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A nitrogen factor for European pike-perch (*Sander lucioperca*), northern pike (*Esox lucius*), and sheatfish (*Silurus glanis*) fillets

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Abstract

Measures for consumer protection against food adulteration and misleading labeling are integrated into EU legislation, including methods for detection of misleading practices. Verification of meat content is available for marine products but not for freshwater fish because of the lack of standard nitrogen factors. The aim of this study was to establish nitrogen factors for European pike-perch *Sander lucioperca* (Linnaeus, 1758), northern pike *Esox lucius* Linnaeus, 1758, and sheatfish *Silurus glanis* Linnaeus, 1758. The study involved analysis of 808 fillet samples obtained in spring (March–April) and autumn (October–November) harvest seasons, 2018–2019, from seven Czech Republic fish rearing facilities. Samples with and without skin were analyzed for nitrogen content, dry matter, protein, ash, and fat according to established ISO methods. The recommended nitrogen factor for European pike-perch with the skin is 3.28 ± 0.09 and without the skin is 3.21 ± 0.09; for northern pike with the skin is 3.18 ± 0.09 and without skin is 3.15 ± 0.09; and for sheatfish with skin is 2.73 ± 0.13 and without skin is 2.75 ± 0.12. The established nitrogen factors will enable analysis of meat content to ensure that consumers are purchasing correctly described and labeled fish products.

Keywords

Adulteration, fish-food fraud, fish products, nitrogen factor, fish

Introduction

Freshwater and marine fishes play a significant role in human nutrition as a source of protein and other nutrients (FAO 2016). They provide crucial elements of a healthy diet including digestibility, low fat content, essential vitamins and minerals, and a higher content of unsaturated fatty acids compared to terrestrial animal fat (Adamkova et al. 2011; Lund 2013; Mraz et al. 2017; Linhartova et al. 2018). The nutritional composition of fish muscle is influenced by many factors such as fish species, age, gender, rearing conditions, breeding technology, and season, etc.(Fajmonova et al. 2003; Buchtova et al. 2008, 2010; Adeniyi et al. 2012).

The annual global per capita consumption of fish products increased from 9.0 kg in 1961 to 20.3 kg in 2017. In this period, fish consumption increased by 3.1 percentage points annually, more than the annual population growth rate of 1.6 percentage points (FAO 2020). The mean consumption of fish and fish products per capita in the EU was 24.4 kg in 2017 (EC 2020). Variation in consumption among EU countries is significant, which is associated with the eating habits.

Pond fish farming has a long tradition in the Czech Republic, dating to the 12th century with the oldest documented pond in Bohemia established in 1115. In the past ten years, the production of freshwater fish in the Czech Republic reached 20 400–21 800 tons per year, of which approximately 10% is represented by processed fish products for foreign and domestic markets (MA 2019).

Some farmed freshwater fish species popular with consumers are among the more costly foodstuffs, motivating fraud among producers. Meat, including fish meat, carries a high potential for economically motivated adulteration (Čížková et al. 2012; Cavin et al. 2018), most commonly consisting of the addition of undeclared substances or their substitution for genuine ingredients (Everstine et al. 2013; Cavin et al. 2018). These illegal practices can endanger the health of consumers. Examples include the addition of melamine to milk powder used for infant formula, Sudan dyes in chili and paprika, the addition of methanol to spirits, the European horsemeat scandal in 2013 or fipronil contamination of eggs. Another example of misleading of consumers can be undeclared added water (Čížková et al. 2012; Cavin et al. 2018; Morin and Lees 2018).

Consumer protection against adulteration and misleading food labeling is contained in EU legislation. Methods for detection of practices misleading the consumer for pork, chicken, and seafoods have been published in European legislation and in standards of Codex Alimentarius. These are primarily procedures to determine meat content or content of absorbed water (EC 2002; CA 2004; EC 2008). The regulation of meat content in farmed freshwater fish products is not possible because of the absence of established species-specific nitrogen content (nitrogen factor) for freshwater fish, with the exception of tilapia (CA 2004).

The aim of this study was to establish nitrogen factors as determined by the Kjeldahl method (ISO 937 1978) within the context of the Codex standard (CA 2004) for European pike-perch *Sander lucioperca* (Linnaeus, 1758); northern pike *Esox lucius* Linnaeus, 1758; and sheatfish, *Silurus glanis* Linnaeus, 1758) the primary predatory fish species farmed and processed in the Czech Republic for commercial markets. The established nitrogen factors have the potential to provide the basis for verifying content of freshwater fish products.

Methods

Experimental animals and experimental protocol

One-hundred-three market-size European pike-perch (385–2025 g weight) were obtained from five Czech aquaculture facilities: The University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters (FFPW USB), Vodnany, and the Blatna, Hodonin, Klatovy, and Lnare fisheries. Two-hundred-seven market-size northern pike (445–3980 g) were obtained from seven Czech aquaculture facilities: FFPW USB and the Chlumec nad Cidlinou, Blatna, Hodonin, Klatovy, Lnare, and Tabor fisheries. Ninety-four market-size sheatfish (505–9265 g) were obtained from FFPW USB and the Chlumec nad Cidlinou, Blatna, Hodonin, and Klatovy fisheries. The location of the farms is shown in Fig. 1. In order to assess any effect of year, season, and location, fish were collected in the spring (March–April) and autumn (October–November) harvesting seasons in 2018 and 2019. The fish were transported live to the laboratory of the Faculty of Fisheries and Protection of Waters, killed by a blow to the head, weighed, measured, and filleted. Two fillets, one with skin removed, from each fish were individually vacuum packed, immediately frozen, and stored at –32°C until chemical analysis. A total of 808 fillets were chemically analyzed.

The study was conducted according to the principles of the Ethical Committee for the Protection of Animals in Research of the University of South Bohemia, Faculty of Fisheries and Protection of Waters, Vodnany.

Chemical analysis

Samples of fish fillets with and without skin were analyzed for dry matter, protein, fat, and ash. After partial thawing to avoid loss of water and soluble protein fractions, samples were homogenized by grinding on the knife mill PULVERISETTE 11 (FRITSCH GmbH, Germany).

The determination of percentage of dry matter was based on the standard method ISO 1442 (1997). The homogenized samples were dried with sand up to constant weight at 103 ± 2°C in the laboratory oven Memmert UE 500 (Memmert GmbH + Co. KG, Germany).

The determination of ash content was based on the standard ISO 936 (1998). The homogenized samples were burned in muffle furnace Nabertherm A11/HR (Nabertherm GmbH, Germany) at 550 ± 25°C to a greyish-white color of ash.

Total fat content was assessed based on the standard ISO 1443 (1973). The homogenized samples were hy-

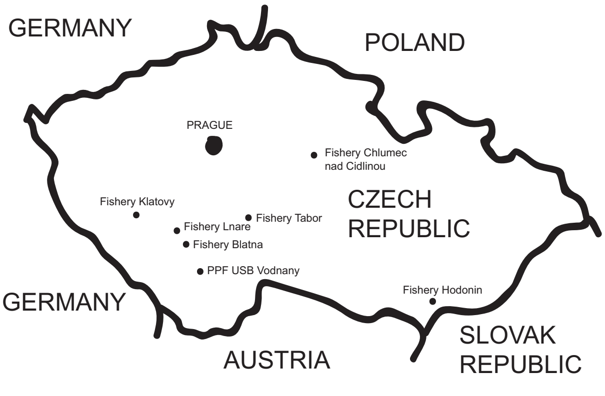

Figure 1. Map of the Czech Republic showing location of aquaculture facilities.

drolyzed by hydrochloric acid, and fat was extracted by light petroleum in the SOXTEC 2050 (FOSS Headquarters, Denmark).

The determination of protein content used the Kjeldahl method based on the standard method ISO 937 (1978). The homogenized samples were digested by sulfuric acid and a catalyzer in the digestion unit KjelROC Digestor 20 (OPSIS AB, Sweden) at 420 ± 10°C. Organically bound nitrogen was determined on the KJELTEC 8400 with KJELTEC sampler 8420 (FOSS Headquarters, Denmark). The coefficient 6.25, the conversion factor for meat, was used for calculation of protein content from the nitrogen content.

Analyses of the dry matter, ash, and total fat were conducted in duplicate, and analysis of protein was performed in triplicate for each sample.

Statistical analysis

Kolmogorov–Smirnov and Bartlett's tests were applied to assess normal distribution data and the homoscedasticity of the variance, respectively. A two-way ANOVA with a subsequent Tukey's test was performed to test the effects of season, weight of fish, place of rearing, and the difference between fillets with and without skin. The significance level was set at $P < 0.05$. Data were expressed as mean ± SD (minimum–maximum). The analysis was performed using STATISTICA v.12.0 for Windows (STATSOFT, Inc.).

Principal component analysis (PCA) of the nitrogen and fat content in fillets with and without skin as response values was applied to describe differences in fish samples from different locations. The analysis was complemented with a redundancy analysis (RDA) with whole fish weight as an independent explanatory variable. The ordination plots were produced using Canoco for Windows v.5.10 (Biometris, The Netherlands and P. Šmilauer, Czech Republic).

Results

European pike-perch

The basic composition of fillets with and without skin of European pike-perch is given in Table 1. Dry matter content was significantly lower ($P < 0.01$) in European pike-perch fillets without skin from Lnare (spring 2019, 21.40 ± 0.81), Hodonin (spring 2019, 21.46 ± 0.31 and autumn 2019, 21.49 ± 0.29) and Klatovy (autumn 2018, 21.24 ± 0.73) fisheries compared to those with skin from Blatna in autumn 2018 (23.18 ± 0.42) and autumn 2019 (23.07 ± 0.46). We found no significant differences ($P > 0.05$) in dry matter content between European pike-perch fillets with and without skin within a sampling period.

The ash content was significantly lower ($P < 0.01$) in European pike-perch fillets with skin from Lnare (spring 2018, 1.17 ± 0.04) and Hodonin (spring 2019, 1.17 ± 0.04) and fillets without skin from Lnare (spring 2018, 1.20 ± 0.03; autumn 2018, 1.20 ± 0.12; and autumn 2019, 1.18 ± 0.05) and Hodonin (spring 2019, 1.20 ± 0.03), compared to fillets with (1.3 ± 0.08) and without (1.38 ± 0.11) skin from Lnare in spring 2019. We found no significant differences ($P > 0.05$) in ash content of European pike-perch fillets with and without skin within a sampling period.

The fat content was significantly lower ($P < 0.01$) in European pike-perch fillets with (0.14 ± 0.10) and without (0.15 ± 0.14) skin from Lnare (spring 2018), with skin (0.21 ± 0.04) from Blatna (autumn 2019), and without skin (0.21 ± 0.07) from Hodonin (autumn 2019) compared to other groups. We found significant differences ($P < 0.01$) in fat content of fillets with skin and those without skin in European pike-perch from FFPW USB in spring 2018 (1.37 ± 0.50 and 0.69 ± 0.20), Blatna in autumn 2018 (0.75 ± 0.35 and 0.54 ± 0.26) and 2019 (0.2 ± 0.04 and 0.31 ± 0.11), Klatovy in autumn 2018 (0.73 ± 0.31 and 0.56 ± 0.37), and Hodonin in autumn 2019 (0.28 ± 0.15 and 0.21 ± 0.07).

We found no significant differences ($P > 0.05$) in protein content of European pike-perch from with respect to farm, season, year, or presence of skin.

The nitrogen content was significantly lower ($P < 0.01$) in European pike-perch fillets without skin from Hodonin (spring 2019, 3.13 ± 0.05) and Klatovy (autumn 2018, 3.05 ± 0.09) than in those fillets with skin from Lnare in autumn 2019 (3.34 ± 0.06) and Blatna in autumn 2018 (3.32 ± 0.06) and 2019 (3.38 ± 0.05). We found no significant differences ($P > 0.05$) in nitrogen content of European pike-perch fillets with and without skin in a single sampling period.

