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Iron content in common Cambodian fish species: Perspectives for dietary iron intake in poor, rural households

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Abstract

Iron deficiency is the most widespread nutritional disorder in Cambodia. Sixteen Cambodian fish species were screened for iron, zinc and calcium contents. *Esomus longimanus* has a higher iron content (451 mg Fe/kg dry matter, SD = 155, $n = 4$) than other species. Iron pools were measured as inorganic iron (I-Fe) by the ferrozine method, haem-bound iron (H-Fe) by the acetone method and total iron (T-Fe) by atomic absorption spectrometry. I-Fe + H-Fe accounted for <50% of T-Fe in *E. longimanus*, indicating a pool of complex bound, probably high-molecular weight non-haem iron (Hm-Fe). In a field study, thirty rural women were interviewed about traditional use of *E. longimanus*; their cleaning and cooking practices were observed and the amounts of fish consumed were recorded and meal samples were collected for iron analysis. Calculations based on the iron content and a high bioavailability of Hm-Fe showed that a traditional fish meal, sour soup, covered 45% of the daily iron requirement for women.

Keywords: Fish; Dietary iron; Zinc; Calcium; Developing countries

1. Introduction

1.1. Micronutrient malnutrition in Cambodia

Cambodia is among the poorest countries in the world and the population is highly vulnerable to malnutrition and poor health, affecting especially women and children (SCN, 2004). Iron deficiency is the most widespread nutritional disorder in Cambodia (Connoly, Panagides, & Bloem, 2001). It is estimated that more that 70% of pregnant women and 74% of children under five years of age suffer from anaemia, mainly caused by iron deficiency (Connoly et al., 2001). Iron deficiency in children causes retarded growth and impaired cognitive development (Moy, 2006) and, in women, has serious impact on health and morbidity. The high prevalence of iron deficiency is therefore regarded as a direct constraint to the overall development in Cambodia (HKI, 1999). In addition to iron deficiency, poor population groups in Cambodia are vulnerable to deficiencies of other micronutrients, such as vitamin A, zinc and calcium (SCN, 2004). Interventions to improve child nutrition in Cambodia only reach a fraction of the poorest segments of the population. Data from ten selected developing countries showed that Cambodian children have the lowest rate of coverage for receiving child-surviving health and nutrition interventions, including micronutrient supplementation (Victora, Fenn, Bryce, & Kirkwood, 2005). The majority of rural households rely on their immediate environment to provide dietary diversity to satisfy micronutrient needs (Halwart, 2006). Thus, to alleviate micronutrient malnutrition in Cambodia, the

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development of simple recommendations, using local foods to enhance the content of bioavailable micronutrients in the diet, is important, in order to reach the most vulnerable population groups.

1.2. Food-based strategies to alleviate micronutrient malnutrition

Food-based strategies to enhance the intake of micronutrients are recognised as the long-term sustainable solution to micronutrient deficiencies in developing countries (Demment, Young, & Sensenig, 2003). Increasing the intake of animal foods, particularly meat, is important for improving iron status (Bæch et al., 2003; Demment et al., 2003). In addition to supplying specific nutrients, animal foods as a whole stimulate physical growth (Grillenberger et al., 2006) and cognitive development in children (Whaley et al., 2003).

Fish plays a fundamental role for the livelihood, income and food security for large population groups in the densely populated Mekong river basin. More than 500 freshwater fish species are found in Cambodia (Rainboth, 1996), and fish from floodplains and rivers is a basic food in the daily diets for millions of people (Van Zalinge, Thouk, Tana, & Loeung, 2000). Small fish species are generally less preferred than large species and therefore have a low market value and are more accessible to the poor. Small, low-valued fish species are therefore likely to be the main or only animal food in the diet of the Cambodian population groups vulnerable to micronutrient deficiencies and therefore of particular interest in food-based strategies for improved nutrition.

In Bangladesh, where fish play a similar dietary role, it was found that small, indigenous fish species are an important source of vitamin A and calcium in poor, rural households, contributing 40% and 31%, respectively, to the total recommended intake on household level in the peak fish production season, while the estimated contribution, from fish, to the recommended intake of iron was 9%, based on assumptions of bioavailability (Roos, Islam, & Thilsted, 2003). Similar estimates of the dietary contribution of specific nutrients from fish in the Cambodian diet are not available.

1.3. Iron compounds in food

In food composition analysis, the content of iron is commonly reported as haem- and non-haem iron (Kongkschuichai, Napatthalung, & Charoensiri, 2002; Lombardi-Boccia, Martinez-Dominguez, & Aguzzi, 2002). In addition to the total amount of iron present in a food, the nutritional value of a dietary iron source will depend mainly on the bioavailability. Non-haem iron, normally with a low bioavailability, is determined as iron soluble, in acidic conditions and detected by a colorometric method with ferrozine after reduction to ferrous iron. However, the ferrozine method does not include the determination of ferritin, a sub-group of the non-haem iron pool with a relatively high bioavailability (Lonnerdal, Bryant, Liu, & Theil, 2006). The present compartmentalization of Fe in foods therefore needs to be adjusted to take into account the heterogeneity of the non-haem iron pool.

