Journal of Oceanology and Limnology
Vol. 36 No. 5, P. 1788-1797, 2018
https://doi.org/10.1007/s00343-018-7205-7

Identification of wild and farmed broadhead catfish (*Clarias macrocephalus* Günther, 1864) based on morphometry, digestive indexes and flesh quality*

Wilawan WHAN-AIR, Karun THONGPRAJUKAEW**, Tasneem SALAEHARAE, Krueawan YOONRAM

Department of Applied Science, *Faculty of Science*, *Prince of Songkla University*, *Songkhla 90112*, *Thailand*

Received Jul. 17, 2017; accepted in principle Sep. 6, 2017; accepted for publication Sep. 29, 2017

Abstract Wild and farmed fish generally differ in their nutritional composition. In this study, adult wild and farmed broadhead catfish (*Clarias macrocephalus* Günther, 1864) were collected and were assessed for various characteristics, namely morphometrics, digestive indexes, and flesh quality. The morphometrics (standard length, body depth, eye width, fin height and tentacle length) and the digestive indexes (intestosomatic index, digestosomatic index, perivisceral fat index and activities of pepsin and lipase) differed significantly between the groups (P<0.05) and can be used to distinguish wild fish from farmed fish. In terms of protein synthesis capacity and color, the flesh quality was similar between the groups. However, radical scavenging activities and reducing power were significantly higher in the wild fish than in the farm-raised group. The thermal transition characteristics of sarcoplasmic proteins, as well as myosin denaturation enthalpy and fatty acid profiles (C18:2n6, C20:0, C22:1n9, C24:0, Σpolyunsaturated fatty acids, and Σn–6) also exhibited potential to enable calls about the fish origin. The proximate chemical composition of whole body did not differ between the two fish populations. Our findings suggest bioindicators, in terms of morphometrics, digestive indexes and flesh quality, that can be used to identify the origin of fish for forensic purposes, of for conservation biology of this near threatened species. The new nutritional information may be of interest to marketing, consumers, and has a connection to nutritional effects on human health.

Keyword: carcass; digestive enzyme; fatty acid; flesh quality; thermal property

1 INTRODUCTION

The annual global production of fish in the genus *Clarias* exceeds 660 000 tonnes, providing economic value in excess of USD 970 million (FAO, 2015). Broadhead catfish (*Clarias macrocephalus* Günther, 1864) is an economically important freshwater fish found in local and commercial fisheries, and is native to Thailand, Lao, Cambodia, Viet Nam and Peninsular Malaysia (Vidthayanon and Allen, 2011). The population of this species is declining due to habitat losses as well as efficient fishing. Moreover, this species is also threatened by the escaped hybrids of female *C. macrocephalus* and introduced male *C. gariepinus* (Na-Nakorn et al., 2004), causing a genetic introgression that could lead to species extinction. Therefore, identification of wild or farmed broadhead catfish is needed for conservation of this near threatened species (Vidthayanon and Allen, 2011).

Substitution of wild fish with farmed fish is motivated by the higher price of the former, which in turn is based on the differences in consumer perceived nutritional images (Claret et al., 2016). In practice, morphometric measurements are used to distinguish the wild fish from the farmed fish (Uglem et al., 2011; Arechavala-Lopez et al., 2012; Fagbuaro et al., 2015). The culturing process or spontaneous induction can have relatively permanent effects on animals (Fjelldal et al., 2009) that persist throughout their entire lifespans. Moreover, observation or measurement of

* Supported by the Research Fund from the Faculty of Science (No. 1-2557-02-005) and the Graduate School Research Support Funding for Thesis of the Prince of Songkla University
** Corresponding author:karun.t@psu.ac.th

fish body is simple, reliable and fast for preliminary screening in fieldwork. Differences in feeding habit can also be investigated by observing the digestive organs and the activities of digestive enzymes (Lemieux et al., 1999; Gildberg, 2004). Farmed fish, such as *C. macrocephalus*, are always fed by a commercial pellet diet containing high amounts of carbohydrate and protein, while the wild fish ingest live prey that are rich in protein and lipid (Thongprajukaew et al., 2013). Routine feeding protocols of the farmed fish, such as regular feeding times and rates, also stand in contrast to the uncertain and irregular spontaneous feeding in the wild.

Man-made and environmental factors can significantly affect the various biochemical processes of fish. Fish flesh is a potent biochemical sample type for identifying differences between farmed and wild cases. Thermal transition properties of the main muscle proteins, actin and myosin, detected by differential scanning colorimetry (DSC), could be used in food research and quality assurance, as well as in forensic investigations (Skipnes et al., 2008; Matos et al., 2011). Fatty acids profiles act as fingerprints due to their uniqueness that differentiates between fish populations (González et al., 2006; Lenas et al., 2011; DePeters et al., 2013; Rincón et al., 2016). Moreover, variations in nutritional composition and sensory, chemical and physical properties of fish have also been observed (González et al., 2006; Johnston et al., 2006; Jensen et al., 2013; O'Neill et al., 2015; Claret et al., 2016; Rincón et al., 2016).

The objective of this study was to determine whether these three bioindicator groups (morphometrics, digestive indexes and flesh quality) can be used to distinguish between wild and farmed broadhead catfish. Findings from the current study might be applied in forensic identification of adulteration, in conservation biology of this near threatened species, or in providing correct nutritional images to the consumers of fish.

