eggs from *H. kuda* fed the enriched mysis were mainly collected between 2 pm and 5 pm (Fig. 1). On the first and third spawning all the eggs were collected between 2 pm and 5 pm. On the third spawning 33% of the eggs were collected between 10 am. and 2 pm (Fig. 1).

From females fed the non-enriched mysis no eggs were ever collected between 10 am and 2 pm (Fig. 1). On the first spawning 60% of the eggs were collected in the morning, whereas in the third spawn 60% were collected between 2 pm and 5 pm. On the second spawning half of the eggs were collected in the morning and the other half between 2 pm and 5 pm (Fig. 1).

The most frequent interval between clutches by the same fish was 17 and 18 days and less frequently 19 days (Fig. 2). There were two clutches which were 36 and 37 days apart (Fig. 2).

3.3. Fecundity, egg viability and size

The number of eggs dropped by the females varied considerably. The average fecundities for females fed the enriched and non-enriched mysis were 62 ± 38 eggs and 48 ± 25 eggs respectively (Mann–Whitney *U*-test; $p = 0.77$). The maximum number of eggs found in the tanks was 120 and the minimum was 10 eggs.

Egg viability varied considerably in all clutches and there were no significant differences between treatments (Fig. 3).

The eggs produced before 10 am (the earliest time they could be observed) had the lowest viability, which was never higher than 20% (Fig. 4). When eggs were collected between 10 am and 2 pm the viability was 40% (just one clutch). When eggs were collected between 2 pm and 5 pm their viability was higher than 60% in the enriched group and higher than 50% in the non-enriched group (Fig. 4).

The minimum egg size found in this study was 1.36 mm and was collected in the first spawning from a female fed the non-enriched mysis. The maximum size was 3.82 mm and was from the first spawning of a female fed the enriched mysis. Eggs originating from females fed the enriched diet were significantly larger in the first spawning compared with eggs from females fed the control diet ($F_{1/4} = 10.13$, $p = 0.033$) (Fig. 5).

3.4. Fatty acid profiles of eggs

The fatty acid composition of the eggs showed significant differences for 22:0 ($F_{2,18} = 4.27$, $p = 0.03$) where the content of this FA in the first spawning of the non-enriched treatment was significantly higher compared to all other spawnings. The content of 24:0 in the first spawn of the non-enriched treatment was significantly higher ($F_{2,18} = 8.21$, $p = 0.003$), except for the first spawning of the enriched treatment. The total mono-unsaturated fatty acids (MUFA) level in the eggs was significantly lower in the first spawning of the non-enriched group compared to the other two spawnings of the same group ($F_{2,18} = 4.70$, $p = 0.02$). There were also differences for the interaction between treatment and spawnings ($F_{2,18} = 3.71$, $p = 0.04$). The 18:3n-3 was also significantly different between spawns ($F_{2,18} = 1.77$, $p = 0.03$), and the total poly-unsaturated fatty acids (PUFA) content of the eggs showed significant differences between the enriched and non-enriched treatments ($F_{2,18} = 4.74$, $p = 0.04$) where the first had a significantly higher content. The sum of the n-3 fatty acid was significantly higher in the group fed the enriched mysis ($F_{1,18} = 5.93$, $p = 0.03$) (Table 1).

4. Discussion

There is limited information on seahorse reproduction and little published information on *H. kuda* eggs. 24 clutches were collected during this study, 83% of which had a periodicity of 17–19 days. The other time interval observed between spawns was 36 and 37 days, which happened when a female skipped a spawn. In reality, 36 is, in fact, 2×18 days. This periodicity of clutches has been reported before for *Hippocampus guttulatus* (Planas et al., 2010), which was found to be approximately 27 days at 20 °C (Planas et al., 2010). The difference between the clutch interval for *H. kuda* and *H. guttulatus* could be related to the temperature, as Planas et al. (2010) showed that the periodicity decreases with a rise of temperature, and *H. kuda* was reared at 25 °C. However, this difference may be explained by differences in the duration of pregnancy in the two species. In this study *H. kuda* pregnancy lasted only 12 days whereas in *H. guttulatus*, male pregnancy usually lasts between 3 and 4 weeks. When the male releases the offspring, it is immediately available to become pregnant again, meaning that the female must be ready to release the eggs earlier.