1.4. Aim of this study

The purpose of the first phase of the present study was to screen common Cambodian fish species for the contents of iron, zinc and calcium, in order to identify nutrient-dense fish species of potential importance in food-based strategies to improve the nutritional status in Cambodia. Based on the screening, the species *Esomus longimanus* was selected for further studies on iron content. In the second phase of the study, the availability and utilisation of *E. longimanus* in poor, rural households in Cambodia were surveyed, and iron content in common dishes prepared with *E. longimanus* was determined.

2. Methods and materials

2.1. Screening

2.1.1. Selection of fish species for screening

From the more than 500 fish species recorded in the Cambodian Mekong river basin, species were selected for iron, zinc and calcium analysis from the following categories: (1) indigenous species common in commercial catches (Loeung & Van Zalinge, 2001); (2) small fish species with low market value (Loeung & Van Zalinge, 2001) and therefore assumed to be commonly consumed in poor households, without entering the market; (3) other small non-commercial species common in rice fields (Rainboth, 1996) and (4) common species of potential interest in aquaculture. Sixteen fish species were selected for screening, based on the above criteria (Table 1).

2.1.2. Sampling of fish species for screening

Three independent fish samples of each species were collected fresh, at landing sites in Kampong Chhnang, Kandal and Phnom Penh local markets, close to the landing sites or directly from fishermen and farmers in October and November, 2001. Each sample contained variable numbers of fish of uniform size (a sample contained either juvenile or full-grown fish, depending on availability). The mean size of fish in each sample was calculated as the total weight of the sample/number of fish in the sample. Raw, cleaned parts were obtained by having rural, Cambodian women to clean the fish according to their traditional practices. The samples were cooled immediately on ice, kept dark and placed in a freezer at −12 °C within 8 h of sampling.

2.1.3. Sample preparation

The samples were handled to minimize the risk of mineral contamination. Before freezing in Cambodia, the

Table 1
Common Cambodian fish species selected for nutrient analyses

Group	Scientific name	Common name
1. Commercially important species	*Channa marulius*	Ros/Ptuok
	Channa micropeltes	Chhdor/Diep
	Cyclocheilichthys apogon	Srawka kdam/Kros
	Dangia sp.	Arch kok
	Osteochilus hasselti	Kros
	Parambassis wollfi	Kantrang preng
	Puntioplites proctozystron	Chra keng
2. Small species with low market value	*Dermogenys pusilla*	Phtong
	Helostoma temmincki	Kanhtrawb
	Parachela siamensis	Chunteas phluk
	Trichogaster microlepis	Kamphleanh phluk
3. Other small, non-commercial species likely to be consumed in poor, rural households	*Esomus longimanus*	Chanwa phlieng
	Euryglossa panoides	Andat chhke
	Rasbora tornieri	Changwe mool
4. Common species of potential interest in aquaculture	*Barbodes altus*	Kahe
	Barbodes gonionotus	Chhpin

samples of raw, cleaned fish were washed in demineralised water. In Denmark, samples of raw fish were homogenised and divided into subsamples for different analyses. The samples were homogenised in a blender (Büchi Mixer B 400) equipped with zirconium oxide ceramic blades. To obtain fully homogenised samples, double-distilled deionised water was added to the samples during blending.

2.2. Field study

2.2.1. Study site and household selection

The species *E. longimanus* was selected for a field study conducted in the Ang Snoul district, Kandal province in October-December, 2004. This district, which is a rice producing area, was selected as *E. longimanus* was abundant. Socio-economic data on the households in the district were not available and identification of poor households for randomised selection was not possible. Thirty-one rural households were selected using the following criteria: (1) visible appearance of having a low socio-economic status; (2) situated within walking distance (maximum 2 km) from a ditch or canal used for fishing and (3) situated at least 3 km apart from each other to avoid influencing each other. All households contacted agreed to participate in the study, which was conducted on the same day as contact with the household was made. The respondent in each household was the woman primarily responsible for cooking.

2.2.2. Conducting the field study

Ingredients for the preparation of sour soup were collected in the morning of each study day, stored on ice and brought to the study household. Each household visit was structured in the following way: first, the woman was requested to participate in an interview, done in two parts, using a semi-structured interview guide developed from pilot interviews. The purpose of the first part of the interview was to discern the woman's familiarity with preparation of *E. longimanus*, including collection of recipes for preparation of the traditional dish, sour soup. After completion of the first part of the interview, the woman was requested to participate in another part of the study on the utilisation of *E. longimanus* and preparation of the traditional sour soup, following the recipes reported in the interview. After agreeing to participate in the second part of the study, the woman was given a portion of *E. longimanus* and other ingredients, which are normally used to prepare a sour soup dish, and she was requested to prepare the sour soup dish. By conducting the study in separate parts, the provision of fish and other ingredients did not have an effect on the first part of the interview.