2 MATERIAL AND METHOD

2.1 Fish sample preparation

Live adult broadhead catfish (*C. macrocephalus*) were purchased from local markets (at 10.00 h) in Songkhla province, Thailand. These fish were detained in black round plastic tank containing low amount of water (~2 folds of water volume per total fish weight) and all fish were sold out within one day of captivity. Although the effects of sex and age were otherwise ignored, purposive sampling of all the fish was requested based on a balanced sex ratio of male:female (1:1). A total of 40 wild and 40 farmed catfish were sampled in six collection rounds (6 rounds×6–7 fish) from different locations (districts Hat Yai, Ronot, Chana, Rattaphum, Khlong Hoi Khong and Meuang). The farmed fish were reared in outdoor pond under semi-intensive farming management and all fish were fed by commercial pellet diet. The wild fish were obtained from rice fields or canals and quatic insects, young shrimp and smaller fishes were the main natural diets. The catfish species was morphologically identified by its large dorsal fin, shorted and rounded occipital process, and presence of white spots on the sides of its black body. The fish were sacrificed by chilling in ice, packed in polyethylene bags and transported to the Department of Applied Science, Faculty of Science, Prince of Songkla University. Morphometric measurements of the individual fish were taken as described in Uglem et al. (2011). The fish (n=30) were dissected on ice in order to carefully remove stomach, intestine, liver, perivisceral fat and white muscle below the dorsal fin (without skin), while another whole fish (n=10) were used for carcass composition analysis. All these samples were kept at -20°C until assaying.

2.2 Digestive enzyme studies

2.2.1 Preparation of crude enzyme extract

Crude enzymes from stomach and intestine were extracted in 0.2 mol/L KCl-HCl buffer (pH 2) and 0.2 mol/L Na_2HPO_4-NaH_2PO_4 buffer (pH 8) at a ratio of 1:3 (w/v), respectively, using a micro-homogenizer (THP-220; Omni International, Kennesaw GA, USA). The homogenates were centrifuged at 15 000×g for 30 min at 4°C and supernatants were collected. Aliquots were kept at -20°C until use.

2.2.2 Determination of protein concentration in crude extract

The protein concentration of a crude enzyme extract was compared to a standard curve of bovine serum albumin (BSA), according to the standard method of Lowry et al. (1951). Normalization by the protein was then used to calculate the specific activities of digestive enzymes (U/mg protein).

2.2.3 Digestive enzyme activity assay

Pepsin (EC 3.4.23.2) activity in the stomach extracts was measured according to the method of

Rungruangsak and Utne (1981), using 2% casein as substrate. The activity was spectrophotometrically measured at 720 nm against *L*-tyrosine standard. For four further enzymes, intestinal extracts were used. Trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) activities were measured as described by Rungruangsak-Torrissen (2007), using 1.25 mmol/L *N*-α-benzoyl-Arg-*p*-nitroanilide (BAPNA) and *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide (SAPNA) as the substrates, respectively. The products of each enzyme were spectrophotometrically measured at 410 nm against *p*-nitroanilide standard. Lipase (EC 3.1.1.3) activity was assayed according to the method of Winkler and Stuckmann (1979), using 0.01 mol/L *p*-nitrophenyl palmitate (*p*-NPP) as substrate. The absorbance at 410 nm was measured against *p*-nitrophenol standard. Amylase (3.2.1.1) activity was assayed as described by Areekijseree et al. (2004), using 5% soluble starch as the substrate. The product was measured at 540 nm against maltose standard.

2.3 Flesh quality

2.3.1 Protein synthesis capacity

Concentrations of RNA and protein in the white muscle were determined as described in Rungruangsak-Torrissen (2007). The extinction coefficients used to calculate RNA and protein were E_{260}=40 μg RNA/mL and E_{280}=2.1 mg protein/mL, respectively. The concentration ratios were calculated from the amounts of RNA and protein in the same sample.

2.3.2 Color

The instrument was first calibrated to a white and black standard. The color coordinates lightness (L^*), redness (a^*) and yellowness (b^*) were measured using a MiniScan EZ (Hunter Associates Laboratory, Reston VA, USA) with small area view (6 mm port and 5 mm view diameter).

2.3.3 Scavenging activity

The frozen flesh was homogenized in 50 mmol/L Tris-HCl (pH 7.5) containing 10 mmol/L ß-mercaptoethanol and 1 mmol/L ethylenediamine tetraacetic acid (EDTA) at the ratio 1:5 (*w*/*v*) using a micro-homogenizer (THP-220; Omni International, Kennesaw GA, USA). The homogenates were centrifuged at 10 000×*g* for 40 min at 10°C and supernatants were collected. The 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging activity (% inhibition) and the reducing power (% inhibition) were determined according to the methods of Thongprajukaew et al. (2015) and Hahor et al. (2016). The radical scavenging activity was calculated as $[(A_o–A_i)/A_o]\times100$, where A_o and A_i are the absorbances of the control sample (extraction buffer in equal volume replacing the actual sample) and the extract, respectively.

2.3.4 Thermal properties

DSC thermograms were determined using a Perkin-Elmer DSC-7 (Perkin Elmer, Waltham, Massachusetts, USA). The frozen fish muscle was defrosted and then approximately 10 mg of sample was dissected. The sample was sealed in an aluminum pan and scanned from 20 to 120°C at a rate of 10°C/min against an unoccupied reference pan. Myosin, actin and sarcoplasmic proteins (SP) were identified by their thermal properties:onset temperature (T_o), denaturation temperature (T_d), conclusion temperature (T_c), and denaturation enthalpy (ΔH), as described by Skipnes et al. (2008) and Matos et al. (2011).