Principal component analysis extracted the first two axes explaining 74.5% of the total variance. It did not explain the differences between functional traits fillets with and without skin, but a negative correlation of nitrogen and fat content with fish weight was revealed (Fig. 2). In the majority of samples of European pike-perch, PCA discriminated along the PC1 axis explaining 44.0% of the total variance in a cluster along the nitrogen gradient. Samples from FFPW USB (spring 2019) and Blatna (autumn 2019) showed a positive correlation of fat with fish weight. Samples from Klatovy (autumn 2018), Lnare (spring 2019), and Hodonin (spring 2019) were inconsistent and negatively correlated with nitrogen content. Redundancy analysis revealed little correlation of fat content with fish weight as an explanatory factor (first canonical axes explained 56.2% of variability, permutation test on all axes: pseudo-$F = 16.3$, $P < 0.002$). Weight was not considered the main factor in nitrogen content.

Northern pike

The basic chemical composition of northern pike fillets with and without skin are given in Table 2. Dry matter content was significantly lower ($P < 0.01$) in fillets with and without skin from Chlumec nad Cidlinou (spring

Table 1. Live weight and length of European pike-perch (*Sander lucioperca*) and chemical composition of fillets with skin and without skin.

Company	Year	Season	Weight [g] x ± SD (min–max)	Total length [cm] x ± SD (min–max)	Fillet	Dry matter [g · 100 g^{-1}] x ± SD (min–max)	Ash [g · 100 g^{-1}] x ± SD (min–max)	Fat [g · 100 g^{-1}] x ± SD (min–max)	Protein [g · 100 g^{-1}] x ± SD (min–max)	N [g · 100 g^{-1}] x ± SD (min–max)
FFPW USB Vodnany	2018	Spring	1359.1 ± 274.9 (1015.0–2025.0)	51.6 ± 3.1 (48.0–57.0)	with skin	22.47 ± 0.33$^{ab(A)}$ (21.77–22.94)	1.35 ± 0.62$^{ab(A)}$ (1.07–3.21)	1.37 ± 0.50$^{d(C)}$ (0.83–2.59)	20.66 ± 0.35$^{a(A)}$ (20.18–21.47)	3.30 ± 0.06$^{ab(A)}$ (3.23–3.43)
					without skin	21.86 ± 0.27$^{ab(A)}$ (19.89–20.58)	1.26 ± 0.06$^{ab(A)}$ (1.16–1.40)	0.69 ± 0.20$^{c(B)*}$ (0.24–0.97)	20.22 ± 0.27$^{a(A)}$ (21.28–22.27)	3.23 ± 0.03$^{ab(A)}$ (3.18–3.29)
	2019	Spring	563.0 ± 119.6 (435.0–805.0)	39.1 ± 2.4 (36.5–44.0)	with skin	22.64 ± 0.26$^{ab(A)}$ (22.36–23.17)	1.25 ± 0.04$^{ab(A)}$ (1.21–1.32)	0.33 ± 0.08$^{b(A)}$ (0.17–0.42)	20.68 ± 0.27$^{a(A)}$ (20.16–21.07)	3.31 ± 0.04$^{ab(A)}$ (3.23–3.37)
					without skin	22.26 ± 0.15$^{ab(A)}$ (21.99–22.46)	1.24 ± 0.03$^{ab(A)}$ (1.20–1.31)	0.37 ± 0.10$^{b(A)}$ (0.16–0.52)	20.37 ± 0.25$^{a(A)}$ (19.99–20.89)	3.26 ± 0.04$^{ab(A)}$ (3.20–3.34)
Fishery Lnare	2018	Spring	437.5 ± 98.9 (330.0–660.0)	35.8 ± 2.1 (36.5–44.0)	with skin	21.72 ± 0.33$^{ab(A)}$ (21.33–22.30)	1.17 ± 0.04$^{a(A)}$ (1.11–1.24)	0.14 ± 0.10$^{a(A)}$ (0.02–0.40)	20.38 ± 0.39$^{a(A)}$ (19.78–21.08)	3.26 ± 0.06$^{ab(A)}$ (3.16–3.37)
					without skin	21.73 ± 0.25$^{ab(A)}$ (21.14–22.00)	1.20 ± 0.03$^{a(A)}$ (1.13–1.24)	0.15 ± 0.14$^{a(A)}$ (0.05–0.57)	20.37 ± 0.31$^{a(A)}$ (19.76–20.81)	3.26 ± 0.05$^{ab(A)}$ (3.16–3.33)
		Autumn	722.0 ± 78.0 (605.0–850.0)	42.3 ± 1.0 (40.5–43.5)	with skin	22.46 ± 0.54$^{ab(A)}$ (20.95–22.98)	1.27 ± 0.04$^{ab(AB)}$ (1.17–1.31)	0.44 ± 0.43$^{b(B)}$ (0.16–1.67)	20.48 ± 0.58$^{a(A)}$ (19.00–21.00)	3.28 ± 0.09$^{ab(A)}$ (3.04–3.36)
					without skin	21.79 ± 0.56$^{ab(A)}$ (20.49–22.44)	1.20 ± 0.12$^{a(A)}$ (0.95–1.48)	0.29 ± 0.18$^{b(B)}$ (0.12–0.76)	19.77 ± 0.47$^{a(A)}$ (18.67–20.27)	3.17 ± 0.07$^{ab(A)}$ (2.99–3.24)
	2019	Spring	562.0 ± 43.6 (500.0–650.0)	38.1 ± 1.4 (36.5–41.0)	with skin	21.91 ± 0.59$^{ab(A)}$ (20.62–22.92)	1.37 ± 0.08$^{b(B)}$ (1.23–1.47)	0.47 ± 0.19$^{b(B)}$ (0.32–0.89)	20.18 ± 0.41$^{a(A)}$ (19.49–20.74)	3.23 ± 0.07$^{ab(A)}$ (3.12–3.32)
					without skin	21.40 ± 0.81$^{a(A)}$ (19.52–22.82)	1.38 ± 0.11$^{b(B)}$ (1.14–1.50)	0.48 ± 0.25$^{b(B)}$ (0.26–1.13	19.75 ± 0.57$^{a(A)}$ (18.30–20.33)	3.17 ± 0.09$^{ab(A)}$ (2.93–3.25)
		Autumn	1044.5 ± 146.0 (805.0–1305.0)	46.2 ± 2.2 (43.0–48.5)	with skin	22.71 ± 0.71$^{ab(A)}$ (21.03–23.63)	1.22 ± 0.04$^{ab(AB)}$ (1.13–1.30)	0.41 ± 0.14$^{b(B)}$ (0.20–0.63)	20.90 ± 0.39$^{a(A)}$ (20.19–21.60)	3.34 ± 0.06$^{b(A)}$ (3.23–3.46)
					without skin	22.34 ± 0.68$^{ab(A)}$ (20.80–23.16)	1.18 ± 0.05$^{a(A)}$ (1.10–1.29)	0.34 ± 0.14$^{b(B)}$ (0.17–0.56)	20.44 ± 0.31$^{a(A)}$ (19.75–20.96)	3.27 ± 0.05$^{ab(A)}$ (3.16–3.35)
Fishery Blatna	2018	Autumn	1230.0 ± 309.2 (765.0–1780.0)	47.3 ± 3.4 (42.0–53.5)	with skin	23.18 ± 0.42$^{b(A)}$ (22.55–23.77)	1.23 ± 0.09$^{ab(A)}$ (1.16–1.47)	0.75 ± 0.35$^{c(C)}$ (0.26–1.36)	20.73 ± 0.40$^{a(A)}$ (20.03–21.60)	3.32 ± 0.06$^{b(A)}$ (3.20–3.46)
					without skin	22.54 ± 0.42$^{ab(A)}$ (22.04–23.28)	1.28 ± 0.07$^{ab(A)}$ (1.20–1.48)	0.54 ± 0.26$^{b(B)*}$ (0.24–1.11)	20.21 ± 0.30$^{a(A)}$ (19.86–20.78)	3.23 ± 0.05$^{ab(A)}$ (3.18–3.33)
	2019	Autumn	627.5 ± 97.8 (385.0–815.0)	39.1 ± 2.8 (32.0–48.0)	with skin	23.07 ± 0.46$^{b(A)}$ (22.31–23.28)	1.30 ± 0.04$^{ab(A)}$ (1.23–1.35)	0.21 ± 0.04$^{a(A)*}$ (0.04–0.37)	21.12 ± 0.29$^{a(A)}$ (20.69–21.67)	3.38 ± 0.05$^{b(A)}$ (3.31–3.47)
					without skin	22.66 ± 0.43$^{ab(A)}$ (21.89–23.28)	1.25 ± 0.06$^{ab(A)}$ (1.17–1.35)	0.31 ± 0.11$^{b(B)}$ (0.12–0.48)	20.63 ± 0.22$^{a(A)}$ (20.33–21.06)	3.30 ± 0.04$^{ab(A)}$ (3.25–3.37)
Fishery Hodonin	2019	Spring	1210.0 ± 386.9 (660.0–1780.0)	49.3 ± 4.8 (41.0–54.5)	with skin	22.04 ± 0.19$^{ab(A)}$ (21.72–22.30)	1.17 ± 0.04$^{a(A)}$ (1.11–1.22)	0.39 ± 0.18$^{b(B)}$ (0.21–0.72)	20.50 ± 0.38$^{a(A)}$ (20.03–20.92)	3.28 ± 0.06$^{ab(A)}$ (3.20–3.35)
					without skin	21.46 ± 0.31$^{a(A)}$ (20.91–21.84)	1.20 ± 0.03$^{a(A)}$ (1.15–1.24)	0.26 ± 0.08$^{b(B)}$ (0.14–0.38)	19.59 ± 0.33$^{a(A)}$ (19.29–20.22)	3.13 ± 0.05$^{a(A)}$ (3.09–3.24)
		Autumn	1053.8 ± 112.0 (910.0–1270.0)	46.3 ± 0.9 (45.0–48.0)	with skin	21.97 ± 0.35$^{ab(A)}$ (21.45–22.55)	1.24 ± 0.07$^{ab(A)}$ (1.11–1.30)	0.28 ± 0.15$^{b(B)}$ (0.05–0.60)	20.44 ± 0.35$^{a(A)}$ (19.86–21.03)	3.27 ± 0.06$^{ab(A)}$ (3.18–3.36)
					without skin	21.49 ± 0.29$^{a(A)}$ (21.12–22.0)	1.27 ± 0.06$^{ab(A)}$ (1.16–1.36)	0.21 ± 0.07$^{a(A)*}$ (0.10–0.29)	19.83 ± 0.14$^{a(A)}$ (19.57–20.03)	3.17 ± 0.02$^{ab(A)}$ (3.13–3.20
Fishery Klatovy	2018	Autumn	1292.0 ± 128.6 (1150.0–1535.0)	49.3 ± 2.1 (46.5–53.0)	with skin	21.76 ± 0.72$^{ab(A)}$ (20.71–22.83)	1.22 ± 0.11$^{ab(A)}$ (1.05–1.43)	0.73 ± 0.31$^{c(B)}$ (0.1–1.37)	19.61 ± 0.61$^{a(A)}$ (18.47–20.88)	3.17 ± 0.10$^{ab(A)}$ (2.95–3.34)
					without skin	21.24 ± 0.73$^{a(A)}$ (20.20–22.38)	1.29 ± 0.12$^{ab(A)}$ (1.07–1.49)	0.56 ± 0.37$^{b(A)*}$ (0.21–1.57)	19.05 ± 0.58$^{a(A)}$ (18.12–19.80)	3.05 ± 0.09$^{a(A)}$ (2.90–3.17)

Data are mean ± standard deviation (minimum value–maximum value), $n = 10$. Values with different small letters in superscripts are significantly ($P < 0.01$) different among the locality groups. Values with different capital letters in superscripts are significantly ($P < 0.01$) different among the season groups in one locality. *Denotes significant differences among fillets with skin or fillets without skin values over one sampling ($P < 0.01$).

2018, 21.29 ± 0.65 and 21.39 ± 0.96), Klatovy (autumn 2019, 21.14 ± 0.62 and 21.35 ± 0.78) and Lnare (autumn 2018, 21.37 ± 0.92 and 21.21 ± 0.95) compared to those from FFPW USB in spring 2019 (23.47 ± 0.33 and 23.19 ± 0.41) and Tabor in spring 2019 (23.52 ± 0.82 and 23.22 ± 0.74). We found no significant differences ($P > 0.05$) between fillets with skin and those without skin in dry matter at a single sampling time.