The cleaning of fish and preparation of sour soup were observed. Amounts and cooking times for each ingredient were recorded. After the preparation of the fish dish and boiled rice – the fish meal – the amounts of the fish dish and rice normally consumed as a fish meal by the woman were recorded by requesting her to serve a meal, identical in size to that which she normally eats. The woman was requested to consume the meal and the plate waste was recorded. After five household visits, this part of the study was discontinued as no plate waste was recorded and it was reported that plate waste never occurred. After completion of this part of the study, the second part of the interview was conducted, covering (1) characteristics of the household, and (2) details about the utilisation of *E. longimanus*. Data on the estimation of the monthly frequency of consumption of *E. longimanus* for the past year in the household were also collected from the woman.

In addition, one woman prepared one of the three other most common dishes with *E. longimanus*, "kob" (a dry paste), "Pha Ork Chamhoy" (a short-term of steamed fermented fish dish) and a deep-fried dish.

2.2.3. Sampling of meals and fish

For each woman, samples of the fish meal, the fish dish and rice proportional to her portion were collected and stored on ice. On returning to the laboratory at the end of the day, the samples were frozen at −18 °C. In addition, a batch of *E. longimanus* collected was sampled and divided into 3 sub-samples: (1) whole, raw fish; (2) raw, cleaned fish, cleaned according to the traditional practice of keeping the head intact as an edible part, and (3) raw, cleaned fish, cleaned with the head discarded. All samples were brought to Denmark for nutrient analyses. The woman's intake of Fe from *E. longimanus* in the fish meal was calculated as the measured total Fe (T-Fe) content in the fish meal, subtracting the T-Fe from the rice (3 μg Fe/g dry matter rice), divided by the T-Fe content in boiled *E. longimanus*.

2.3. Nutrient analyses

2.3.1. Total iron, zinc and calcium

For the screening, subsamples of freeze-dried homogenised sample were dry-ashed at 550 °C in quartz crucibles. Ashes were dissolved in 65% nitric acid and the contents of total iron (T-Fe), zinc (Zn) and calcium (Ca) were measured by atomic absorption spectrometry (PU 9400 X, Pye Unicam, Philips Scientific, England) according to (Larsen & Sandström, 1993). Calibration curves were created using Tritisol® Standards (Merck, Darmstadt, Germany), and control solutions were independently prepared, using iron sulphate, zinc and calcium carbonates.

Meal samples and samples of raw and cleaned *E. longimanus* collected during field study were wet-ashed in a MES 1000 Microwave Solvent Extraction system with 65% nitric acid. Heat treatment for determination of iron compartments in *E. longimanus* and in an in-house pork meat standard were performed in DigiTUBEs (SCP Science, Quebec, Canada), in a DigiPREP MS Digestion System (SCP Science), and T-Fe, Zn and Ca were determined by atomic absorption spectrometry (Spektr-AA 200; Varian, Zug, Switzerland). A typical diet, containing standard reference material 1548a (National Institute of Standards and Technology, Gaithersburg, MD), was used as the reference material.

2.3.2. Inorganic iron

Classical non-heme iron, termed inorganic iron (I-Fe), was determined by the ferrozine method according to Ahn, Wolfe, and Sim (1993). Aliquots of freeze-dried samples were homogenised with citrate buffer at pH 5.5, followed by incubation with ascorbic acid, addition of trichloroacetic acid, centrifugation, addition of ammonium acetate, ferrozine colour reagent and filtration, before absorption was measured at 562 nm. The content of I-Fe was calculated from a standard curve.

2.3.3. High molecular non-haem iron and haem iron

The iron fraction not quantified as I-Fe by the ferrozine method was termed a pool of complex-bound high molecular non-haem Fe and haem iron (Hm-Fe) and calculated as the difference between T-Fe and I-Fe.

2.3.4. Haem-iron

Haem-iron (H-Fe), a sub-pool of Hm-Fe, was measured in selected samples of *E. longimanus* by the method described by Carpenter and Clark (1995), based on the principles of Hornsey (1956) and a standard curve prepared from haematin (Merck).

2.4. Iron compartments in fish

The effect of heating on the content of I-Fe in *E. longimanus* was investigated. Several freeze-dried sub-samples were homogenised to a slurry by adding deionisized water to a solution of dry matter of 20–30% for better heat transfer. The samples were heated in DigiTUBEs in a DigiPREP MS at 120 °C for different times, ranging from 0–240 min. An in-house standard of freeze-dried minced pork was included and I-Fe and T-Fe were measured, while H-Fe was measured in some samples of heat-treated *E. longimanus*, and unheated pork.

3. Results

3.1. Mineral content in fish

3.1.1. Screening

The contents of iron (T-Fe, I-Fe and Hm-Fe), Zn and Ca in the 16 selected fish species are shown in Table 2. One species, *E. longimanus,* had significantly higher contents of T-Fe and Hm-Fe than have the other screened species Also, one sample of juvenile *Dermogenys pusilla* had a high T-Fe content while, in two samples of full-grown *D. pusilla*, the T-Fe content was at the level of those in the other screened species, apart from *E. longimanus*. The Zn content in *E. longimanus* was the highest among the screened species, whereas the Ca content was similar in all 16 species.