2.3.5 Fatty acid composition

Lipid from the white muscle was extracted as described by Kates (1986). Fatty acid methyl esters (FAME) of the extracted lipid were separated and analyzed using a 6890 gas chromatograph with flame ionization detection (Agilent Technologies, Santa Clara, CA, USA) capillary column (0.32 mm inside diameter×30 m length, 0.25 μm film thickness), using helium as the carrier gas at a flow rate of 1 mL/min and with split ratio 50:1. The detector temperature was set at 300°C to achieve optimal separation. The column temperature was ramped from 210°C to 250°C at 20°C/min. The FAMEs were identified by comparing with retention time of each individual standard. The fatty acids were categorized as saturated fatty acids (SFA) and unsaturated fatty acids (USFA), and the latter group consisted of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA).

2.4 Proximate chemical composition of carcass

The whole body was minced and then moisture, crude protein, crude lipid and crude ash were determined according to the standard methods of AOAC (2005).

Table 1 Morphometries of wild and farmed broadhead catfish

Parameter	Wild	Farmed	*P*-value
Body weight (g)	158.16±4.05	150.50±4.73	0.223
Total length (cm)	25.58±0.19	25.05±0.27	0.123
Standard length (cm)	22.77±0.17[a]	22.10±0.26[b]	0.036
Fulton's condition index (g/cm^3)	0.94±0.01	0.94 ±0.01	0.755
Snout length (cm)	1.72±0.01	1.73±0.01	0.887
Eye diameter (cm)	0.37±0.01	0.38±0.01	0.320
Head length (cm)	6.00±0.05	5.93±0.07	0.485
Body depth (cm)	3.69±0.07[a]	3.39±0.05[b]	0.003
Body width (cm)	3.90±0.04	3.88±0.05	0.770
Eye width (cm)	0.35±0.01[b]	0.37±0.01[a]	0.018
Dorsal fin height (cm)	1.68±0.02[b]	1.78±0.03[a]	0.037
Pectoral fin length (cm)	3.15±0.03	3.20±0.03	0.335
Tentacle length (cm)	5.87±0.11[b]	6.22±0.10[a]	0.031
Distance from snout to eye center (cm)	1.82±0.02	1.83±0.02	0.608
Distance from eye center to end of gill cover (cm)	2.82±0.03	2.76±0.03	0.178
Lower jaw length (cm)	0.97±0.01	0.97±0.02	0.889
Upper jaw length (cm)	1.04±0.01	1.04±0.01	0.836
Cephalic index	0.23±0.01	0.23±0.01	0.335
Relative profile index	0.86±0.02	0.78±0.03	0.081

Data are expressed as mean±SEM (n=30). Significant differences between groups are indicated by different superscripts (P<0.05).

Table 2 Visceral indexes and specific activities of the main digestive enzymes for the wild and the farmed broadhead catfish

Parameter	Wild	Farmed	*P*-value
Index			
SSI (%)	0.50±0.02	0.44±0.01	0.061
ISI (%)	0.45±0.02[a]	0.36±0.01[b]	0.004
HSI (%)	1.12±0.08	1.14±0.06	0.787
DSI (%)	0.96±0.04[a]	0.80±0.02[b]	0.006
Relative intestinal length	0.86±0.02	0.78±0.03	0.081
Perivisceral fat index (%)	3.42±0.29[a]	2.33±0.16[b]	0.002
Digestive enzyme			
Pepsin (mU/mg protein)	12.40±0.73[b]	17.08±0.60[a]	0.001
Trypsin (U/mg protein)	1.32±0.11	1.09±0.07	0.092
Chymotrypsin (U/mg protein)	1.01±0.09	0.90±0.07	0.374
Amylase (U/mg protein)	13.56±0.47	13.29±0.40	0.668
Lipase (mU/mg protein)	5.23±0.31[a]	4.08±0.23[b]	0.005
Amylase/trypsin ratio	12.11±0.88	13.41±0.72	0.261

SSI: stomasomatic index; ISI: intestosomatic index; HSI: hepatosomatic index; DSI: digestosomatic index. Data are expressed as mean±SEM (n=30). Significant differences between groups are indicated by different superscripts (P<0.05).

2.5 Statistical analysis and calculations

All data analyses were performed using SPSS Version 20 (SPSS Inc., Chicago, USA). The percentages were checked for normality after arcsine transformations. Data were analyzed using independent sample t-test. All values are expressed as mean±SEM. Significant differences between groups are indicated by different superscripts (P<0.05). The investigated parameters were calculated as follows:

Fulton's condition index (K, g/cm^3)=100×[live body weight (g)/total body length (cm)^3];

Cephalic index (CI)=[head length (cm)/total body length (cm)];

Relative profile index=[maximum body height (cm)/total body length (cm)];

Stomasomatic index (SSI, %)=100×[stomach weight (g)/live body weight (g)];

Intestosomatic index (ISI, %)=100×[intestinal weight (g)/live body weight (g)];

Hepatosomatic index (HSI, %)=100×[liver weight (g)/live body weight (g)];

Digestosomatic index (DSI, %)=100×[gastrointestinal tract weight (g)/live body weight (g)];

Relative intestinal length=[intestinal length (cm)/standard length (cm)];

Perivisceral fat index (%)=100×[perivisceral fat weight (g)/live body weight (g)].

3 RESULT

3.1 Morphometric measurement

Five of the observed nineteen parameters were significantly different between wild and farm-raised broadhead catfish (P<0.05, Table 1). Standard length and body depth were larger in the wild than in the farmed fish, and ditto to eye width, dorsal fin height, and tentacle length.