The ash content was significantly lower ($P < 0.01$) in northern pike fillets with skin (1.17 ± 0.06) from Chlumec nad Cidlinou (autumn 2018) and in fillets with (1.13 ± 0.12) and without (1.16 ± 0.12) skin from Lnare (autumn 2019) compared to fillets with skin from FFPW USB in spring 2018 (1.52 ± 0.45) and from Tabor in autumn 2018 (1.42 ± 0.06). We found no significant differences ($P > 0.05$) in ash content of fillets with and without skin at a single sampling time.

The fat content was significantly lower ($P < 0.01$) in northern pike fillets with skin from Klatovy (autumn 2019, 0.28 ± 0.19), Lnare (spring 2018, 0.33 ± 0.19; autumn 2018, 0.45 ± 0.20; and autumn 2019, 0.41 ± 0.16), and Tabor (spring 2018, 0.43 ± 0.27), and in northern pike fillets without skin from Lnare (autumn 2018, 0.38 ± 0.28 and autumn 2019, 0.44 ± 0.21) and Klatovy (spring 2018, 0.39 ± 0.29 and autumn 2019, 0.40 ± 0.26) compared to other groups. We found significant differences ($P < 0.01$) between fillets with skin and fillets without skin in the fat content of northern pike from Klatovy in spring 2018 (0.64 ± 0.28 and 0.39 ± 0.29), Chlumec nad Cidlinou in autumn 2018 (0.68 ± 0.19 and 0.91 ± 0.32) and Fishery Lnare in spring 2018 (0.33 ± 0.19 and 0.63 ± 0.34).

No significant differences ($P > 0.05$) were observed in protein content of northern pike fillets with respect to farm, season, year, and presence/absence of skin.

The nitrogen content was significantly lower ($P < 0.01$) in northern pike fillets with (3.08 ± 0.08) and without (3.06 ± 0.06) skin from Chlumec nad Cidlinou (spring 2018) and fillets without skin (3.06 ± 0.11) from Blatna

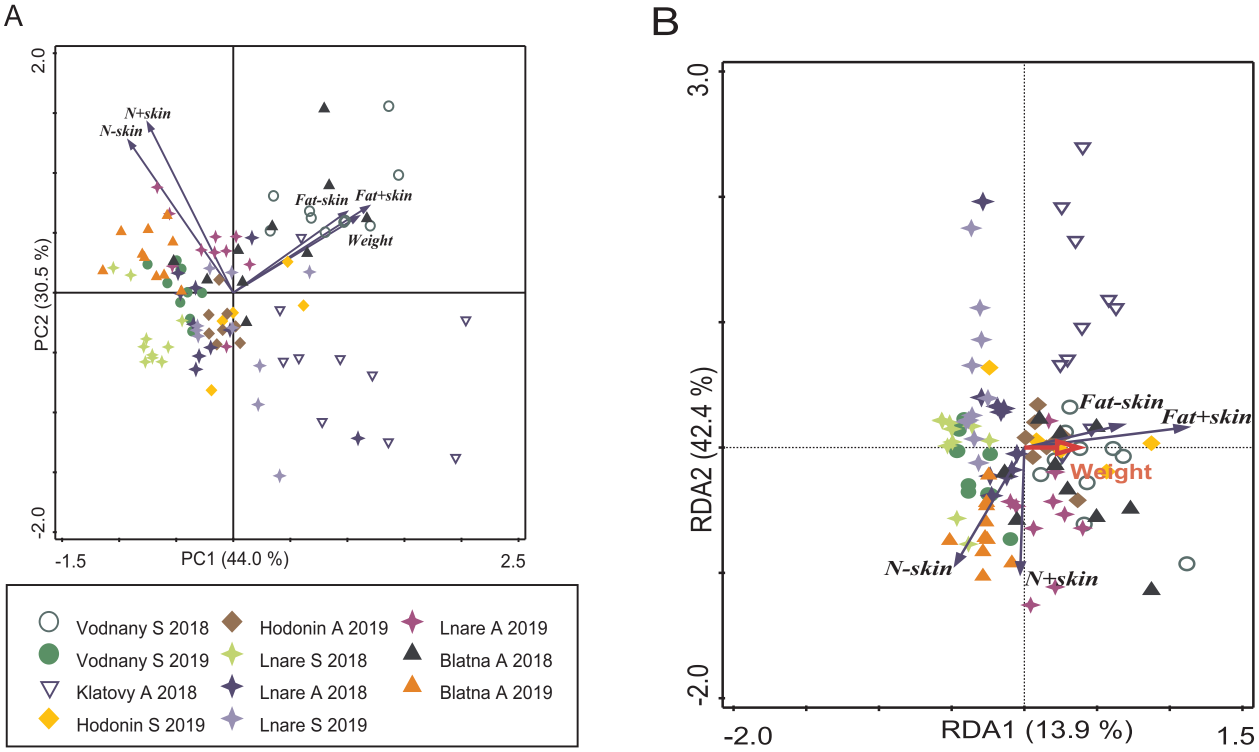

Figure 2. Ordination plots of sample distribution after principal component analysis (PCA) of functional traits as response variables (**A**) and redundancy analysis (RDA) (**B**) of functional traits as response variables and weight of European pike-perch, *Sander lucioperca* as explanatory variable. N+skin = nitrogen concentration in fillet with skin, N-skin = nitrogen concentration in fillet without skin, Fat+skin = fat percentage in fillet with skin, Fat-skin = fat percentage in fillet without skin, Weight = body weight of individual fish; abbreviations used in legend: S = spring sampling, A = autumn sampling). The length of the arrow reflects the power of the variable to differentiate the samples.

(autumn 2019) compared to fillets with skin from Chlumec nad Cidlinou (autumn 2019, 3.22 ± 0.07), Lnare (spring 2018, 3.23 ± 0.09), and fillets with and without skin from Tabor (autumn 2018, 3.22 ± 0.03 and 3.22 ± 0.05 and spring 2019, 3.25 ± 0.07 and 3.22 ± 0.07) and Klatovy (spring 2018, 3.24 ± 0.07 and 3.22 ± 0.06). We found no significant differences ($P > 0.05$) in nitrogen content of northern pike fillets with and without skin at a single sampling.

The PCA distribution of samples in northern pike did not show separate clusters, and the samples were discriminated against both axes (Fig. 3). The first two axes explained 75.42% of total variance. No association of nitrogen content with farm source or season or in functional traits of fillets with and without skin was observed. The RDA showed that fish weight slightly positively explained the funcional traits (first two canonical axes explained 53.79% of variability, permutation test on all axes, pseudo-$F = 11.5$, $P < 0.002$).

Sheatfish

The basic chemical composition of sheatfish fillets with and without skin is provided in Table 3. The dry matter content was significantly lower ($P < 0.01$) in sheatfish fillets with skin from FFPW USB (spring 2018, 19.07 ± 0.57 and spring 2019, 19.91 ± 0.91), from Blatna (autumn 2018, 21.97 ± 0.58 and autumn 2019, 20.77 ± 2.06) and those without skin from FFPW USB (spring 2018, 18.84 ± 0.58) and Blatna (autumn 2018, 21.15 ± 0.36 and autumn 2019, 19.86 ± 1.81) compared to the remaining samples. We found significant differences ($P < 0.01$) in dry matter of sheatfish fillets with skin and those without from FFPW USB in spring 2019 (19.91 ± 0.91 and 25.64 ± 2.49) and from Chlumec nad Cidlinou in spring 2019 (30.56 ± 2.01 and 27.25 ± 2.22).

The ash content was significantly lower ($P < 0.01$) in sheatfish fillets without skin from Chlumec nad Cidlinou (spring 2019, 0.98 ± 0.07 and autumn 2019, 0.97 ± 0.09), Klatovy (autumn 2019, 0.98 ± 0.05), and Hodonin (spring 2019, 0.98 ± 0.08) and fillets with skin from Chlumec nad Cidlinou (spring 2019, 0.90 ± 0.05 and autumn 2019, 0.92 ± 0.06), Blatna (autumn 2019, 0.95 ± 0.05), Klatovy (autumn 2019, 0.92 ± 0.05), and Hodonin (spring 2019, 0.92 ± 0.08 and autumn 2019, 0.95 ± 0.05) compared to sheatfish fillets without skin (1.15 ± 0.05) from Blatna in autumn 2018. We found significant differences ($P < 0.01$) in ash content of fillets with skin and those without skin from Chlumec nad Cidlinou in spring 2019 (0.90 ± 0.05 and 0.98 ± 0.07).

The fat content was significantly lower ($P < 0.01$) in sheatfish fillets with skin from FFPW USB (spring 2018,

Table 2. Live weight and length of northern pike (*Esox lucius*) and chemical composition of fillets with skin and without skin.