3.1.2. Iron compartments in E. longimanus and pork

The effect of heating on the content of I-Fe as a proportion of T-Fe is shown in Fig. 1. In *E. longimanus*, as well as in pork, I-Fe content increased with the time of heating, levelling out at 45% and 68% I-Fe of T-Fe, respectively, with 60–120 min of heating. The measured iron compartments, I-Fe, H-Fe and T-Fe, for heat-treated *E. longimanus* and unheated pork are shown in Fig. 2.

3.2. Field study

3.2.1. Household characteristics

The mean (SD) age of the women was 45 (13) years, the mean (SD) number of household members was 6.4 (2.9) and the mean (SD) household income was 81 (71) USD/month. Thirty seven percent of the household members > 10 years of age were illiterate. One woman, out of the 31 households contacted, responded to being unfamiliar with

Table 2
Contents of iron, zinc and calcium in raw, cleaned parts of common Cambodian fish species

Fish species	n[a]	Size of fish g/fish[e] Mean (range)	T-Fe[b] (mg Fe/kg DM)[f] Mean (SD)	I-Fe[c] (mg Fe/kg DM) Mean (SD)	Calculated Hm-Fe[d] (mg Fe/kg DM) Mean (SD)	Hm-Fe of T-Fe (%) Mean (SD), n	Zn (mg Zn/kg DM) Mean (SD)	Ca (g Ca/kg DM) Mean (SD)
Barbodes altus	3	72 (18–134)	33.6 (14.9)	7.0 (1.3)	26.6 (14.0)	76 (10.7)	40.7 (11.7)	21.6 (0.7)
Barbodes gonionotus	3	94 (82–107)	33.7 (6.1)	7.8 (1.0)	25.8 (6.9)	76 (6.3)	44.2 (3.8)	20.4 (2.0)
Channa marulius	3	91 (38–170)	62.3 (34.3)	13.4 (3.7)	48.9 (31.3)	77 (3.6)	61.1 (3.6)	60.4 (9.2)
Channa micropeltes	3	93 (36–178)	51.5 (2.3)	11.6 (2.3)	39.8 (2.0)	76 (6.7)	60.1 (2.3)	45.3 (4.5)
Cyclocheilichthys apogon	3	22 (8–36)	29.1 (10.9)	7.6 (1.2)	21.6 (4.6)	71 (2.6)	86.7 (10.9)	48.3 (6.1)
Dangila sp.	3	33 (28–36)	76.1 (15.0)	22.4 (2.2)	53.7 (13.3)	70 (3.7)	70.5 (15.0)	32.5 (4.9)
Dermogenys pusilla[g]	2	21 (18–24)	36.0 (8.4)	14.5 (3.5)	20.9 (5.5)	56 (1.6)	110.0 (3.9)	41.6 (1.4)
Dermogenys pusilla, juv.[g]	1	8	218.0	64.0	154.5	70	135.0	50.0
Esomus longimanus	4	0.3 (0.2–0.5)	451 (155)	91.4 (56.5)	360.0 (149)	78 (10.0)	203.0 (19.0)	35.0 (8.2)
Euryglossa panoides	3	21 (18–24)	52.4 (12.6)	13.9 (12.6)	38.5 (11.4)	72 (11.4)	71.3 (17.1)	43.9 (11.5)
Helostoma temmincki	3	45 (38–50)	52.9 (26.0)	15.8 (26.0)	37.1 (18.1)	71 (3.9)	64.5 (8.1)	43.2 (8.9)
Osteochilus hasselti	3	64 (37–85)	41.5 (5.0)	19.1 (5.0)	22.4 (4.1)	54 (7.4)	68.0 (2.2)	41.4 (3.5)
Parachela siamensis	3	11 (2–16)	49.9 (12.2)	15.9 (12.2)	34.0 (11.8)	67 (6.8)	91.4 (5.4)	24.3 (3.7)
Rasbora tornieri	3	16 (13–20)	27.2 (5.2)	7.5 (5.2)	19.7 (3.9)	72 (5.8)	114.3 (8.5)	30.4 (0.8)
Trichogaster microlepis	3	8 (3–11)	49.5 (4.4)	16.3 (4.4)	33.2 (2.4)	67 (3.2)	65.0 (4.7)	37.3 (3.1)
Parambassis wollfi	3	18 (5–35)	57.1 (19.7)	11.2 (19.7)	46.0 (20.5)	78 (10.8)	67.3 (10.7)	46.6 (3.0)
Puntioplites proctozysron	3	68 (61–74)	34.4 (9.7)	11.3 (2.5)	23.1 (7.4)	66 (3.9)	52.3 (13.3)	26.7 (7.1)

[a] n = number of samples collected from separate sampling sites. Each sample contained at least 50 g of fish. Number of fish in a sample was 1–130, depending on the size of fish.
[b] T-Fe = total iron.
[c] I-Fe = inorganic iron.
[d] Hm-Fe = High molecular non-haem iron and haem iron.
[e] Size of fish in a samples with more than one fish was calculated as: weight of sample (g)/number of fish in the sample.
[f] DM = dry matter.
[g] One of three samples of *Dermogenus pusilla* was juvenile fish. This sample had a significantly higher content of T-Fe and I-Fe than had the two samples of full-grown fish.