3.2 Visceral index and specific activity of digestive enzymes

ISI, DSI and perivisceral fat index were significantly higher in the wild catfish as compared to farm-raised catfish (Table 2), while no differences were observed in the other indexes (SSI, HSI, and relative intestinal

Table 3 Flesh protein synthesis capacity, color coordinates, and radical scavenging activities of the wild and the farmed broadhead catfish

Parameter	Wild	Farmed	*P*-value
Protein synthesis capacity			
RNA (μg/g)	1 126±28	1 071±27	0.157
Protein (mg/g)	184.03±3.59	181.18±3.78	0.586
RNA/protein ratio (μg/mg)	6.18±0.19	5.97±0.17	0.400
Color			
*L**	38.77±0.71	39.16±0.55	0.669
*a**	-0.91±0.38	–1.06±0.32	0.763
*b**	9.31±0.63	9.42±0.51	0.890
Scavenging activity (% inhibition)			
DPPH	60.37±1.16^{a}	43.28±1.09^{b}	<0.001
Reducing power	67.57±0.42^{a}	56.38±0.75^{b}	<0.001

DPPH, 2,2-diphenylpicrylhydrazyl. Data are expressed as mean±SEM (n=30). Significant differences between groups are indicated by different superscripts (P<0.05).

Table 4 Thermal transition characteristics of flesh proteins in the wild and the farmed broadhead catfish

Parameter	Wild			Farmed		
	T_o (°C)	T_d (°C)	T_c (°C)	T_o (°C)	T_d (°C)	T_c (°C)
Myosin	46.72	51.57	55.91	46.97	51.48	56.14
Actin	70.56	74.23	76.70	71.13	75.03	77.16
SP I	28.09	33.52	36.25	ND	ND	ND
SP II	39.90*	42.70**	44.68	41.57*	43.79**	45.49
SP III	44.32	46.00	48.04	ND	ND	ND
SP IV	78.66	81.79	83.15	78.82	81.16	84.33
SP V	99.24	107.47	112.95	101.04	107.69	113.60
SP VI	117.34	117.58	118.15	ND	ND	ND

The data given are means. T_o: onset temperature; T_d: denaturation peak temperature; T_c: conclusion temperature; SP: sarcoplasmic protein; ND: not detected.*, **: significant differences between wild and farmed groups (P<0.05).

length). Significantly increased pepsin specific activity was found in the farm-raised catfish relative to the wild catfish, and vice versa for the lipase specific activity (Table 2). There were no differences in specific activity of trypsin, chymotrypsin, amylase, or in amylase/trypsin ratio between the two groups.

3.3 Protein synthesis capacity and color

There were no differences in flesh parameters relating to protein synthesis capacity (RNA, protein and RNA/protein ratio) or color (*L**, *a** and *b**) between the wild and the farm-raised catfish (Table 3).

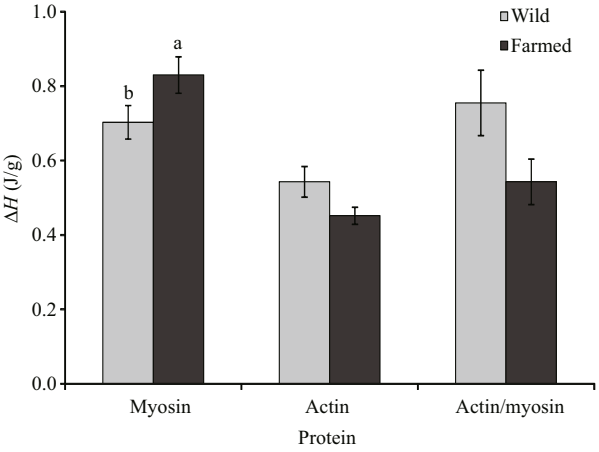

Fig.1 Denaturation enthalpy (J/g) of muscle myosin, actin and actin/myosin in wild and farmed broadhead catfish

Data are expressed as mean±SEM (n=30). Independent sample t-test was used to compare for significant differences between groups (P<0.05).

3.4 Scavenging activity

Radical scavenging activities and reducing power were significantly higher in the wild fish than in the farmed fish (Table 3).

3.5 Thermal transition properties

There were no differences in thermal characteristics of muscle actin and myosin between wild and farmed broadhead catfish (Table 4). SPs I, III and VI were only detected in the wild fish, but overall six SPs were present in the farm-raised fish. Thermal properties in terms of T_o and T_d were significantly lower in the wild samples relative to the farmed samples, but not so for T_c. The myosin ΔH in farmed fish was higher than in the wild cases, while actin ΔH and ΔH for actin/myosin were similar (Fig.1).

3.6 Fatty acid profiles

C18:2*n*6, C20:0, ΣPUFA and Σ*n*–6 were significantly higher in the wild fish as compared to the farmed cases (Table 5). C22:1*n*9 was only detected in the wild fish. Other fatty acids did not differ between the wild and the farmed cases, and the same goes for the groupings ΣSFA, ΣMUFA, Σ*n*–3, *n*–3/*n*–6, *n*–6/*n*–3, PUFA/SFA and USFA/SFA.

3.7 Carcass proximate composition

There were no differences in moisture, crude protein, crude lipid and crude ash between the wild and the farmed fish (Table 6).