Company	Year	Season	Weight [g] x ± SD (min–max)	Total length [cm] x ± SD (min–max)	Fillet	Dry matter [g · 100 g^{-1}] x ± SD (min–max)	Ash [g · 100 g^{-1}] x ± SD (min–max)	Fat [g · 100 g^{-1}] x ± SD (min–max)	Protein [g · 100 g^{-1}] x ± SD (min–max)	N [g · 100 g^{-1}] x ± SD (min–max)
FFPW USB Vodnany	2018	Spring	1721.4 ± 469.1 (1260.0–2550)	63.1 ± 4.5 (59.0–74.0)	with skin	21.63 ± 1.03$^{ab(A)}$ (19.79–23.36)	1.52 ± 0.45$^{b(A)}$ (1.17–2.60)	1.13 ± 0.63$^{c(B)}$ (0.49–2.61)	19.73 ± 0.61$^{a(A)}$ (18.59–20.52)	3.16 ± 0.10$^{ab(A)}$ (2.97–3.28)
					without skin	21.78 ± 1.12$^{ab(A)}$ (19.84–23.59)	1.30 ± 0.14$^{ab(A)}$ (1.20–1.70)	1.18 ± 0.40$^{c(B)}$ (0.67–1.78)	19.79 ± 0.60$^{a(A)}$ (18.57–20.79)	3.17 ± 0.10$^{ab(A)}$ (2.97–3.33)
	2019	Spring	1609.0 ± 473.6 (945.0–2470.0)	55.5 ± 4.0 (48.0–60.0)	with skin	23.47 ± 0.33$^{b(A)}$ (22.84–24.05)	1.27 ± 0.06$^{ab(A)}$ (1.17–1.40)	0.72 ± 0.30$^{b(A)}$ (0.26–1.31)	20.12 ± 0.26$^{a(A)}$ (19.66–20.49)	3.21 ± 0.04$^{ab(A)}$ (3.15–3.28)
					without skin	23.19 ± 0.41$^{b(A)}$ (22.53–23.90)	1.31 ± 0.07$^{ab(A)}$ (1.21–1.47)	0.59 ± 0.22$^{b(A)}$ (0.27–0.99)	20.00 ± 0.30$^{a(A)}$ (19.51–20.51)	3.20 ± 0.05$^{ab(A)}$ (3.12–3.28)
Fishery Chlumec nad Cidlinou	2018	Spring	1728.5 ± 464.81 (880.0–2415.0)	64.9 ± 5.5 (53.0–72.0)	with skin	21.29 ± 0.65$^{a(A)}$ (20.30–22.33)	1.24 ± 0.15$^{ab(A)}$ (1.06–1.64)	0.60 ± 0.36$^{b(A)}$ (0.18–1.48)	19.24 ± 0.48$^{a(A)}$ (18.21–20.03)	3.08 ± 0.08$^{a(A)}$ (2.91–3.20)
					without skin	21.39 ± 0.96$^{a(A)}$ (20.27–23.40)	1.24 ± 0.05$^{ab(A)}$ (1.13–1.32)	0.61 ± 0.47$^{b(A)}$ (0.06–1.52)	19.10 ± 0.37$^{a(A)}$ (18.37–19.63)	3.06 ± 0.06$^{a(A)}$ (2.94–3.14)
		Autumn	1459.0 ± 457.4 (530.0–2405.0)	57.1 ± 5.9 (43.0–67.5)	with skin	22.10 ± 0.55$^{ab(A)}$ (20.96–22.99)	1.17 ± 0.06$^{a(A)}$ (1.07–1.29)	0.68 ± 0.19$^{b(A)*}$ (0.39–1.08)	19.62 ± 0.47$^{a(A)}$ (18.65–20.34)	3.14 ± 0.07$^{ab(AB)}$ (2.98–3.25)
					without skin	22.24 ± 0.79$^{ab(A)}$ (20.80–23.20)	1.24 ± 0.07$^{ab(A)}$ (1.14–1.34)	0.91 ± 0.32$^{b(B)}$ (0.45–1.45)	19.51 ± 0.52$^{a(A)}$ (18.33–19.99)	3.12 ± 0.08$^{ab(AB)}$ (2.93–3.20)
	2019	Spring	1125.0 ± 271.1 (615.0–1545.0)	55.0 ± 4.3 (46.0–63.0)	with skin	21.92 ± 0.90$^{ab(A)}$ (20.54–23.27)	1.34 ± 0.09$^{ab(A)}$ (1.28–1.61)	1.02 ± 0.39$^{b(B)}$ (0.46–1.78)	19.56 ± 0.57$^{a(A)}$ (18.49–20.72)	3.13 ± 0.09$^{ab(AB)}$ (2.96–3.31)
					without skin	21.64 ± 1.02$^{ab(A)}$ (20.00–23.24)	1.35 ± 0.07$^{ab(A)}$ (1.26–1.51)	0.96 ± 0.38$^{b(B)}$ (0.48–1.77)	19.39 ± 0.58$^{a(A)}$ (18.38–20.64)	3.10 ± 0.09$^{ab(AB)}$ (2.94–3.30)
		Autumn	1653.5 ± 278.5 (1240.0–2075.0)	59.5 ± 3.6 (54.5–66.0)	with skin	22.51 ± 0.51$^{ab(A)}$ (21.55–23.00)	1.24 ± 0.02$^{ab(A)}$ (1.21–1.28)	0.59 ± 0.20$^{b(A)}$ (0.25–0.95)	20.13 ± 0.42$^{a(A)}$ (19.34–20.59)	3.22 ± 0.07$^{b(B)}$ (3.09–3.29)
					without skin	22.62 ± 0.54$^{ab(A)}$ (21.72–23.44)	1.29 ± 0.04$^{ab(A)}$ (1.23–1.35)	0.65 ± 0.26$^{b(A)}$ (0.38–1.05)	20.00 ± 0.27$^{a(A)}$ (19.49–20.54)	3.20 ± 0.04$^{ab(AB)}$ (3.12–3.29)
Fishery Blatna	2018	Autumn	1143.3 ± 361.2 (685.0–1820.0)	54.7 ± 4.4 (46.5–60.0)	with skin	22.77 ± 0.41$^{ab(A)}$ (22.12–23.45)	1.33 ± 0.10$^{ab(A)}$ (1.33–1.52)	0.67 ± 0.20$^{b(A)}$ (0.38–1.01)	20.03 ± 0.26$^{a(A)}$ (19.60–20.32)	3.20 ± 0.04$^{ab(A)}$ (3.14–3.25)
					without skin	22.68 ± 0.43$^{ab(A)}$ (21.99–23.31)	1.37 ± 0.10$^{ab(A)}$ (1.20–1.55)	0.71 ± 0.38$^{b(A)}$ (0.14–1.33)	19.87 ± 0.18$^{a(A)}$ (19.60–20.12)	3.18 ± 0.03$^{ab(A)}$ (3.14–3.22)
	2019	Autumn	1141.5 ± 322.9 (675.0–1850.0)	56.4 ± 4.31 (48.5–64.0)	with skin	21.50 ± 0.78$^{ab(A)}$ (19.47–22.57)	1.21 ± 0.04$^{ab(A)}$ (1.14–1.27)	0.65 ± 0.29$^{b(A)}$ (0.17–1.06)	19.35 ± 0.70$^{a(A)}$ (18.13–20.67)	3.10 ± 0.11$^{ab(A)}$ (2.90–3.31)
					without skin	21.60 ± 0.95$^{ab(A)}$ (19.11–22.76)	1.28 ± 0.05$^{ab(A)}$ (1.22–1.38)	0.80 ± 0.42$^{b(A)}$ (0.25–1.88)	19.13 ± 0.65$^{a(A)}$ (17.66–19.69)	3.06 ± 0.11$^{a(A)}$ (2.83–3.15)
Fishery Hodonin	2019	Spring	1378.0 ± 384.2 (865.0–2030.0)	59.3 ± 5.8 (50.5–68.5)	with skin	21.83 ± 0.70$^{ab(A)}$ (20.55–22.87)	1.28 ± 0.05$^{ab(A)}$ (1.20–1.36)	0.62 ± 0.19$^{b(B)}$ (0.21–0.88)	19.74 ± 0.77$^{a(A)}$ (18.16–21.03)	3.16 ± 0.12$^{ab(A)}$ (2.91–3.36)
					without skin	21.62 ± 0.59$^{ab(A)}$ (20.30–22.19)	1.28 ± 0.06$^{ab(A)}$ (1.19–1.38)	0.53 ± 0.22$^{b(B)}$ (0.24–1.05)	19.43 ± 0.61$^{a(A)}$ (18.21–20.33)	3.11 ± 0.10$^{ab(A)}$ (2.91–3.26)
		Autumn	1450.6 ± 240.0 (1075.0–1790.0)	59.8 ± 4.4 (52.0–67.5)	with skin	21.79 ± 0.60$^{ab(A)}$ (20.62–22.58)	1.31 ± 0.04$^{ab(A)}$ (1.27–1.42)	0.34 ± 0.14$^{a(A)*}$ (0.11–0.57)	20.23 ± 0.68$^{a(A)}$ (18.65–20.83)	3.24 ± 0.11$^{b(A)}$ (2.98–3.33)
					without skin	22.05 ± 0.81$^{ab(A)}$ (20.74–23.28)	1.39 ± 0.05$^{ab(A)}$ (1.27–1.45)	0.56 ± 0.29$^{b(B)}$ (0.17–1.10)	20.00 ± 0.61$^{a(A)}$ (19.13–20.94)	3.20 ± 0.10$^{ab(A)}$ (3.06–3.35)
Fishery Klatovy	2018	Spring	1360.0 ± 551.1 (725.0–2795.0)	57.1 ± 5.9 (48.5–70.0)	with skin	22.26 ± 0.87$^{ab(A)}$ (20.98–23.92)	1.28 ± 0.05$^{ab(A)}$ (1.18–1.35)	0.64 ± 0.28$^{b(B)}$ (0.14–2.28)	20.23 ± 0.44$^{a(A)}$ (19.44–20.80)	3.24 ± 0.07$^{b(A)}$ (3.11–3.33)
					without skin	22.04 ± 0.60$^{ab(A)}$ (21.11–23.21)	1.29 ± 0.04$^{ab(A)}$ (1.22–1.36)	0.39 ± 0.29$^{a(A)*}$ (0.12–1.19)	20.13 ± 0.40$^{a(A)}$ (19.19–20.63)	3.22 ± 0.06$^{b(A)}$ (3.07–3.30)
		Autumn	1537.5 ± 461.4 (510.0–2200.0)	59.0 ± 7.4 (42.0–70.0)	with skin	22.16 ± 1.19$^{ab(A)}$ (19.10–23.93)	1.23 ± 0.31$^{ab(A)}$ (0.59–1.59)	0.50 ± 0.17$^{b(B)}$ (0.14–0.68)	19.75 ± 0.89$^{a(A)}$ (17.43–21.06)	3.16 ± 0.14$^{ab(A)}$ (2.79–3.37)
					without skin	22.08 ± 1.34$^{ab(A)}$ (18.48–23.38)	1.30 ± 0.24$^{ab(A)}$ (0.82–1.68)	0.62 ± 0.19$^{b(B)}$ (0.09–0.83)	19.70 ± 0.81$^{a(A)}$ (17.53–20.68)	3.15 ± 0.13$^{ab(A)}$ (2.80–3.31)
	2019	Autumn	1375.0 ± 385.9 (755.0–2115.0)	57.4 ± 4.9 (50.5–66.5)	with skin	21.14 ± 0.62$^{a(A)}$ (19.77–21.93)	1.26 ± 0.05$^{ab(A)}$ (1.18–1.34)	0.28 ± 0.19$^{a(A)}$ (0.08–0.58)	19.43 ± 0.47$^{a(A)}$ (18.70–20.22)	3.11 ± 0.07$^{ab(A)}$ (2.99–3.24)
					without skin	21.35 ± 0.78$^{a(A)}$ (19.56–22.24)	1.36 ± 0.10$^{ab(A)}$ (1.21–1.50)	0.40 ± 0.26$^{a(A)}$ (0.08–0.90)	19.44 ± 0.42$^{a(A)}$ (18.43–19.81)	3.11 ± 0.07$^{ab(A)}$ (2.95–3.17)
Fishery Lnare	2018	Spring	1322.5 ± 431.1 (615.0–1960.0)	57.2 ± 7.8 (44.5–68.5)	with skin	22.32 ± 0.94$^{ab(A)}$ (19.69–23.09)	1.27 ± 0.10$^{ab(A)}$ (1.16–1.51)	0.33 ± 0.19$^{a(A)*}$ (0.07–0.67)	20.17 ± 0.54$^{a(A)}$ (19.16–21.07)	3.23 ± 0.09$^{b(A)}$ (3.07–3.37)
					without skin	22.61 ± 0.78$^{ab(A)}$ (20.59–23.37)	1.32 ± 0.13$^{ab(A)}$ (1.18–1.67)	0.63 ± 0.34$^{b(B)}$ (0.27–1.35)	19.97 ± 0.60$^{a(A)}$ (18.48–20.83)	3.20 ± 0.10$^{ab(A)}$ (2.96–3.33)
		Autumn	1327.0 ± 524.1 (805.0–2105.0)	58.9 ± 7.5 (50.0–70.0)	with skin	21.37 ± 0.92$^{a(A)}$ (19.44–22.80)	1.35 ± 0.07$^{ab(A)}$ (1.25–1.50)	0.45 ± 0.20$^{a(A)}$ (0.14–0.99)	19.65 ± 0.42$^{a(A)}$ (19.14–20.54)	3.13 ± 0.07$^{ab(A)}$ (3.06–3.29)
					without skin	21.21 ± 0.95$^{a(A)}$ (19.15–22.73)	1.35 ± 0.11$^{ab(A)}$ (1.09–1.53)	0.38 ± 0.28$^{a(A)}$ (0.12–0.93)	19.42 ± 0.56$^{a(A)}$ (18.35–20.52)	3.11 ± 0.09$^{ab(A)}$ (2.94–3.28)
Fishery Lnare	2019	Spring	981.0 ± 434.4 (445.0–1650.0)	49.7 ± 6.2 (40.0–58.5)	with skin	22.65 ± 0.71$^{ab(A)}$ (21.23–23.56)	1.31 ± 0.05$^{ab(A)}$ (1.22–1.38)	0.72 ± 0.30$^{b(B)}$ (0.22–1.27)	19.48 ± 0.27$^{a(A)}$ (19.47–20.24)	3.17 ± 0.04$^{ab(A)}$ (3.12–3.24)
					without skin	22.72 ± 0.87$^{ab(A)}$ (21.00–23.86)	1.37 ± 0.06$^{ab(A)}$ (1.29–1.49)	0.71 ± 0.36$^{b(B)}$ (0.26–1.52)	19.85 ± 0.49$^{a(A)}$ (19.07–20.66)	3.18 ± 0.08$^{ab(A)}$ (3.05–3.31)
		Autumn	1136.0 ± 20.9.9 (715.0–1435.0)	54.5 ± 3.01 (47.0–58.0)	with skin	22.02 ± 0.68$^{ab(A)}$ (20.50–23.03)	1.13 ± 0.12$^{a(A)}$ (0.99–1.28)	0.41 ± 0.16$^{a(A)}$ (0.15–0.70)	19.95 ± 0.34$^{a(A)}$ (19.51–20.46)	3.19 ± 0.05$^{ab(A)}$ (3.12–3.27)
					without skin	22.05 ± 0.61$^{ab(A)}$ (21.20–23.05)	1.16 ± 0.12$^{a(A)}$ (0.99–1.32)	0.44 ± 0.21$^{a(A)}$ (0.12–0.78)	20.02 ± 0.29$^{a(A)}$ (19.44–20.45)	3.20 ± 0.05$^{ab(A)}$ (3.11–3.27)

Table 2 continues on next page.