Another factor which did not seem to occur randomly was the time of the day when females dropped their eggs. In the enriched treatment, females most commonly dropped their egg between 2 pm and 5 pm, whereas in the non-enriched treatment it either happened in the morning or in the afternoon but rarely between 10 am and 2 pm. This is also interesting as in the wild the "seahorse dance" occurs at dawn, during which time the female transfers her eggs to the male (Vincent and Sadler, 1995).

Egg viability varied considerably between spawnings and between treatments. The minimum egg viability was

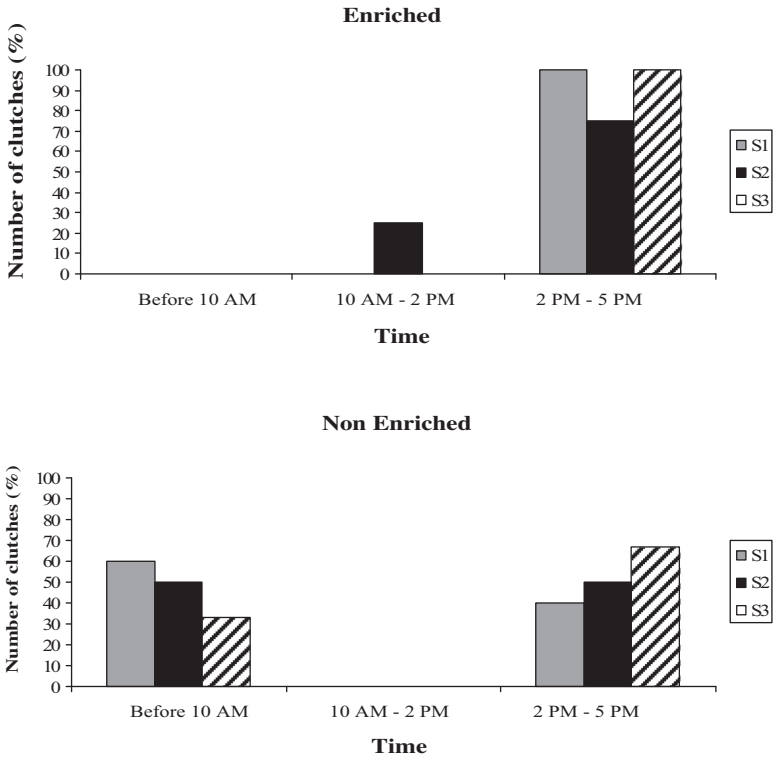

Fig. 1. Time of day that clutches from *Hippocampus kuda* were collected from the tanks. S1, S2 and S3 correspond to the first, second and spawn clutches from the same fish.

observed in two spawns of the non-enriched group (5%) and egg viability of 100% was observed in spawns of both treatments. Generally, females which dropped poor quality eggs in the first spawn did not show an improvement in eggs quality in the following spawns. On the contrary, females which had good quality in the first spawn either kept or decreased egg quality (never below 20%) on the following spawns. The variability in egg viability was so high that no significant differences were found, meaning that the enrichment did not improve the egg quality in terms of the number of viable eggs. This fluctuation in egg quality may explain why sometimes it is so difficult to breed seahorses. A male aborting the eggs was often observed during this study, possibly because of the poor quality of the eggs transferred from the female. The eggs found in the morning always showed lower viability, which could have been due to the amount of time in the tank before collection.