Table 5 Flesh fatty acid profiles (in % dry weight) for the wild and the farmed broadhead catfish

Fatty acid	Wild	Farmed	*P*-value
C12:0	0.28±0.11	0.74±0.30	0.180
C14:0	1.26±0.08	1.54±0.24	0.313
C15:0	0.22±0.01	0.27±0.06	0.435
C16:0	20.27±0.47	19.19±0.16	0.054
C16:1*n*9	1.85±0.12	1.74±0.13	0.532
C18:0	7.76±0.25	7.38±0.39	0.443
C18:1*n*9	28.69±1.36	27.84±1.56	0.688
C18:2*n*6	14.42±0.48^{a}	12.45±0.64^{b}	0.032
C18:3*n*3	1.13±0.06	1.18±0.09	0.634
C20:0	0.18±0.01^{a}	0.16±0.01^{b}	0.010
C20:1*n*9	0.616±0.05	0.54±0.04	0.279
C22:0	0.08±0.01	0.09±0.01	0.731
C22:1*n*9	0.17±0.04	ND	-
C24:1*n*9	2.16±0.19	1.60±0.18	0.061
ΣSFA	30.07±0.68	29.42±0.89	0.573
ΣMUFA	33.43±1.48	31.73±1.50	0.439
ΣPUFA	15.55±0.46^{a}	13.63±0.65^{b}	0.034
Σ*n*–3	1.13±0.06	1.18±0.09	0.634
Σ*n*–6	14.42±0.48^{a}	12.45±0.64^{b}	0.032
n–3/*n*–6	0.07±0.01	0.09±0.01	0.177
n–6/*n*–3	13.11±1.17	10.89±0.93	0.166
PUFA/SFA	0.51±0.02	0.46±0.02	0.137
USFA/SFA	1.63 ±0.06	1.54 ±0.03	0.244

ND: not detected; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; USFA: unsaturated fatty acids. Data are expressed as mean±SEM (n=30). Significant differences between groups are indicated by different superscripts (P<0.05).

Table 6 Proximate chemical composition (% wet weight) of the whole carcass for the wild and the farmed broadhead catfish

Chemical composition	Wild	Farmed	*P*-value
Moisture	65.61±0.59	65.81±0.81	0.841
Crude protein	16.13±0.33	15.89±0.31	0.592
Crude lipid	10.99±0.62	10.77±0.71	0.788
Crude ash	4.24±0.10	4.43±0.20	0.455

Data are expressed as mean±SEM (n=10). Significant differences between groups are indicated by different superscripts (P<0.05).

4 DISCUSSION

Morphometric measurement is simple, not demanding great scientific expertise, and it is reliable and fast for distinguishing the wild from the farmed population of various fish species (Uglem et al., 2011; Arechavala-Lopez et al., 2012; Fagbuaro et al., 2015). The observed caracteristics are results from the culture process or the spontaneous induction, with relatively permanent nature (Fjelldal et al., 2009) so they persist throughout the entire lifespan. In the current study, standard length, body depth, eye width, dorsal fin height and tentacle length exhibited significant differences for segregating the two populations. In European seabass (*Dicentrarchus labrax*), CI and relative profile index are widely applicable in the identification of wild and farmed fish (Arechavala-Lopez et al., 2012), while dorsal fin size, neck curvature and lower jaw length are similarly used for Atlantic cod, *Gadus morhua* (Uglem et al., 2011). Relative profile index and body proportions (standard length/total length, eye diameter/head length, and postocular distance/head length) are also used for gilthead seabream, *Sparus aurata* (Arechavala-Lopez et al., 2012). On the other hand, there were no significant differences in the geometrical morphometry between lagoon caught and cultured cases of this species (Çoban et al., 2008). Within the same genus as *C. macrocephalus*, wild and farmed population of *C. gariepinus* can be discriminated by nine parameters, as reported by Fagbuaro et al. (2015). Although some discriminating parameters for various species match our findings in the current study, still the most commonly informative parameters showed no differences in this study. This may be due to similarity of the environmental conditions of the wild and the cultured populations (Çoban et al., 2008), or may be a result of sufficient fertility or low competition level in the wild population (Fagbuaro et al., 2015). In addition, genetic and environmental factors may affect morphometric differentiation, and could be investigated further. Preliminary screening in fieldwork by morphometric measurements appears practical as it does not require chemicals or sensitive equipment, while the other assessed characteristics are only appropriate to laboratory studies.

Five visceral organ indexes and activities of digestive enzymes were significantly different between the wild and the farmed fish. However, some effects on the harvested live fish might be due to gut emptying from food starvation during a few days of captivity, reducing gastrointestinal functionality. The higher gastrointestinal weight relative to body weight of the wild fish probably relates to the larger sized preys in the wild, such as aquatic insects, young

shrimp and smaller fishes (Teugels et al., 1999), while the farmed fish were receiving the dry pellet diet with smaller size. Therefore, direct harvest from the fish sources might provide different trends than sampling from the markets. However, Gildberg (2004) proposed that the carnivorous species can retain their gastrointestinal functionality, with high levels of digestive enzymes, even during long starvation periods. Perivisceral fat index was higher in the wild fish than in the farmed fish. The high amount of lipid in live diet is reasonable, increasing the lipase activity for digestion. Jobling (1988) suggested that farmed fish have poorer stomach digestion of pelleted diet than of wild-captured preys. Significant increase in pepsin specific activity might improve the capacity to digest dietary protein. Regarding trypsin and chymotrypsin, no differences in the specific activities of these enzymes were observed between the wild and the farmed fish. This finding is in agreement with the observations on wild captured and farmed cod, *G. morhua* (Lemieux et al., 1999). Since glucose is an essential energy source for a number of tissues, it is particularly important to maintain the glucose levels throughout starvation (Romijn et al., 1990). Therefore, maintaining the amylase activity level appears to be reasonable, as well as maintaining the amylase to trypsin ratio.

Color is among the most important characteristics used to evaluate the quality of fishing products. The black skin of *C. macrocephalus* is removed as it looks unattractive for the canned fish industry, and this reveals a clear yellow color that is appreciated by the Asian consumers (Cacot and Hung, 2009). Based on our investigation of the flesh, no differences in the color coordinates were observed for the two fish populations. Possibly the environmental conditions were rather similar in the pond cultivation of farmed fish and in the wild habitats, such as marshes, canals, ricefields, stagnant pools and rivers. Similar findings are also reported in Atlantic salmon, *Salmo salar* (Johnston et al., 2006). On the other hand, Rincón et al. (2016) reported differences in b^* and C^* (chroma) values for the flesh of wild and farmed blackspot seabream (*Pagellus bogaraveo*), as well as in a^* values for yellow perch, *Perca flavescens* (González et al., 2006). The variation in color within various fish species relates not only to rearing conditions but also to diet composition and the specific mechanisms of coloration in the species.