Table 2. cont.

Company	Year	Season	Weight [g] x ± SD (min–max)	Total length [cm] x ± SD (min–max)	Fillet	Dry matter [g · 100 g⁻¹] x ± SD (min–max)	Ash [g · 100 g⁻¹] x ± SD (min–max)	Fat [g · 100 g⁻¹] x ± SD (min–max)	Protein [g · 100 g⁻¹] x ± SD (min–max)	N [g · 100 g⁻¹] x ± SD (min–max)
Fishery Tabor	2018	Spring	937.0 ± 202.2 (685.0–1315.0)	52.3 ± 4.0 (46.0–60.0)	with skin	$21.69 \pm 0.58^{ab(A)}$ (20.94–22.75)	$1.28 \pm 0.06^{ab(A)}$ (1.18–1.38)	$0.43 \pm 0.27^{a(A)}$ (0.06–1.00)	$19.82 \pm 0.29^{a(A)}$ (19.38–20.28)	$3.17 \pm 0.05^{ab(A)}$ (3.10–3.24)
					without skin	$21.80 \pm 0.65^{ab(A)}$ (20.69–22.71)	$1.34 \pm 0.07^{ab(A)}$ (1.22–1.48)	$0.51 \pm 0.24^{ab(AB)}$ (0.08–0.95)	$19.53 \pm 0.36^{a(A)}$ (18.74–20.04)	$3.12 \pm 0.06^{ab(A)}$ (3.00–3.21)
		Autumn	677.0 ± 80.7 (560.0–800.0)	46.0 ± 1.6 (43.0–48.5)	with skin	$22.42 \pm 0.45^{ab(A)}$ (21.38–22.97)	$1.42 \pm 0.06^{b(A)}$ (1.35–1.57)	$0.47 \pm 0.13^{ab(AB)}$ (0.30–0.74)	$20.10 \pm 0.18^{a(A)}$ (19.83–20.44)	$3.22 \pm 0.03^{b(A)}$ (3.17–3.27)
					without skin	$22.57 \pm 0.45^{ab(A)}$ (21.46–23.03)	$1.38 \pm 0.07^{ab(A)}$ (1.30–1.50)	$0.55 \pm 0.20^{ab(AB)}$ (0.20–0.92)	$20.07 \pm 0.31^{a(A)}$ (19.48–20.58)	$3.22 \pm 0.05^{b(A)}$ (3.12–3.29)
	2019	Spring	1565.0 ± 889.1 (760.0–3980.0)	57.7 ± 9.7 (48.0–82.0)	with skin	$23.52 \pm 0.82^{b(A)}$ (22.50–25.00)	$1.26 \pm 0.05^{ab(A)}$ (1.18–1.34)	$0.91 \pm 0.48^{c(B)}$ (0.34–2.02)	$20.30 \pm 0.43^{a(A)}$ (19.36–20.87)	$3.25 \pm 0.07^{b(A)}$ (3.10–3.34)
					without skin	$23.22 \pm 0.74^{b(A)}$ (21.92–24.39)	$1.26 \pm 0.06^{ab(A)}$ (1.16–1.36)	$0.74 \pm 0.42^{b(B)}$ (0.20–1.62)	$20.05 \pm 0.41^{a(A)}$ (19.45–20.81)	$3.22 \pm 0.07^{b(A)}$ (3.11–3.33)
		Autumn	981.0 ± 269.9 (540.0–1650.0)	51.0 ± 4.2 (43.5–60.0)	with skin	$21.84 \pm 0.38^{ab(A)}$ (21.07–22.50)	$1.35 \pm 0.13^{ab(A)}$ (1.20–1.57)	$0.68 \pm 0.22^{b(B)}$ (0.27–1.13)	$19.94 \pm 0.39^{a(A)}$ (19.37–20.37)	$3.19 \pm 0.06^{ab(A)}$ (3.10–3.26)
					without skin	$21.86 \pm 0.45^{ab(A)}$ (20.94–22.57)	$1.37 \pm 0.14^{ab(A)}$ (1.22–1.63)	$0.79 \pm 0.19^{b(B)}$ (0.60–1.18)	$19.71 \pm 0.28^{a(A)}$ (19.38–20.16)	$3.15 \pm 0.05^{ab(A)}$ (3.10–3.23)

Data are mean ± standard deviation (minimum value–maximum value), $n = 10$. Values with different small letters in superscripts are significantly ($P < 0.05$) different among the locality groups. Values with different capital letters in superscripts are significantly ($P < 0.05$) different among the season groups in one locality. *Denotes significant differences among fillets with skin or fillets without skin values over one sampling ($P < 0.01$).

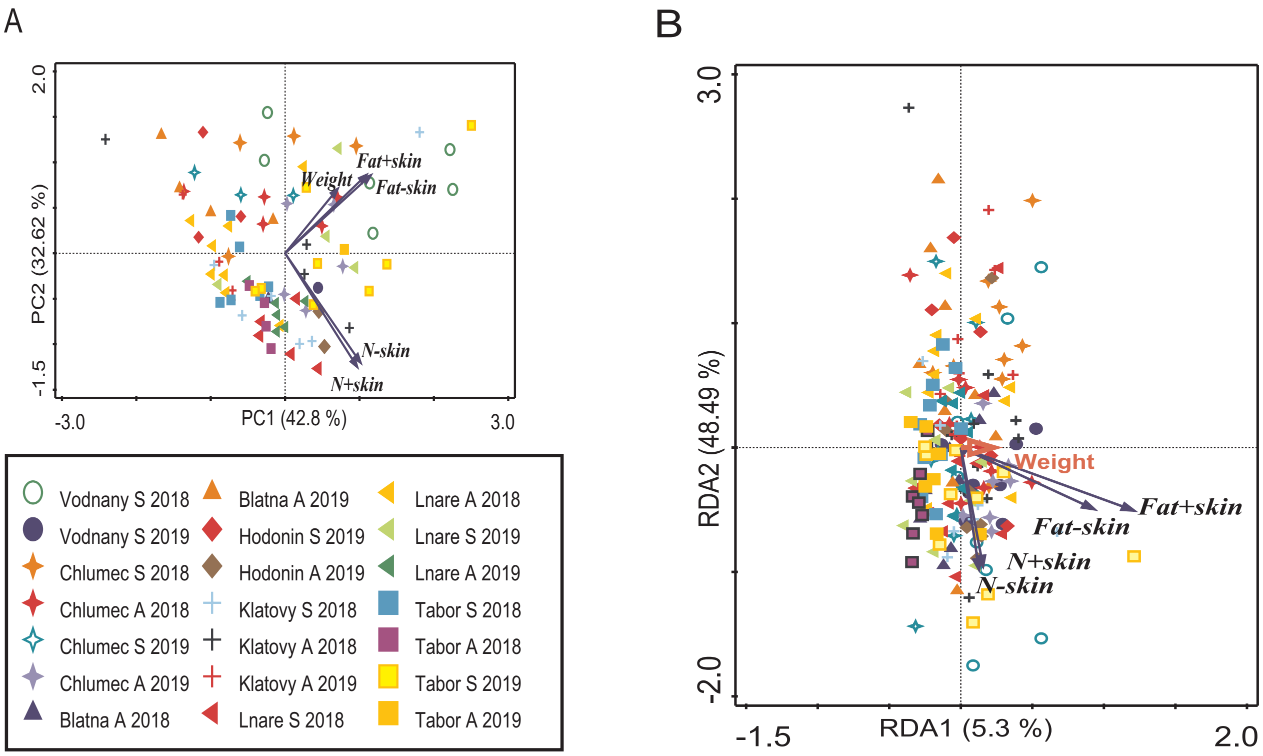

Figure 3. Ordination plots of sample distribution after principal component analysis (PCA) of functional traits as response variables (**A**) and redundancy analysis (RDA) (**B**) of functional traits as response variables and weight of northern pike, *Esox lucius* as explanatory variable. N+skin = nitrogen concentration in fillet with skin, N-skin = nitrogen concentration in fillet without skin, Fat+skin = fat percentage in fillet with skin, Fat-skin = fat percentage in fillet without skin, Weight = body weight of individual fish; abbreviations used in legend: S = spring sampling, A = autumn sampling). The length of the arrow reflects the power of the variable to differentiate the samples. Sample scores were limited to 90 points for better fit.

1.17 ± 0.36 and spring 2019, 0.74 ± 0.54) and Blatna (autumn 2018, 1.83 ± 0.72 and autumn 2019, 2.42 ± 2.34) and without skin from FFPW USB (spring 2018, 0.79 ± 0.31 and spring 2019, 0.54 ± 0.48) and Blatna (autumn 2018, 1.20 ± 0.59 and autumn 2019, 1.53 ± 2.16) compared to other groups. We found significant differences ($P < 0.01$) in fat content of fillets with and without skin in sheatfish from FFPW USB in spring 2018 (1.17 ± 0.36 and 0.79 ± 0.31), Chlumec nad Cidlinou in spring 2019 (13.41 ± 2.67 and 9.33 ± 2.89), Blatna in autumn 2019 (2.42 ± 2.34 and 1.53 ± 2.16), and Klatovy in autumn 2019 (8.63 ± 2.80 and 6.92 ± 2.41).

Table 3. Live weight and length of sheatfish (*Silurus glanis*) and chemical composition of fillets with skin and without skin.