Female fecundity was considerably lower compared to other studies (Lin et al., 2006; Faleiro and Narciso, 2010; Planas et al., 2010) with other species of Hippocampus that are able to produce several hundreds of eggs at once. This is because the *H. kuda* used in this study were small in relation to other Hippocampus species and the fact that they were very young (6–12 months). Moreover, there are several sub-species of *H. kuda* and it is

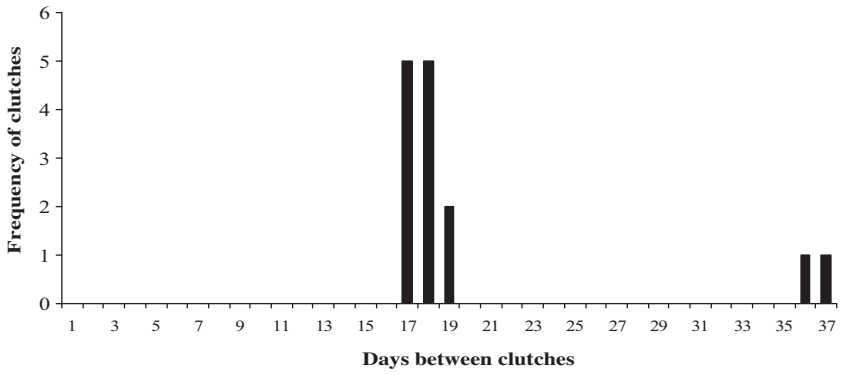

Fig. 2. Periodicity (days) of clutches by same *Hippocampus kuda* female.

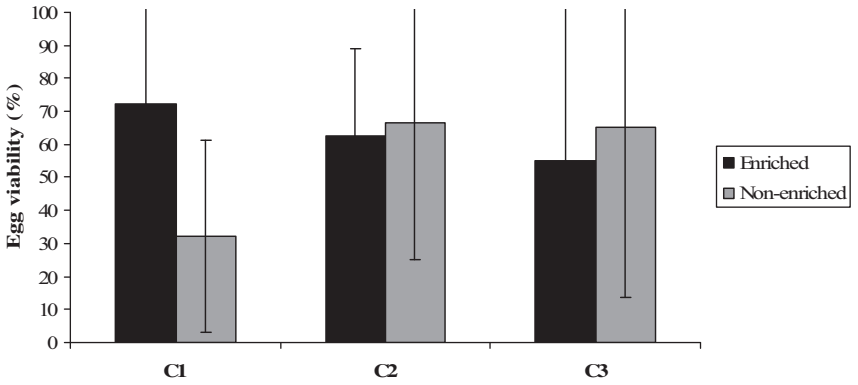

Fig. 3. Viability of eggs from *Hippocampus kuda* female fed enriched mysis and non-enriched mysis. C1, C2 and C3 correspond to the first, second and third clutch from the same fish. Values are mean and standard deviation.

believed that the species used here was a dwarf kuda. A new classification of the several *H. kuda* sub-species would improve the identification of different individuals. The size of seahorse eggs varies considerably. The most common size of egg was between 2.2 and 2.9 mm, although a minimum of 1.36 mm and a maximum 3.82 mm were found in this study. This variability in egg size was also found for *H. guttulatus* (Faleiro and Narciso, 2010; Planas et al., 2010). The average egg size observed across the whole study (2.77 ± 0.10 mm in the enriched treatment and 2.61 ± 0.33 mm for the non-enriched) was higher than those reported for *H. guttulatus* (2.44 ± 0.37 mm (Faleiro and Narciso, 2010) and 2.49 ± 0.16 mm (Planas et al., 2010). Seahorse eggs have a pear shape and are distinct from the typical spherical eggs of other marine fish (Foster and Vincent, 2004). The elongated shape is thought to increase the egg surface for gas exchange when incubating in the male's pouch (Carcupino et al., 2002). The larger dimension of seahorse eggs is related to the high amount of yolk (Foster and Vincent, 2004; Faleiro and Narciso, 2010; Planas et al., 2010). Reports from the seahorse trade in Asia and Africa over the last decade have mentioned a significant decline in seahorse catches and in the size of the individuals caught (McPherson. and Vincent, 2004; Perry et al., 2010).