Higher radical scavenging activity was observed in the wild fish than in the farmed fish. This finding conflicts with the reported concentrations of vitamin E in wild and farmed Atlantic salmon (Johnston et al., 2006). High vitamin E doses in the commercial diet (Baker, 2001) as well as colorant supplementation may improve the scavenging activity in farmed salmon. In contrast, colorants are not necessary in the commercial diet of broadhead catfish while the live diets contain various pigments, increasing the capacity against oxidation of free radicals. In addition, higher welfare animal products often contain higher levels of antioxidants than intensively produced animal products (Compassion in World Farming, 2012). This reduces the risk of quality loss by oxidation, extending shelf life, and also potentially has positive effects on health of the consumer. From the health food products perspective, improving the radical scavenging activity in farmed fish flesh appears to be a neglected opportunity.

Protein synthesis capacity is a biochemical marker of the growth quality of fish (Rungruangsak-Torrissen, 2007; Thongprajukaew et al., 2013). Regardless of sex and age recorded, no differences were observed between the two groups of broadhead catfish, indicating no effects of fish origins on these flesh qualities. However, the proteins that control biological processes, molecular function and cellular components have different expression profiles in wild and farmed gilthead sea bream (Piovesana et al., 2016). In fish, myofibrillar proteins are the major component (39%–56%), followed by sarcoplasmic proteins (21%–25%) and stromal or connective tissue proteins (6%–21%) (Chaijan et al., 2010). In the current study, there were no differences in thermal characteristics (T_o, T_d and T_c) of the major muscle proteins, myosin and actin, between wild and farmed *C. macrocephalus* populations. Since ΔH is a measure of the protein amount left in its native state, the higher ΔH of myosin in the muscle of farmed fish may relate to this. Coughlin et al. (2016) have reported effects of rearing condition on myosin heavy chain expression, causing changes in swimming performance and muscle contractile properties. That only the ΔH of myosin changed is not surprising, since it is the most abundant myofibrillar protein that contributes 50%–60% of the total (Shahidi, 1994). There were no differences in ΔH of actin or actin/myosin between the two groups. Regarding sarcoplasmic proteins, the wild broadhead catfish exbibited six thermal characters of which four could be used to distinguish the populations by qualitative and quantitative determinations. Variation in dietary food items and activities in the wild may

affect these proteins in both form and function. Therefore, the proteomics of muscle appears to be a powerful tool to identify the origin of fish.

The fatty acid profile can be used as a fingerprint due to substantial differences between wild and farmed fish (Jensen et al., 2013). In the current study, differences between the populations were apparent in both individual fatty acids (C18:2*n*6, C20:0, C22:1*n*9 and C24:0) and their groups (ΣPUFA and Σ*n*–6). In white sturgeon (*Acipenser transmontanus*), the most notable difference was the concentration of C18:2*n*6 (linoleic acid), while other differences between fatty acids were smaller (DePeters et al., 2013). The use of C18:2*n*6 as a potent marker was also reported in European seabass (Lenas et al., 2011) and blackspot seabream (Rincón et al., 2016), but did not serve well in the case of yellow perch (González et al., 2006). For C20:0 (arachidic acid), the significantly elevated concentration in the wild population of this study is in agreement with observations in yellowtail, *Seriola lalandi* (O'Neill et al., 2015). However, the concentration of this fatty acid is relatively low in comparison to the main SFA, C16:0 (palmitic acid). The C22:1*n*9 (erucic acid) can serve as marker with presence only in the wild population of broadhead catfish. Erucic acid is produced across a great range of green plants. For industrial purposes, low erucic acid rapeseed (LEAR) has been developed (canola), which contains fats derived from oleic acid instead of erucic acid (Anneken et al., 2006). This fatty acid is not detectable in aquafeed, while very low amounts can be detected in wild catfish due to the ingested food. Higher amount of C22:1*n*9 has also been observed in fillet and perivisceral fat of wild seabass, relative to the farmed cases (Lenas et al., 2011). These differences contributed to the higher ΣPUFA and Σ*n*–6 in the wild fish relative to the farmed fish. While statistical differences was detected, the changes in fatty acid composition of broadhead catfish were smallish. This might be due to the small-scale aquaculture of fish farming in Thailand, in ponds that also provide live food. Expanding the study by sampling food items and observing environmental parameters could inform about the phenotypical effects in this fish species; the current sampling of fish from markets could not exclude genotypic effects.

Generally, differences in proximate compositions of farmed and wild fish are expected (González et al., 2006; Rincón et al., 2016) due to a variety of factors, including type of available food, dietary ingredients (commercial diets are usually high in fat content and also include dietary carbohydrate) and higher energy consumption of the farmed fish than of the wild fish (Grigorakis et al., 2002). However, in the current study there were no significant differences in the carcass proximate composition between the two catfish populations. This is in agreement with the flesh composition study by O'Neill et al. (2015). The similarity of nutritional composition of catfish from the two populations from the current study might affect the nutritional images in the minds of fish consumers, and help preserve the wild stock of this near threatened species and to improve the sustainability of aquaculture. However, habitat and seasonal variations could affect the observed trends, and further studies would be warranted to ensure wider generality than what the current study could provide.