Company	Year	Season	Weight [g] x ± SD (min–max)	Total length [cm] x ± SD (min–max)	Fillet	Dry matter [g · 100 g^{-1}] x ± SD (min–max)	Ash [g · 100 g^{-1}] x ± SD (min–max)	Fat [g · 100 g^{-1}] x ± SD (min–max)	Protein [g · 100 g^{-1}] x ± SD (min–max)	N [g · 100 g^{-1}] x ± SD (min–max)
FFPW USB Vodnany	2018	Spring	900.0 ± 76.9 (750.0–1000.0)	50.5 ± 1.2 (48.0–52.0)	with skin	$19.07 \pm 0.57^{a(A)}$ (18.22–20.27)	$0.99 \pm 0.01^{ab(A)}$ (0.97–1.00)	$1.17 \pm 0.36^{a(B)}$ (16.42–17.61)	$17.03 \pm 0.36^{a(A)}$ (16.42–17.61)	$2.73 \pm 0.06^{ab(A)}$ (2.63–2.82)
					without skin	$18.84 \pm 0.58^{a(A)}$ (17.72–20.02)	$1.03 \pm 0.04^{ab(A)}$ (0.98–1.13)	$0.79 \pm 0.31^{a(A)*}$ (0.45–1.40)	$16.88 \pm 0.51^{a(A)}$ (15.69–17.60)	$2.70 \pm 0.08^{ab(A)}$ (2.51–2.82)
	2019	Spring	744.0 ± 161.0 (505.0–985.0)	48.8 ± 3.1 (44.0–53.0)	with skin	$19.91 \pm 0.91^{a(A)}$ (18.83–21.38)	$1.04 \pm 0.74^{ab(A)}$ (0.98–1.09)	$0.74 \pm 0.54^{a(A)}$ (0.16–1.79)	$17.52 \pm 0.69^{a(A)}$ (16.58–18.62)	$2.80 \pm 0.11^{ab(A)}$ (2.65–2.98)
					without skin	$25.64 \pm 2.49^{b(B)*}$ (21.64–31.63)	$1.07 \pm 0.04^{ab(A)}$ (0.95–1.12)	$0.54 \pm 0.48^{a(A)}$ (0.16–1.48)	$17.52 \pm 0.70^{a(A)}$ (18.40–20.79)	$2.80 \pm 0.11^{ab(A)}$ (2.68–3.01)
Fishery Chlumec nad Cidlinou	2018	Autumn	2027.0 ± 1170.9 (885.0–4115.0)	59.95 ± 9.31 (48.0–75.5)	with skin	$24.82 \pm 2.42^{b(A)}$ (22.52–30.48)	$0.99 \pm 0.07^{ab(AB)}$ (0.89–1.07)	$6.61 \pm 2.98^{b(A)}$ (3.06–13.33)	$16.59 \pm 0.58^{a(A)}$ (15.71–17.67)	$2.66 \pm 0.09^{ab(AB)}$ (2.51–2.83)
					without skin	$24.82 \pm 3.45^{b(A)}$ (20.67–30.38)	$1.05 \pm 0.08^{ab(B)}$ (0.84–1.12)	$7.21 \pm 4.00^{b(A)}$ (3.08–15.02)	$16.35 \pm 0.67^{a(A)}$ (14.67–17.18)	$2.62 \pm 0.11^{a(A)}$ (2.35–2.75)
	2019	Spring	3220.0 ± 426.3 (2435.0–3725.0)	76.9 ± 3.6 (70.0–82.0)	with skin	$30.56 \pm 2.01^{d(C)}$ (26.27–32.55)	$0.90 \pm 0.05^{a(A)*}$ (0.81–0.98)	$13.41 \pm 2.67^{c(B)}$ (7.71–16.50)	$16.31 \pm 0.61^{a(A)}$ (15.73–17.66)	$2.62 \pm 0.10^{a(A)}$ (2.52–2.83)
					without skin	$27.25 \pm 2.22^{c(B)*}$ (23.25–30.68)	$0.98 \pm 0.07^{a(A)}$ (0.83–1.06)	$9.33 \pm 2.89^{c(B)*}$ (4.76–13.93)	$17.33 \pm 0.65^{a(A)}$ (16.12–18.00)	$2.77 \pm 0.01^{ab(B)}$* (2.58–2.88)
		Autumn	3892.5 ± 732.0 (2515.0–4815.0)	77.8 ± 6.8 (55.5–91.0)	with skin	$25.48 \pm 1.32^{b(A)}$ (23.46–27.19)	$0.92 \pm 0.06^{a(A)}$ (0.82–1.06)	$7.60 \pm 1.62^{b(A)}$ (5.16–10.03)	$16.33 \pm 0.62^{a(A)}$ (14.95–17.22)	$2.61 \pm 0.10^{a(A)}$ (2.39–2.76)
					without skin	$24.24 \pm 1.60^{b(A)}$ (22.31–27.34)	$0.97 \pm 0.09^{a(A)}$ (0.85–1.15)	$5.93 \pm 1.65^{b(A)}$ (2.91–8.68)	$16.77 \pm 0.44^{a(A)}$ (16.05–17.66)	$2.68 \pm 0.07^{a(A)}$ (2.57–2.81)
Fishery Blatna	2018	Autumn	2552.5 ± 1290.3 (1005.0–4870.0)	72.0 ± 12.3 (55.5–91.0)	with skin	$21.97 \pm 0.58^{a(A)}$ (21.16–23.09)	$1.08 \pm 0.13^{ab(AB)}$ (0.76–1.29)	$1.83 \pm 0.72^{a(A)}$ (1.04–3.03)	$18.23 \pm 0.32^{a(A)}$ (17.60–18.67)	$2.92 \pm 0.05^{b(A)}$ (2.82–2.99)
					without skin	$21.15 \pm 0.36^{a(A)}$ (20.42–21.77)	$1.15 \pm 0.05^{b(B)}$ (1.08–1.26)	$1.20 \pm 0.59^{a(A)}$ (0.39–2.52)	$18.12 \pm 0.36^{a(A)}$ (17.60–18.62)	$2.90 \pm 0.06^{b(A)}$ (2.82–2.98)
	2019	Autumn	2527.5 ± 1481.6 (515.0–6115.0)	72.4 ± 11.01 (60.0–100.0)	with skin	$20.77 \pm 2.06^{a(A)}$ (19.11–26.50)	$0.95 \pm 0.05^{a(A)}$ (0.87–1.03)	$2.42 \pm 2.34^{a(A)}$ (0.81–9.05)	$17.26 \pm 0.64^{a(A)}$ (16.17–18.15)	$2.76 \pm 0.10^{ab(A)}$ (2.59–2.90)
					without skin	$19.86 \pm 1.81^{a(A)}$ (18.57–24.94)	$1.01 \pm 0.07^{ab(AB)}$ (0.93–1.12)	$1.53 \pm 2.16^{a(A)*}$ (0.22–7.71)	$17.21 \pm 0.33^{a(A)}$ (16.65–17.59)	$2.75 \pm 0.05^{ab(A)}$ (2.66–2.81)
Fishery Hodonin	2019	Spring	5892.9 ± 1055.4 (4805.0–7780.0)	93.3 ± 6.8 (87.0–105.0)	with skin	$26.09 \pm 3.05^{b(A)}$ (21.57–30.37)	$0.92 \pm 0.08^{a(A)}$ (0.83–1.04)	$7.94 \pm 3.79^{b(A)}$ (2.51–13.69)	$17.35 \pm 0.81^{a(A)}$ (16.00–18.42)	$2.78 \pm 0.13^{ab(A)}$ (2.56–2.95)
					without skin	$25.17 \pm 3.19^{b(A}$ (21.14–29.78)	$0.98 \pm 0.08^{a(A)}$ (0.88–1.11)	$6.84 \pm 3.78^{b(A)}$ (2.39–12.97)	$17.52 \pm 0.74^{a(A)}$ (16.17–0.74)	$2.80 \pm 0.12^{ab(A)}$ (2.59–2.92)
		Autumn	4979.0 ± 1798.8 (3120.0–9265.0)	83.5 ± 9.5 (72.0–101.0)	with skin	$27.07 \pm 2.47^{b(A)}$ (23.18–32.86)	$0.95 \pm 0.05^{a(A)}$ (0.83–1.01)	$8.95 \pm 3.01^{b(A)}$ (4.60–16.57)	$17.06 \pm 0.62^{a(A)}$ (15.80–18.04)	$2.73 \pm 0.10^{ab(A)}$ (2.53–2.89)
					without skin	$25.64 \pm 2.49^{b(A)}$ (21.64–31.63)	$1.03 \pm 0.05^{ab(A)}$ (0.95–1.13)	$7.11 \pm 2.72^{b(A)}$ (3.16–13.82)	$17.29 \pm 0.54^{a(A)}$ (16.21–18.08)	$2.77 \pm 0.09^{ab(A)}$ (2.59–2.89)
Fishery Klatovy	2019	Autumn	5220.0 ± 2247.8 (1955.0–8615.0)	86.7 ± 13.9 (64.0–105.0)	with skin	$26.66 \pm 2.08^{b(A)}$ (22.77–30.97)	$0.92 \pm 0.05^{a(A)}$ (0.84–1.00)	$8.63 \pm 2.80^{b(A)}$ (4.16–13.10)	$16.99 \pm 0.72^{a(A)}$ (15.61–18.51)	$2.72 \pm 0.12^{ab(A)}$ (2.50–2.96)
					without skin	$26.04 \pm 2.44^{b(A)}$ (22.14–31.28)	$0.98 \pm 0.05^{a(A)}$ (0.90–1.05)	$6.92 \pm 2.41^{b(A)*}$ (3.23–11.10)	$17.17 \pm 0.46^{a(A)}$ (16.17–17.74)	$2.75 \pm 0.07^{ab(A)}$ (2.59–2.84)

Data are mean ± standard deviation (minimum value–maximum value), $n = 10$. Values with different small letters in superscripts are significantly ($P < 0.05$) different among the locality groups. Values with different capital letters in superscripts are significantly ($P < 0.05$) different among the season groups in one locality. *Denotes significant differences among fillets with skin or fillets without skin values over one sampling ($P < 0.01$).

There were no significant differences ($P > 0.05$) in protein content of sheatfish fillets of different farms, seasons, years, or with/without skin.

The nitrogen content was significantly lower ($P < 0.01$) in sheatfish fillets with skin from Chlumec nad Cidlinou (spring 2019, 2.62 ± 0.10 and autumn 2019, 2.61 ± 0.10), and fillets without skin from Chlumec nad Cidlinou (autumn 2018, 2.62 ± 0.11 and autumn 2019, 2.68 ± 0.07) compared to those with (2.92 ± 0.05) and without (2.90 ± 0.06) skin from Blatna in autumn 2018. Nitrogen content of fillets with skin (2.62 ± 0.10) significantly ($P < 0.01$) differed from those without skin (2.77 ± 0.01) in sheatfish from Chlumec nad Cidlinou in spring 2019.

The first two axes of PCA in sheatfish explained 92.80% and the PC1 axis explained 62.9% of the total variance (Fig. 4). The samples were not clearly separated into specific clusters, but a gradient of samples negatively correlated with fat content. RDA distribution showed that fish weight was a stronger explanatory factor for functional traits data (first two canonical axes explained 77.80% of variability, permutation test on all axes: pseudo-$F = 25.7$, $P < 0.002$) in sheatfish than in European pike-perch and northern pike.

Nitrogen factors

Nitrogen factor indicates the content of nitrogen determined by Kjeldahl (ISO 937 1978). The term was chosen in the context of the Codex standard (CA 2004). The recommended nitrogen factor was determined from all samples of each fish species and expressed as mean and standard deviation. The recommended nitrogen factors for European pike-perch, northern pike, and sheatfish fillets with skin and without skin determined by Kjeldahl methods are given in Table 4. The recommended nitrogen factor for European pike-perch with skin is 3.28 ± 0.09 and 3.21 ± 0.09 without skin, for northern pike with skin is 3.18 ± 0.09 and without skin is 3.15 ± 0.09, for sheatfish with skin 2.73 ± 0.13 and without skin 2.75 ± 0.12. Codex Alimentarius recommended value ± 10% of variation (CA 2004).

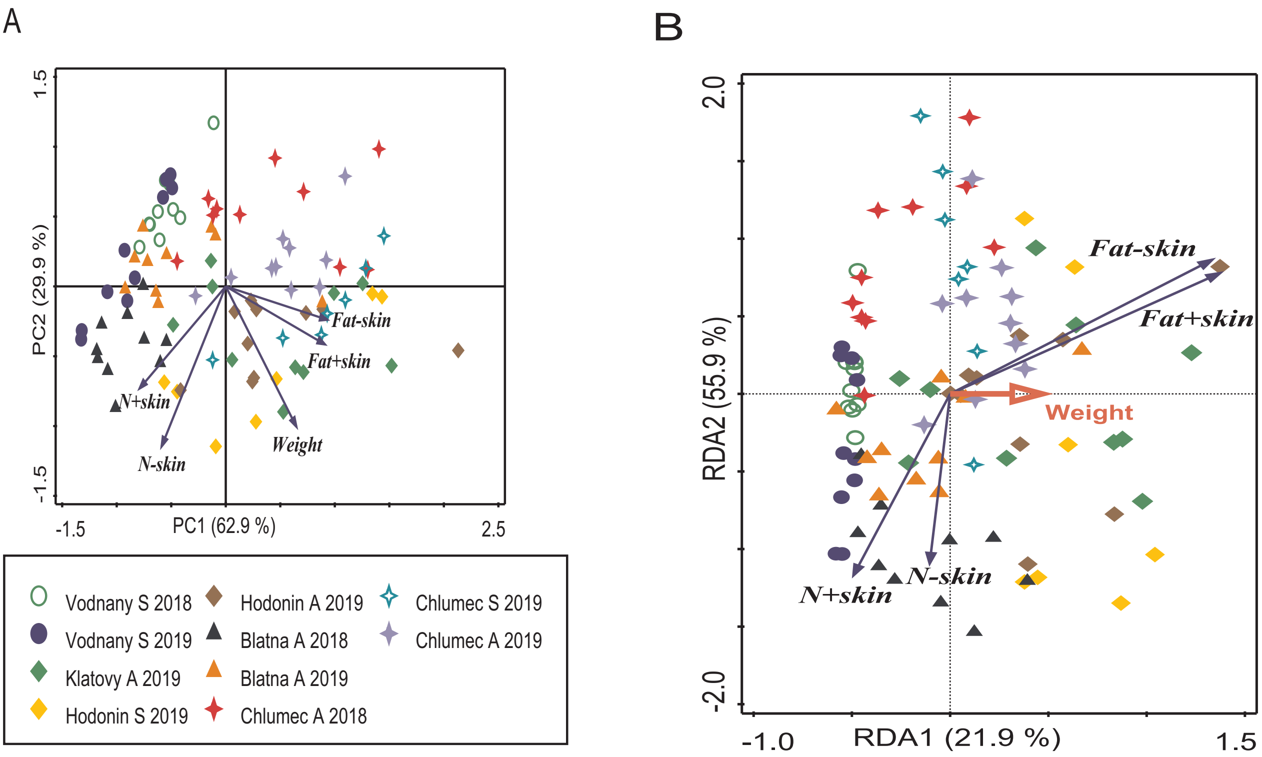

Figure 4. Ordination plots of sample distribution after principal component analysis (PCA) of functional traits as response variables (**A**) and redundancy analysis (RDA) (**B**) of functional traits as response variables and weight of sheatfish, *Silurus glanis* as explanatory variable. N+skin = nitrogen concentration in fillet with skin, N-skin = nitrogen concentration in fillet without skin, Fat+skin = fat percentage in fillet with skin, Fat-skin = fat percentage in fillet without skin, Weight = body weight of individual fish; abbreviations used in legend: S = spring sampling, A = autumn sampling). The length of the arrow reflects the power of the variable to differentiate the samples.