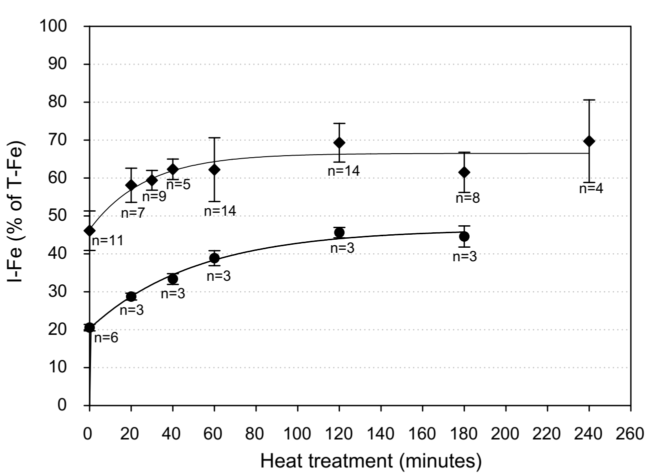

Fig. 1. The effect of heating at 120 °C on the proportion of inorganic iron (I-Fe) of the total iron (T-Fe) in *Esomus longimanus* (●) and pork (♦). Mean T-Fe in the sample of *Esomus longimanus* used for heat treatment was 616 μg Fe/g dry matter and in pork 17 μg Fe/g dry matter.

cooking *E. longimanus* and she was eliminated for the study and therefore 30 households were included in the study.

3.2.2. Traditional utilisation of E. longimanus

All women habitually cooked sour soup with *E. longimanus* and 70% of the women reported sour soup to be the most common dish cooked with *E. longimanus*. The dishes, kob, pra ork and deep-fried *E. longimanus*, were prepared by 93%, 87% and 60% of the women, respectively. The key reason for using *E. longimanus* was ease of availability, either by catching the fish nearby or buying in the local market, where *E. longimanus* was reported to be cheap. The women reported that they usually prepared sour soup with a mixture of available small fish species, with *E. longimanus* contributing about 50% of the total weight of all fish. The frequency of consuming *E. longimanus* was reported as being highest in July–November, with a mean (25th, 75th percentile) frequency of 7 times/month (2,12) in August and 8 times/month (2,12) in September ($n = 30$).

3.2.3. Traditional cleaning of E. longimanus

Eighty percent of the women reported that they cleaned *E. longimanus* leaving the head intact.

The weight losses of raw, whole *E. longimanus* by the two traditional cleaning methods were 12%, with the head intact and 34% with the head cut off. The effect of cleaning on the Fe content in one batch of *E. longimanus* is shown in Table 3. With the head intact, half of the T-Fe in the raw, whole fish was lost, whereas 60% was lost with the head cut off. The loss of T-Fe in boiled, cleaned fish with the head intact was 35% of the amount in the raw, whole fish.

3.2.4. Iron contents in traditional dishes with E. longimanus and intakes

The measured contents of T-Fe and I-Fe and the calculated content of Hm-Fe in meals prepared with *E. longimanus* are shown in Table 4. The weights of the portions of fish meals – fish dish and rice – for sour soup and the three