5 CONCLUSION

Morphometric measurements (standard length, body depth, eye width, fin height and tentacle length) and digestive indexes (intestosomatic index, digestosomatic index, perivisceral fat index and activities of pepsin and lipase) and flesh quality in terms of thermal characteristics of sarcoplasmic proteins and myosin denaturation enthalpy, and fatty acid profiles (C18:2*n*6, C20:0, C22:1*n*9, C24:0, Σpolyunsaturated fatty acids, and Σ*n*–6) were assessed for distinguishing between wild and farmed broadhead catfish. These measured characteristics were informative with sufficient capacity to discriminate between the two fish populations, while flesh protein synthesis capacity and color did not differ. Flesh radical scavenging activities and reducing power were significantly higher in the wild fish than in the farm-raised group, while no differences in carcass proximate composition were observed across two fish sources. Findings from the current study can be applied in forensic identification when high priced wild catfish are substituted for by lower priced farmed catfish, as well as in identifying fish escapees. Conservation biology of this near threatened species and providing correct nutritional images to the consumers of fish and marketing are also applicable.

6 ACKNOWLEDGEMENT

We acknowledge Assoc. Prof. Dr. Seppo Karrila and the Publication Clinic, Research and Development Office, Prince of Songkla University, for advice in manuscript preparation.

7 DATA AVAILABILITY STATEMENT

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

References

Anneken D J, Both S, Christoph R, Fieg G, Steinberner U, Westfechtel A. 2006. Fatty acids. *In*: Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH, Weinheim.

AOAC. 2005. Official Methods of Analysis of AOAC International. 18th edn. Association of Official Analytical Chemists, Arlington VA.

Arechavala-Lopez P, Sanchez-Jerez P, Bayle-Sempere J T, Sfakianakis D G, Somarakis S. 2012. Morphological differences between wild and farmed Mediterranean fish. *Hydrobiologia*, **679**(1): 217-231.

Areekijseree M, Engkagul A, Kovitvadhi U, Thongpan A, Mingmuang M, Pakkong P, Rungruangsak-Torrissen K. 2004. Temperature and pH characteristics of amylase and proteinase of adult freshwater pearl mussel, *Hyriopsis* (*Hyriopsis*) *bialatus* Simpson 1900. *Aquaculture*, **234**(1-4): 575-587.

Baker R T M. 2001. The effect of certain micronutrients on fish flesh quality. *In*: Kestin S C, Warriss P D eds. Farmed Fish Quality. Blackwell Science, Oxford. p.180-191.

Cacot P, Hung L T. 2009. Overview of the catfishes aquaculture. *In*: Safran P ed. Fisheries and Aquaculture Vol. III. EOLSS Publishers, Oxford. p.250-277.

Chaijan M, Jongjareonrak A, Phatcharat S, Benjakul S, Rawdkuen S. 2010. Chemical compositions and characteristics of farm raised giant catfish (*Pangasianodon gigas*) muscle. *LWT-Food Science and Technology*, **43**(3): 452-457.

Claret A, Guerrero L, Gartzia I, Garcia-Quiroga M, Ginés R. 2016. Does information affect consumer liking of farmed and wild fish? *Aquaculture*, **454**: 157-162.

Çoban D, Saka Ş, Firat K. 2008. Morphometric comparison of cultured and lagoon caught gilthead seabream (*Sparus aurata* L. 1758). *Turkish Journal of Zoology*, **32**(3): 337-341.

Compassion in World Farming. 2012. Nutritional Benefits of Higher Welfare Animal Products. Compassion in World Farming, Surrey.

Coughlin D J, Shiels L P, Nuthakki S, Shuman J L. 2016. Thermal acclimation to cold alters myosin content and contractile properties of rainbow smelt, *Osmerus mordax*, red muscle. *Comparative Biochemistry and Physiology Part A*: *Molecular & Integrative Physiology*, **196**: 46-53.

DePeters E J, Puschner B, Taylor S J, Rodzen J A. 2013. Can fatty acid and mineral compositions of sturgeon eggs distinguish between farm-raised versus wild white (*Acipenser transmontanus*) sturgeon origins in California? Preliminary report. *Forensic Science International*, **229**(1-3): 128-132.

Fagbuaro O, Oso J A, Olurotimi M B, Akinyemi O. 2015. Morphometric and meristic characteristics of *Clarias gariepinus* from controlled and uncontrolled population from southwestern Nigeria. *Journal of Agriculture and Ecology Research International*, **2**(1): 39-45.

FAO. 2015. Global Aquaculture Production Database. FAO, Rome.

Fjelldal P G, van der Meeren T, Jørstad K E, Hansen T J. 2009. A radiological study on vertebral deformities in cultured and wild Atlantic cod (*Gadus morhua* L.). *Aquaculture*, **289**(1-2): 6-12.

Gildberg A. 2004. Digestive enzyme activities in starved pre-slaughter farmed and wild-captured, Atlantic cod (*Gadus morhua*). *Aquaculture*, **238**(1-4): 343-353.

González S, Flick G J, O'Keef S F, Duncan S E, McLean E, Craig S R. 2006. Composition of farmed and wild yellow perch (*Perca flavescens*). *Journal of Food Composition and Analysis*, **19**(6-7): 720-726.

Grigorakis K, Alexis M N, Taylor K D A, Hole M. 2002. Comparison of wild and cultured gilthead sea bream (*Sparus aurata*); composition, appearance and seasonal variations. *International Journal of Food Science and Technology*, **37**(5): 477-484.

Hahor W, Thongprajukaew K, Yoonram K, Rodjaroen S. 2016. Gastrointestinal functionality of aquatic animal (*Oreochromis niloticus*) carcass in water allows estimating time of death. *Journal of Forensic Sciences*, **61**(6): 1 647-1 655.