Discussion

Fillet samples with and without skin from European pike-perch, *Sander lucioperca*; northern pike, *Esox lucius*; sheatfish, *Silurus glanis* from several rearing locations and different harvest seasons and years were analyzed for dry matter, protein, fat, and ash content. To date, there are no established nitrogen factors determined by the Kjeldahl method for European pike-perch, northern pike, and sheatfish with and without skin.

The basic nutrient values obtained in this study are similar to those reported by Policar et al. (2016) in European pike-perch, Salama and Davies (1994) and Modzelewska-Kapituła et al. (2017) in northern pike, and Jankowska et al. (2007) in sheatfish. The water percentage decreased, and the fat and protein percentages increased, with increasing body weight and length, whereas the ash content remained fairly constant. Our findings are in agreement with those reported for other fish species (McComish et al. 1974; Elliott 1976; Costopoulos and Fond 1989; Brown and Murphy 1991; Clawson et al. 1991; Salama and Davies 1994).

Obtained nitrogen factors, taking into account the fat content of fish with skin and without skin, for European pike-perch, northern pike, and sheatfish determined by Kjeldahl method are given in Table 4. All these values take into account the fat contents of the fish fillets with skin and without skin. The fat content of European pike-perch and northern pike fillets was low as shown in Table 1 and Table 2. The sheatfish fillets contained higher fat percentage with larger inter-sample differences as shown in Table 3 nevertheless fat content showed no significant influence ($P > 0.05$) on protein (nitrogen) content. We found no association of nitrogen content with rearing location, sampling season, or presence of skin in European pike-perch and northern pike. Nitrogen and fat content in sheatfish were negatively correlated. It is not necessary to establish nitrogen factors on a fat-free basis as is the case for some fish species (Colwell et al. 2011).

Table 4. Recommended nitrogen factors.

Species	Fillet	Nitrogen factor (Kjeldahl)
European pike-perch (*Sander lucioperca*)	with skin	3.28 ± 0.09
	without skin	3.21 ± 0.09
Northern pike (*Esox Lucius*)	with skin	3.18 ± 0.09
	without skin	3.15 ± 0.09
Sheatfish (*Silurus glanis*)	with skin	2.73 ± 0.13
	without skin	2.75 ± 0.12

Note: The recommended nitrogen factor was determined from all samples each fish species and mentioned as mean and standard deviation. Codex Alimentarius apply the recommended value ± 10% of variation (CA 2004).

The established nitrogen factors allow analysis of products from European pike-perch, northern pike, and sheatfish, in accordance with the EU legislation. Regulation (EU) No 1169/2011 of the European Parliament and of the Council on the provision of food information to consumers (EC 2011), including the requirement for a Quantitative Ingredients Declaration (QUID) label specifying the quantity of fish content. On the basis of recommended nitrogen factors is possible to calculate the value of QUID (CA 2004) for analyzed samples which are verified for labeled QUID information.

There are limitations in the use of nitrogen factors. They are calculated as mean values with standard deviations, and it is important to bear in mind the effects of season, weight, fishery location, and nutritional status on natural values and the analytical variability of their determination and to apply the recommended value of ± 10% to the factor (CA 2004).

Conclusions

Determined nitrogen factors for European pike-perch, northern pike, and sheatfish would help to ensure that consumers are buying correctly labeled or described fish products. The recommended nitrogen factor for European pike-perch with skin is 3.28 ± 0.09 and without the skin is 3.21 ± 0.09, for northern pike with skin is 3.18 ± 0.09 and without the skin is 3.15 ± 0.09, for sheatfish with skin 2.73 ± 0.13 and without the skin 2.75 ± 0.12. Codex Alimentarius recommend allowing ± 10% variation (CA 2004).

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Alteration of the feeding behavior of an omnivorous fish, *Scardinius acarnanicus* (Actinopterygii: Cypriniformes: Cyprinidae), in the presence of fishing lights

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Abstract

Fishing with light is an old and common practice yielding a substantial catch volume globally. Despite the popularity of the method and the efforts to improve it, there is a lack of field studies on the effects of light on the feeding preferences of the attracted fishes. A previous report suggested that purse seine fishing lights can differentiate the feeding preferences of the approaching fishes, such as *Atherina boyeri* Risso, 1810 in Lake Trichonis (Greece). The presently reported study aims to verify these findings by investigating the diet of the endemic *Scardinius acarnanicus* Economidis, 1991. The feeding behavior of *S. acarnanicus* was studied from 2016 to 2019 through gut content analysis, in specimens from Lake Trichonis that came from purse seining with light and specimens caught without light. The same investigation was carried out comparatively in specimens taken by gillnets from two nearby lakes (lakes Ozeros and Amvrakia), where *S. acarnanicus* is present, but no fishing with light is exercised. The stomach content analysis conducted on 699 *S. acarnanicus* specimens revealed the intense effect of light on its diet resulting in the alteration of its feeding habits towards fish predation and especially *Atherina boyeri*. On the contrary, the specimens taken with the use of gillnets, from the three lakes, showed a typical omnivorous feeding behavior. The findings of the presently reported study support the assumption that the elevated concentration of fish close to fishing lights alters the feeding behavior of certain species making them predators. Considering that fishing with light is practiced worldwide, this could be of great ecological significance to the ichthyofauna not only of inland waters but also of marine areas, affecting perhaps several commercial species.

Keywords

Atherina boyeri, feeding, fishing lights, purse seine, *Scardinius acarnanicus*

Introduction

Artificial light sources have been used to aid fishing for thousands of years, ever since humans first observed that many fish species exhibit a positive phototactic response to light sources. Historical records show that the earliest applications of light-assisted fishing include the use of large beach bonfires to attract fish; these simple techniques have slowly been developed and refined so that large numbers of fish can now be caught with impressive efficiency. Kerosene and electric lamps were introduced in the 20th century and, more recently, light-emitting diode (LED) lamps have entered the field as the light-source of choice due to their strong luminosity, high energy efficiency, long lifespan, chromatic performance, and reduced environmental impact compared to non-LED lamps (Nguyen and Winger 2019).

Zooplankton exhibits a positive phototactic response to light, and its behavior attracts fish larvae and zooplanktivorous fishes, as well as larger fish species and top predators, initiating a trophic chain reaction (Maeda 1951; Ben-Yami 1976). Although light-assisted fishing has enabled large catches of fish around the world, and significant efforts have been made to improve fishing gear and techniques, there is poor understanding of how the presence of artificial light influences fish feeding behavior and diet preferences.

A common marine and freshwater fishing technique around the world is to combine the use of purse seine fishing gear with artificial light (Ben-Yami 1976; Acros and Oro 2002). In this technique, rafts of lamps are anchored and turned on to attract large schools of different fish species. Once the desired concentration of the target species is achieved, the main fishing boat, assisted by a small skiff, encircles the school by releasing the seine net. The base of the seine net is then closed to form a 'bag' that traps the fish so that the net can then be hauled into the main fishing boat and the fish collected (Tsagarakis et al. 2012).

Light-assisted purse seine fishing is a common technique in Lake Trichonis, the largest natural lake in Greece, where the landlocked and zooplanktivorous species *Atherina boyeri* Risso, 1810 is targeted by fishermen. Previous behavioral studies conducted in Lake Trichonis by Kehayias et al. (2018a, 2018b) found that the presence of artificial light alters the dietary preferences of species such as *A. boyeri* (see Doulka et al. 2013). Specifically, artificial light causes *A. boyeri* to prey heavily on *Economidichthys trichonis* Economidis et Miller, 1990 larvae, an endangered fish that is endemic to Lake Trichonis. During those field expeditions, it was determined that artificial light attracts not only *A. boyeri*, but also *Scardinius acarnanicus,* which encircle the *A. boyeri* schools. *Scardinius acarnanicus* Economidis, 1991, Trichonis rudd or 'Tseroukla' in Greek, is an endemic fish species of the freshwater ecosystems of western Greece. In the only previous study of its feeding behavior, which was performed nearly 40 years ago in Lake Trichonis and the nearby Lake Lysimachia, *S. acarnanicus* was found to be herbivorous, with a diet of only aquatic vegetation and phytoplankton (Iliadou 1991). More recently, however, Tsounis (2016) reported that, in Lake Trichonis and nearby lakes, S. *acarnanicus* feeds not only on aquatic vegetation and phytoplankton but also on small invertebrates and insects, as well as fishes, suggesting it to be omnivorous. Thus, the observation that dense schools of *S. acarnanicus* swarm around *A. boyeri* during light-assisted purse seine fishing suggests that, in the presence of artificial light, S. *acarnanicus* is a predator of *A. boyeri*.

The presently reported study, which continues recent investigations by Kehayias et al. (2018a, 2018b), further supports the hypothesis that the presence of artificial light influences fish feeding preferences, with a tendency to make fish more predatory. This study investigates the feeding behavior of *S. acarnanicus* using gut content analysis. *Scardinius acarnanicus* specimens from Lake Trichonis were obtained both from purse seining with light, and without light with the use of gillnets. In addition, a similar investigation was carried out comparatively in specimens from two nearby lakes (Lakes Ozeros and Amvrakia), where *S. acarnanicus* is present, but no fishing with light is exercised. The issues addressed are a) Whether the presence of light can alter the diet of *S. acarnanicus* and in which degree concerning season and size; b) Are these results valid given the parallel feeding investigation in other lake ecosystems of the area? c) Which could be the possible local as well as global ecological consequences of the light-driven alteration of the fish diet towards predatory.

Materials and methods

Study area

The study was conducted at three natural lakes of western Greece: Lake Trichonis, Lake Ozeros, and Lake Amvrakia (Fig. 1). Lake Trichonis is Greece's largest natural lake, covering an area of 98.6 km^2 and having a catchment area of 421 km^2. It is a warm monomictic lake with oligotrophic to mesotrophic characteristics and a maximum depth of 57 m (Doulka and Kehayias 2008; Kehayias and Doulka 2014). Lake Ozeros is a karstic lake covering an area of 10.1 km^2 and having a catchment area of 59 km^2. It is a typical shallow Mediterranean lake with a maximum depth of 5.6 m. The trophic state of the lake can be characterized as mesotrophic (Chalkia and Kehayias 2013). Finally, Lake Amvrakia is the second largest lake in the area, covering an area of 14.5 km^2 and having a catchment area of 177 km^2, with a maximum depth of about 50 m. Lake Amvrakia can be characterized as mesotrophic (Chalkia et al. 2012).