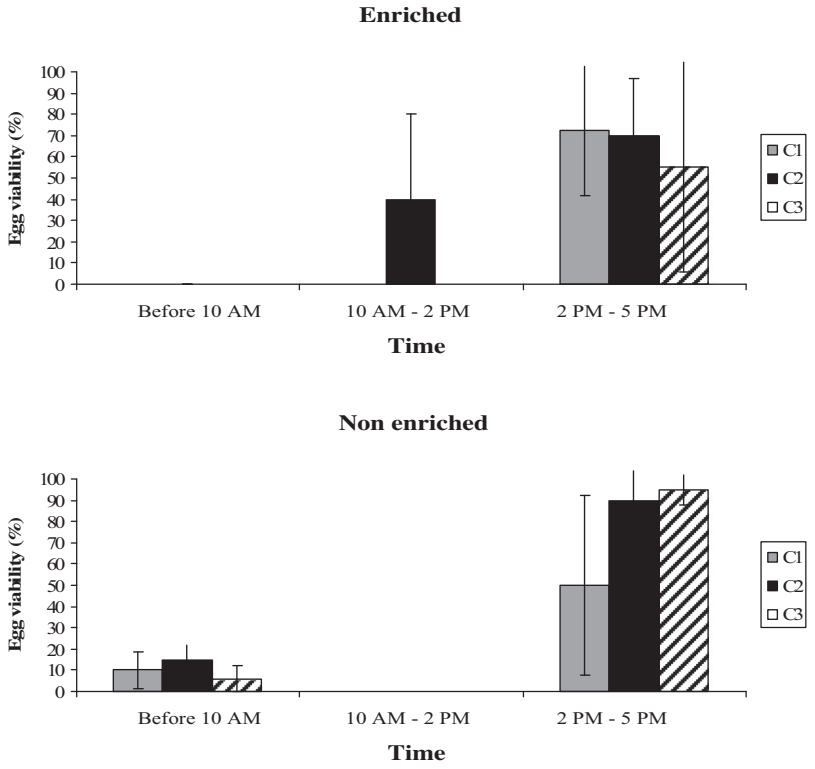

Fig. 4. *Hippocampus kuda* unfertilised eggs viability according to the time of collection. C1, C2 and C3 correspond to the first, second and third clutches from the same fish.

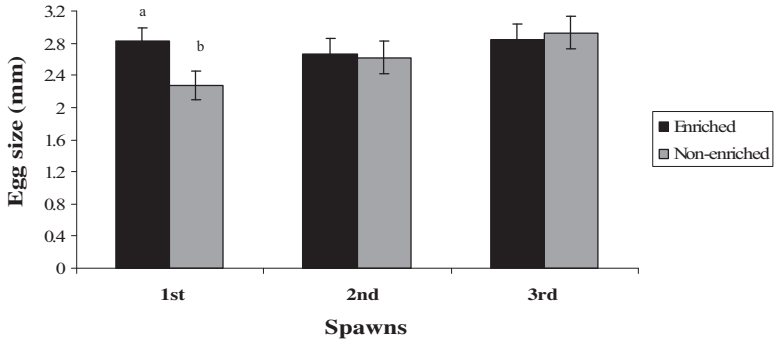

Fig. 5. *Hippocampus kuda* average egg size from females fed enriched mysis (E) and non-enriched mysis (NE) in the first, second and third spawning. Values are mean and standard error of the mean. Different letters represent significant differences ($p < 0.05$).