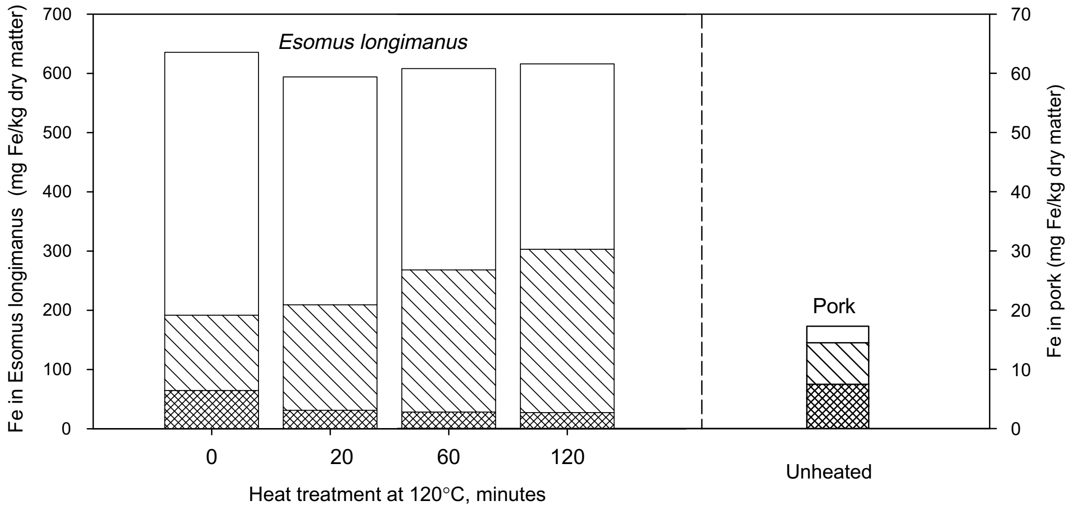

Fig. 2. Iron compartments in *Esomus longimanus* following heating and in unheated pork. ▩: H-Fe, $n = 2$ per heat treatment, SEM < 10%; ▧: I-Fe, $n = 3$–6 per heat treatment, SEM < 10%; ☐: T-Fe, minus I-Fe and H-Fe, $n = 3$–11per heat treatment, SD < 10%.

Table 3
Effect of cleaning and boiling on iron content in *Esomus longimanus*, measured on sub-samples of one single batch

Esomus longimanus	Moisture (%)	T-Fe[a] (mg/kg DM)[d]	I-Fe[b] (mg Fe/kg DM)	Hm-Fe[c] (mg/kg DM)	Hm-Fe of T-Fe (%)
Raw, whole	77	1100	180	921	84
Raw, cleaned with head intact	82	573	35	538	94
Raw, cleaned with head cut off	83	457	28	429	94
Boiled in water (9 min), cleaned with head intact	85	365	86	279	76

[a] T-Fe = total iron.
[b] I-Fe = inorganic iron.
[c] Hm-Fe = High molecular non-haem iron and haem iron.
[d] DM = dry matter.

other traditional dishes ($n = 1$ for each dish) normally eaten by the women are presented, along with the calculated intakes of iron.

4. Discussion

4.1. Iron content in Cambodian fish species

4.1.1. Screening

There was relatively little variation in T-Fe content between the screened fish species, except for the species *E. longimanus*. T-Fe content in *E. longimanus* was 6-fold higher than in the other species, except for one of three samples of the species *D. pusilla*. In a study of the contents of nutrients in Bangladeshi fish species, it was found that the indigenous *Esomus* species, *Esomus danricus* had a high T-Fe content (600 mg Fe/kg dry matter in raw, cleaned fish) compared to other screened Bangladeshi fish species (Roos et al., 2003), indicating that the level of T-Fe in fish is a genetic characteristic. In the field survey on utilisation of *E. longimanus* in rural households, it was observed that *E. longimanus* was highly perishable compared to other species. For conducting the study on the preparation of *E. longimanus*, the batches of *E. longimanus* which were provided to the households had to be collected fresh and delivered to the household on the same day. In the preparatory phase of the field study, it was found that frozen *E. longimanus* underwent changes in texture and taste which were unacceptable to women. The frozen and thawed fish were reported to be soft and bitter. This effect of freezing may be associated with the high Fe content as cell damage due to freezing and thawing, in tissues with high Fe contents, may stimulate oxidation processes, as shown in studies on different storage conditions of fish (Benjakul & Bauer, 2001).

There is no obvious biological explanation for the higher Fe content found in one sample of juvenile *D. pusilla*, than in samples of full-grown fish of the same species.

The Zn content in *E. longimanus* was higher than that in any other of the screened species, though the inter-species difference was less than that for the Fe content. The Ca content in *E. longimanus* was similar to the level in other screened species (Table 2). The study on the consumption of the fish meal of sour soup prepared with *E. longimanus* and boiled rice showed that there was no plate waste; all

Table 4
Iron contents in common fish meals (fish dish and boiled rice)[a] prepared with *Esomus longimanus* and the intakes of fish meal and iron fractions by women

Fish dish	Iron content in fish meal samples					Intake by women					
	Moisture (%)	T-Fe[b] (µg Fe/g DM)	I-Fe[c] (µg Fe/g DM)	Calculated Hm-Fe[d] (µg Fe/g DM)	Hm-Fe of T-Fe (%)	Intake of fish dish (g/meal)	Intake of rice (g/meal)	Intake of T-Fe (mg Fe/meal)	Intake I-Fe (mg/meal)	Calculated intake of Hm-Fe[d] (mg/meal)	Calculated intake of *Esomus longimanus* (g/meal)[e]
Sour soup ($n = 30$)[f]	78 (2.7)	23 (9.5)	9 (4)	14 (7.6)	62 (14)	257 (71)	357 (48)	3.12 (1.85)	1.18 (0.75)	1.94 (1.42)	49 (31)
Kob ($n = 1$)	70	43	14	29	67	197	292	6.42	2.13	4.30	110
Pra ork ($n = 1$)	71	20	9	11	55	67	277	1.94	0.88	1.06	29
Deep fried ($n = 1$)	78	24	14	10	43	32	151	0.99	0.56	0.43	15