Jensen I J, Larsen R, Rustad T, Eilertsen K E. 2013. Nutritional content and bioactive properties of wild and farmed cod (*Gadus morhua* L.) subjected to food preparation. *Journal of Food Composition and Analysis*, **31**(2): 212-216.

Jobling M. 1988. A review of the physiological and nutritional energetics of cod, *Gadus morhu*a L., with particular reference to growth under farmed conditions. *Aquaculture*, **70**(1-2): 1-19.

Johnston I A, Li X J, Vieira V L A, Nickell D, Dingwall A, Alderson R, Campbell P, Bickerdike R. 2006. Muscle and flesh quality traits in wild and farmed Atlantic salmon. *Aquaculture*, **256**(1-4): 323-336.

Kates M. 1986. Techniques of Lipidology: Isolation, Analysis and Identification of Lipids. 2nd edn. Elsevier, Amsterdam.

Lemieux H, Blier P, Dutil J D. 1999. Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in the Atlantic cod (*Gadus morhua*)? *Fish Physiology and Biochemistry*, **20**(4): 293-303.

Lenas D, Chatziantoniou S, Nathanailides C, Triantafillou D. 2011. Comparison of wild and farmed sea bass (*Dicentrarchus labrax* L) lipid quality. *Procedia Food Science*, **1**: 1 139-1 145.

Lowry O H, Rosenbrough N J, Farr A L, Randall R J. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**(1): 265-275.

Matos E, Silva T S, Tiago T, Aureliano M, Dinis M T, Dias J. 2011. Effect of harvesting stress and storage conditions on protein degradation in fillets of farmed gilthead seabream (*Sparus aurata*): a differential scanning calorimetry study.

Food Chemistry, **126**(1): 270-276.

Na-Nakorn U, Wongpathom K, Thawatchai N. 2004. Genetic diversity of walking catfish, *Clarias macrocephalus*, in Thailand and evidence of genetic introgression from introduced farmed *C. gariepinus*. *Aquaculture*, **240**(1-4): 145-163.

O'Neill B, Le Roux A, Hoffman L C. 2015. Comparative study of the nutritional composition of wild versus farmed yellowtail (*Seriola lalandi*). *Aquaculture*, **448**: 169-175.

Piovesana S, Capriotti A L, Caruso G, Cavaliere C, La Barbera G, Chiozzi R Z, Laganà A. 2016. Labeling and label free shotgun proteomics approaches to characterize muscle tissue from farmed and wild gilthead sea bream (*Sparus aurata*). *Journal of Chromatography A*, **1428**: 193-201.

Rincón L, Castro P L, Álvarez B, Hernández M D, Álvarez A, Claret A, Guerrero L, Ginés R. 2016. Differences in proximal and fatty acid profiles, sensory characteristics, texture, colour and muscle cellularity between wild and farmed blackspot seabream (*Pagellus bogaraveo*). *Aquaculture*, **451**: 195-204.

Romijn J A, Godfried M H, Hommes M J T, Endert E, Sauerwein H P. 1990. Decreased glucose oxidation during short-term starvation. *Metabolism*, **39**(5): 525-530.

Rungruangsak K, Utne F. 1981. Effect of different acidified wet feeds on protease activities in the digestive tract and on growth rate of rainbow trout (*Salmo gairdneri* Richardson). *Aquaculture*, **22**: 67-79.

Rungruangsak-Torrissen K. 2007. Digestive efficiency, growth and qualities of muscle and oocyte in Atlantic salmon (*Salmo salar* L.) fed on diets with krill meal as an alternative protein source. *Journal of Food Biochemistry*, **31**(4): 509-540.

Shahidi F. 1994. Seafood proteins and preparation of protein concentrates. *In*: Shahidi F, Botta J R eds. Seafoods: Chemistry, Processing Technology and Quality. Springer, Boston, MA. p.3-9.

Skipnes D, van der Plancken I, van Loey A, Hendrickx M E. 2008. Kinetics of heat denaturation of proteins from farmed Atlantic cod (*Gadus morhua*). *Journal of Food Engineering*, **85**(1): 51-58.

Teugels G G, Diego R C, Pouyaud L, Legendre M. 1999. Redescription of *Clarias macrocephalus* (Siluriformes: Clariidae) from South-East Asia. *Cybium*, **23**(3): 285-295.

Thongprajukaew K, Kovitvadhi U, Kovitvadhi S, Engkagul A, Rungruangsak-Torrissen K. 2013. Evaluation of growth performance and nutritional quality of diets using enzymatic markers and *in vitro* digestibility in Siamese fighting fish (*Betta splendens* Regan, 1910). *African Journal of Biotechnology*, **12**(14): 1 689-1 702.

Thongprajukaew K, Rodjaroen S, Yoonram K, Sornthong P, Hutcha N, Tantikitti C, Kovitvadhi U. 2015. Effects of dietary modified palm kernel meal on growth, feed utilization, radical scavenging activity, carcass composition and muscle quality in sex reversed Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, **439**: 45-52.

Uglem I, Berg M, Varne R, Nilsen R, Mork J, Bjørn P A. 2011. Discrimination of wild and farmed Atlantic cod (*Gadus morhua*) based on morphology and scale-circuli pattern. *ICES Journal of Marine Science*, **68**(9): 1 928-1 936.

Vidthayanon C, Allen D J. 2011. *Clarias macrocephalus*. The IUCN Red List of Threatened Species 2011:e. T166020A6170044. International Union for Conservation of Nature and Natural Resources, Gland. https://doi.org/10.2305/IUCN.UK.2011-1.RLTS.T166020A6170044.en.

Winkler U K, Stuckmann M. 1979. Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. *Journal of Bacteriology*, **138**(3): 663-670.