This decline is probably due to overexploitation but, most importantly, the destruction of their shallow coastal habitats. Seagrass beds, mangroves and coral reefs are amongst the most threatened habitats in the world (McPherson. and Vincent, 2004). Considering that the destruction of habitats will probably affect seahorse prey availability it is possible that wild seahorses may have a reduced feed diversity and that feed imbalances may occur. These nutritional imbalances may be reflected in poorer egg quality and smaller eggs. Seahorse development is dependent upon the high nutritional components of the eggs as they are release as juveniles and they need the yolk reserve to last for a longer period compared to other marine fish species which hatch as larvae (Planas et al., 2010). If the eggs yolk does not provide enough to fulfil the embryo's nutritional need then smaller juveniles will be released, decreasing not only their chances of overcoming more adverse environmental conditions but also their survival and growth in the wild (Foster and Vincent, 2004). In this study the size of the egg originating from the enriched females was significantly higher in the first spawn compared to the eggs from females fed the non-enriched mysis, suggesting a higher yolk content according to Faleiro and Narciso (2010). The fact that this increase was not observed in the second and third spawn may suggest that the enrichment given to the females prior to spawning was insufficient to last more than one spawning and that the effect of enrichment was thereby reduced in the second and third spawnings. Also, the number of females dropping eggs in the second and third spawning was lower (five in the first, four in the second and three in the third) and therefore fewer eggs were observed. The size of the eggs from females fed enriched mysis was relatively constant in the three spawns and significantly increased in the females fed the non-enriched mysis. This is probably because the females which had lowest egg quality only spawned twice so, in reality, there was no increase in the size of the eggs. The absence of the smallest eggs originating from poor quality clutches thus probably explains this effect. This is consistent with the viability results which also increased in the two last spawns in the non-enriched treatment.

The nutritional requirements of seahorses are still largely unknown (Wong and Benzie, 2003) but it has been suggested that they need a food input up to 15% of their body weight per day (Forteeth, 1996) (in this study approximately 11% body weight was given to the fish daily) and considerable amounts of live feed. Copepods and live mysis are usually considered to be the most suitable feed for seahorses due to their higher content of PUFA, but they are difficult to produce on a large scale and their source is unreliable. For this reason, the most common feed given to seahorses are frozen mysis and live Artemia as they are easier to produce or obtain commercially (Payne and Rippingale, 2000). As shown in this study, fish fed frozen mysis without any enrichment had a higher content of some SFA (22:0 and 24:0). However, when the enrichment was added to the diet some improvements were registered and a better fatty acid profile of the eggs was obtained. The eggs from females fed the enriched diet showed a higher content of PUFA and the sum of n-3 fatty acids was also higher. This is the first study with describes the fatty acid profiles of *H. kuda*. Faleiro and Narciso (2010) and Planas et al. (2010) have some of the few published works reporting information on fatty acid profiles of seahorse eggs. Those two studies were undertaken using *H. guttulatus*, which is a temperate marine species. The egg profiles obtained in the present study showed a lower content of SFA and MUFA for *H. kuda eggs* compared to *H. guttulatus* (data from Faleiro and Narciso, 2010 and Planas et al., 2010). On the contrary, the content of PUFA is higher, especially the percentage of DHA, which is three times higher than the one stated by Planas et al. (2010). The differences obtained for these two species are probably due to the diet, as in both studies *H. guttulatus* were fed on Adult Artemia whereas *H. Kuda* were fed on mysis and one meal of live adult Artemia. According to Woods (2003) frozen mysis is a more suitable diet to seahorse and this is reflected on the fatty acid profile of the eggs.

In conclusion, *H. kuda* females fed on mysis alone showed relatively poor egg quality with lower PUFA contents and a general poor egg viability, which will probably have an impact on embryo development and offspring survival. However, when females were given a supplement or enrichment together with the mysis the egg size increased and the egg quality was improved. In this study the females were given the enriched mysis over seven weeks, but if the enrichment were given on a regular basis, better results would probably be achieved as egg size was higher in females fed the enriched diet in the first spawning but not in the second or third spawning. The

results obtained in this study are also relevant in terms of explaining how the destruction of seahorse habitats might affect populations. It is not enough to have conservation strategies for seahorses alone. It is crucial to have a balanced ecosystem that provides conditions for the continuity of the species.

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