[a] Meals with sour soup were prepared by 30 women, and three of the women in addition prepared and consumed three other common fish dishes, "kob", "pra ork" and deep-fried fish. The fish meal samples contained fish dish and rice in amounts proportional to the observed servings for the women.
[b] T-Fe = total iron.
[c] I-Fe = inorganic iron.
[d] Hm-Fe = (T-Fe) − (I-Fe).
[e] Calculated from the content of T-Fe content in sour soup meal and the content of Hm-Fe in boiled fish (Table 3), minus the T-Fe contribution from rice.
[f] Values are mean (SD).

fish bones were consumed and therefore the calcium intake from fish was proportional to the measured calcium content. In a study in rural households in Bangladesh, it was observed that most of the calcium in large fish with hard bones is lost as the bones form the plate waste (Roos et al., 2003). In contrast, small fish with soft, edible bones are very good sources of calcium which has also been shown to be highly bioavailable (Larsen, Thilsted, Kongsbak, & Hansen, 2000).

4.1.2. Iron compounds in fish

The commonly used terminology, defining non-haem iron as the fraction determined by the ferrozine method, is not valid, since a high molecular sub-pool of complex-bound non-haem iron, such as ferritin, is not measured by this method. In this study, iron measured colorometrically with ferrozine is therefore referred to as inorganic iron (I-Fe) and iron in complexes with low molecular organic compounds, such as citrate or fumarate, is also characterised as inorganic.

The major proportion of iron in *E. longimanus* is present in forms not detected by the ferrozine method and hence assumed to be as complex bound compounds, either as haem iron or a non-haem iron form, probably with a relatively high molecular weight. The heat treatment of *E. longimanus* and the in-house standard of pork showed an increase in the fraction of I-Fe which levelled off after 60–120 min of heating, 45% T-Fe was found as I-Fe for *E. longimanus* and nearly 70% for pork. In fish and pork, a fraction of the complex bound iron remained separate from the I-Fe pool, even after heating for 240 min at 120 °C. This is in accordance with the results of Lombardi-Boccia et al. (2002), showing that commonly practised pan-frying of pork reduced the H-Fe content by up to 18% of the initial content, and that the maximum loss of H-Fe in various meats was 43%, found in chicken wings fried at 180 °C for 50 min. Han et al. (1993) found that H-Fe was released during heating, following the cleavage of the porphyrin ring, but a fraction of the iron bound in high molecular denaturation products of haemoglobin, and possibly ferritin, remained unreleased to the I-Fe pool.

In the present study, the content of H-Fe was measured in selected samples, using the acidified acetone extraction method, following the principals of Hornsey (1956). The acidified acetone method is widely used and has been found to be reproducible but the accuracy of the method is questioned due to critical factors under the analytical conditions (Lombardi-Boccia, Martinez-Dominguez, Aguzzi, & Rincon-Leon, 2002). The measured H-Fe content may therefore not be exact but serves as an indication of the magnitude of this iron compartment in *E. longimanus* compared to pork. The measurements of I-Fe, H-Fe and T-Fe in pork showed that I-Fe and H-Fe accounted for 85% of the measured T-Fe, leaving a minor Fe fraction bound in either high molecular denaturation products or ferritin. This confirmed that I-Fe and H-Fe are the major iron compartments in meat, as previously shown (Buchowski, Mahoney, Carpenter, & Cornforth, 1988) and it is therefore a reasonable assumption that I-Fe + H-Fe = T-Fe in meat. However, in *E. longimanus*, the distribution pattern of iron was quite different. First, the T-Fe content in the sample of *E. longimanus* used for the heat treatment was 30 times higher than that in pork. The absolute contents of I-Fe and H-Fe were also higher in fish, than in pork. In raw fish, I-Fe + H-Fe accounted for 32% of T-Fe in *E. longimanus*, increasing to 50% after 120 min of heating. The remaining fraction of iron is assumed to be complex bound, probably high molecular compounds, such as ferritin. Methods for ferritin determination based on immunological reaction with anti-ferritin are widely used, but immunological cross-reaction between species is limited (Suryakala & Deshpande, 1999) and anti-ferritin for immunoassay in fish is not available. Therefore, quantitative determination of the ferritin was not done in the present study and the amount of ferritin in *E. longimanus* and other fish species remains to be established.

Nutritional contribution of dietary iron from *E. longimanus* in the diet of poor, rural households in Cambodia depends mainly on iron bioavailability. It is assumed that a significant fraction of the high molecular iron fraction (T-Fe minus I-Fe) is ferritin or ferritin-like compounds, in addition to H-Fe. The bioavailability of ferritin in soyabean was determined to be 30% in non-anaemic individuals (Lonnerdal et al., 2006) and is it assumed that ferritin in animal foods is at least as bioavailable. The bioavailability of H-Fe is 20–25% (Martinez-Torres et al., 1986). It is therefore assumed that bioavailability of Fe from the high molecular fraction, including H-Fe is 25% and this value is used in the calculated contribution of iron from *E. longimanus* to the daily iron requirement of women.

It should be noted that the distribution of iron compounds found in the analysed samples of *E. longimanus* may have been influenced by the collection, storage and transport procedures. During storage on ice, H-Fe bound in fish may be released and also, haemo-proteins may undergo oxidation following temperature fluctuations (Benjakul & Bauer, 2001). The samples were kept on ice for up to 8 h from the time of collection and temperature fluctuations during the transport from the collection sites in Cambodia to the laboratory in Denmark were unavoidable. As discussed above, the eating quality of *E. longimanus* was affected by freezing and thawing. The distribution of iron compounds in the analysed samples may therefore not be identical to that in the freshly collected fish. However, the total iron content is unaffected by the history of the samples.

4.2. Field survey

4.2.1. Households

The households included in the field study were not randomly selected, due to practical limitations. However, the surveyed households were considered to be typical poor, rural households in Cambodia. Thirty of 31 contacted households were familiar with *E. longimanus*, indicating that *E. longimanus* is commonly consumed in rural households when locally available. This was confirmed with the women reporting that ease of availability is the main reason for the frequent consumption of *E. longimanus*.

4.2.2. Traditional utilisation and cleaning of E. longimanus

The reported frequency of consumption of *E. longimanus* showed that consumption peaked in July–November. This indicates that the availability pattern of *E. longimanus* is different from that of capture fisheries in Cambodia which peaks in November to early February (Van Zalinge et al., 2000). The peak in availability of *E. longimanus*, prior to the peak of other captured fish, contributes to extending the season of fish availability and consumption in rural areas. This emphasises that protection of the habitats of *E. longimanus* and other small, low-valued fish species, in rice fields and canals, is important for ensuring food security of the rural, poor population in Cambodia.

The traditional cleaning method for *E. longimanus,* which leaves the head intact and therefore as an edible part, has a nutritional advantage as more iron is made available in the diet. The head of the fish may also contribute to other nutrients in the diet. It has previously been found that the Bangladeshi small indigenous fish species, *Amblypharyngodon mola,* is very rich in vitamin A, with more than half of the vitamin A located in the eyes (Roos, Leth, Jakobsen, & Thilsted, 2002).

4.2.3. Nutritional contribution from E. longimanus

The most common dish with *E. longimanus* was sour soup. A serving of the sour soup dish for women contained, on average, 49 g of *E. longimanus*. The women reported that, traditionally, sour soup is prepared with a mixture of available fish species, including *E. longimanus,* as well as other aquatic animals, such as frogs and snails. The evaluation of the iron contribution was made for dishes prepared exclusively with *E. longimanus* in order to demonstrate the potential of *E. longimanus* as a source of dietary iron. The recorded serving size and the measured iron content of sour soup meals – sour soup plus rice – showed that the mean intake of Hm-Fe from the meals was 1.94 mg/meal. The bioavailability of Fe in the high-molecular iron pool is assumed to be 25%, hence proving that 0.49 mg of Fe absorbed is from a *E. longimanus* sour soup. This amount of iron covers 36% of the daily median iron requirement of women of 55 kg, 1.35 mg Fe/d (FAO/WHO, 2004). The I-Fe content provides 0.12 mg of absorbed iron additionally, assuming 10% bioavailability, and thereby covers 9% of the daily requirement. In total, a daily fish meal with a sour soup prepared with *E. longimanus* has the potential to cover 45% of the daily median iron requirement of women of childbearing age.

In addition to being a rich iron source, *E. longimanus* also provides other micronutrients, for example zinc (Table 2), known to be deficient in the diet of poor, rural households in Cambodia. A screening of common Cambodian fish species, for the content of vitamin A, showed that raw, whole, *E. longimanus* ($n = 1$) is characterised as having a "medium" vitamin A content (Roos, Chamnan, Loeung, Jakobsen, & Thilsted, in press). Later replicate analysis, using the same analytical procedures as in Roos et al. (in press) showed that the vitamin A content of *E. longimanus* was 639 retinol activity equivalents (RAE)/100 g raw, whole fish (SD = 176, $n = 7$), with on average 68% of the total content present as all-*trans* retinol.

In western diets, there is a focus on the health benefits of the long chain $n - 3$ fatty acids found in fish. However, the contents and nutritional significance of $n - 3$ fatty acids in fish species in developing countries remain to be investigated.

In conclusion, for Cambodian population groups vulnerable to micronutrient deficiencies, the accessibility of nutrient-dense fish species is important for their food and nutrition security. As large proportions of the population have poor access to preventive health services (Victora et al., 2005), it makes it more important that these groups have the potential to improve their nutrition by optimising the use of locally available nutrient-dense foods. Rice-based aquatic ecosystems have a high biodiversity and are a rich source of nutrient-dense local foods (Halwart, 2006). This study demonstrates that the locally available fish species, *E. longimanus,* which is accessible to the poor due to its low market value, has the potential to contribute to food-based strategies to reduce the risk of iron deficiency in rural Cambodians, particularly women who are a high-risk group.

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