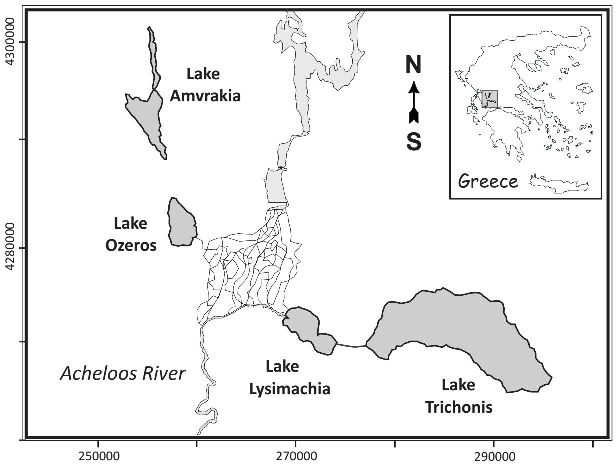

Figure 1. Geographic location of the three lakes, Trichonis, Ozeros, and Amvrakia

Field operations

A total of 699 *S. acarnanicus* specimens were collected and analyzed from 2016 to 2019. All specimens, which were selected at random from fish catches at the three study lakes, were preserved by immersing them immediately in 10% formalin solution; this minimized post-capture digestion of gut content.

In the period June 2016 to July 2019, 151 specimens were collected from Lake Trichonis using Nordic multi-mesh gillnets (length: 30 m, height: 1.5 m, mesh sizes: 43 mm, 19.5 mm, 6.25 mm, 10 mm, 55 mm, 8 mm, 12,5 mm, 24 mm, 15.5 mm, 5 mm, 35 mm, and 29 mm), while 138 specimens were collected using purse seine nets (length: 150 m, height: 20 m, mesh size: 6 mm). Purse seine fishing was performed monthly (in May, July, August, October, and November 2017) on moonless nights, assisted by rafts of green LED lamps. The purse seine lamp rafts, which were anchored at roughly 200 m intervals, were switched on just before dusk and remained on for 6–8 h, depending on the time of year. The LED lamps, which were powered by 12 V/60 A lead-acid batteries to give a luminosity of 2400 lm, were positioned above the surface of the water. In contrast, no light was used in the case of the gillnets, which were set late at night and were picked up early in the morning. A total of 196 and 214 specimens were collected from Lake Ozeros and Lake Amvrakia, respectively; all these specimens were collected using Nordic multi-mesh gillnets, due to the lack of purse seine fishing in those lakes. Specimens were collected from Lake Ozeros in the period May 2016 to August 2018, and from Lake Amvrakia from April 2016 to July 2019.

Laboratory measurements

Once transferred to the laboratory, whole specimen lengths [cm] and weights [g] were recorded before then weighing and analyzing gut content of the entire intestine using a stereomicroscope. For specimens that were collected using light-assisted purse seine fishing, it was necessary to eliminate food that had been ingested after the specimens were confined within the purse seine nets; this was done by disregarding food that was (i) still in the specimens' mouths, or (ii) had been ingested but had not yet reached the stomach. Food content was grouped into the following food categories: (i) plant material, (ii) fish remains, (iii) mollusks, and (iv) insects. The 'plant material' category included all macrophytic vegetation and phytoplankton; it was frequently difficult to identify macrophytic vegetation in the gut content due to maceration by the pharyngeal teeth. The 'fish remains' category included all identifiable fish species as well as unidentified fish bones, fins, and other fish parts. The 'mollusks' category included bivalves and gastropods, and the 'insects' category included all terrestrial insects. The percentage contribution by weight of each food category was roughly estimated and then recalculated into real weights based on the weight of the total gut content. The vacuity index (VI) was calculated as the number of specimens with empty stomachs divided by the total number of specimens, multiplied by 100 (Molinero and Flos 1992). Due to frequent difficulties in numerically estimating whole food items, the relative frequency of occurrence (*F*%) for each of the four food categories, was selected as the best way to present the results, focusing on the presence of fish remains (Hyslop 1980; Silveira et al. 2020). To investigate seasonal trends in the diet of *S. acarnanicus*, *F*-values were pooled, irrespective of sampling year, and divided by season. Seasons were defined as follows: (i) summer covered June, July, and August; (ii) autumn covered September, October, and November; (iii) winter covered December, January, and February; and (iv) spring covered March, April, and May. Statistical significance between the two fishing methods was tested using the Chi-square test.

To determine the feeding strategy, the modified Costello graphical method was used. According to this method, the prey-specific abundance (*Pi*), which is defined as the percentage of a prey item over the total of prey items in only those predators in which the actual prey occurs, is plotted against the frequency of occurrence (*Fi*) on a two-dimensional graph. Information about prey importance and feeding strategy of the predator is provided by the distribution of points along the diagonals and the axes of the diagram (Amundsen et al. 1996).

Results

The dietary analysis of *S. acarnanicus* specimens collected from the three study lakes revealed five macrophyte species (*Vallisneria spiralis*, *Najas marina*, *Phragmites australis*, *Ceratophyllum demersum*, and *Myriophyllum spicatum*), one bivalve mollusk species (*Dreissena blanci*), two gastropod mollusk species (*Theodoxus varius*, *Valvata piscinalis*) and one fish species (*Atherina boyeri*). The vacuity index (VI) was calculated for each lake: Lake Trichonis had a VI of 18.5% for gillnet specimens and 11.3% for light-assisted purse seine specimens, while Lake Ozeros and Lake Amvrakia had VI's of 20.4% and 24.8%, respectively.

Specimens collected from gillnet fishing in Lake Trichonis were observed to have a diet of macrophytes and phytoplankton, which were categorized as plant material ($F = 82.1\%$), fish remains ($F = 22.7\%$), terrestrial insects ($F = 2.4\%$), and mollusks ($F = 0.8\%$). The overall F values for the fish remains found were remarkably higher in the samples taken by purse seining with the use of light ($F = 73.1\%$), instead of those taken without light using gillnets (Chi-square test, $P < 0.05$). The samples taken using gillnets had slightly higher F values for prey types such as the terrestrial insects and bivalves, in contrast to the samples taken with the use of light, where the F value for the insects was 1.6%, while no mollusks were present in the diet (Fig. 2). The diet of specimens collected from light-assisted purse seine fishing included a large,

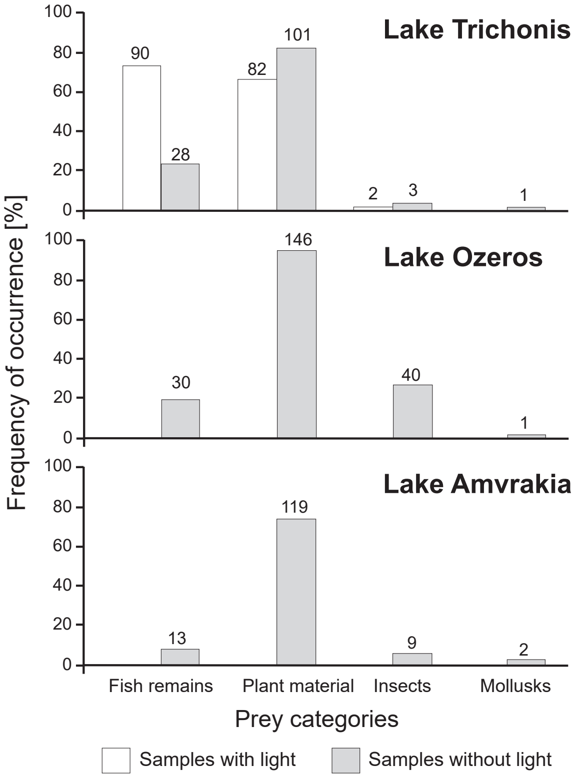

Figure 2. The overall frequency of occurrence of the main food types found in the gut contents of the *Scardinius acarnanicus* specimens collected in Lake Trichonis (separately for the samples taken with or without light), Lake Ozeros and Lake Amvrakia. The numbers of the *Scardinius acarnanicus* individuals with the specific type of food are given.

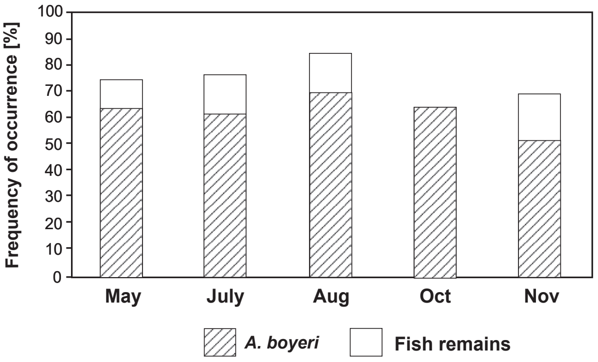

Figure 3. The frequency of occurrence of unidentified fish remains and *Atherina boyeri* found in the gut contents of the *Scardinius acarnanicus* specimens taken from Lake Trichonis using light between May and November 2017.

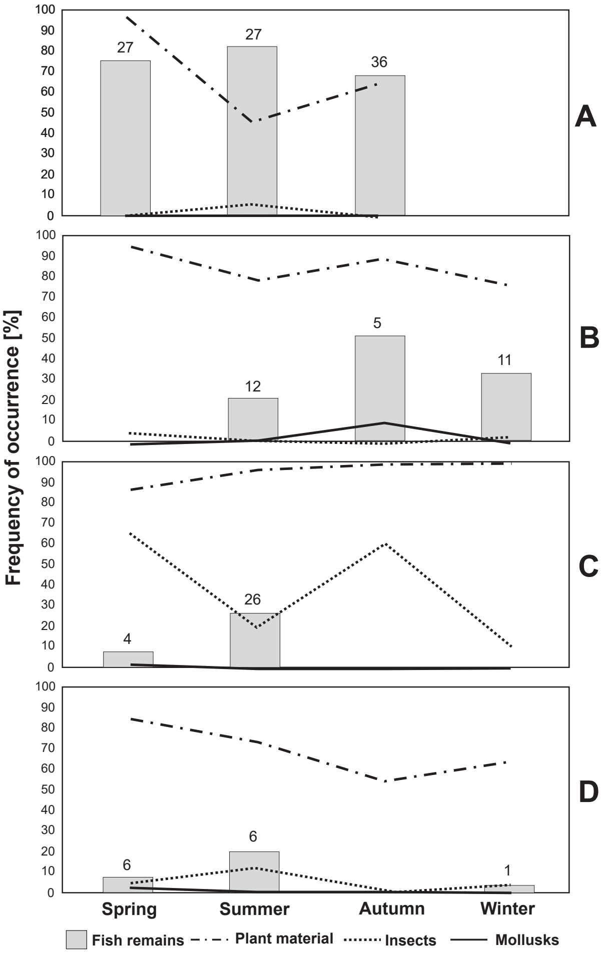

Figure 4. The frequency of occurrence of the main food types found in the gut contents of the *Scardinius acarnanicus* specimens collected during the four seasons from **A**: Lake Trichonis (with light), **B**: Lake Trichonis (without light), **C**: Lake Ozeros and **D**: Lake Amvrakia. The numbers of the *Scardinius acarnanicus* individuals with fish remains in their gut content are given.

but seasonally diverse, amount of *A. boyeri*, with *F* values ranging from 52.4% in November to 70% in August (Fig. 3). In contrast, the diet of specimens collected from gillnet fishing did not include *A. boyeri*; this can probably be explained by the longer time-lag between collection and preservation for these specimens, resulting in some of their gut content having been digested before preservation.

The dietary analysis of the *S. acarnanicus* specimens from Lake Ozeros revealed a lower *F* value for the fish remains (*F* = 19.2%) in comparison to Lake Trichonis, while the respective value for Lake Amvrakia was the lowest among the 3 lakes (*F* = 8.1%). The overall *F* value for the plant material for Lake Ozeros was 93.5%, for the terrestrial insects 25.6% and for the bivalve mollusks 0.6%, while for Lake Amvrakia the overall *F* value for the plant material was 74.8%, for the terrestrial insects 5.6% and 1.2% for the bivalve and gastropod mollusks.

In Lake Trichonis, the highest *F* value for the fish remains in the samples taken with the use of light was recorded in summer (81.8%) and the lowest in autumn (66.6%), while no samples were taken in winter (Fig. 4). In contrast, in the samples taken with the use of gillnets, the *F* values

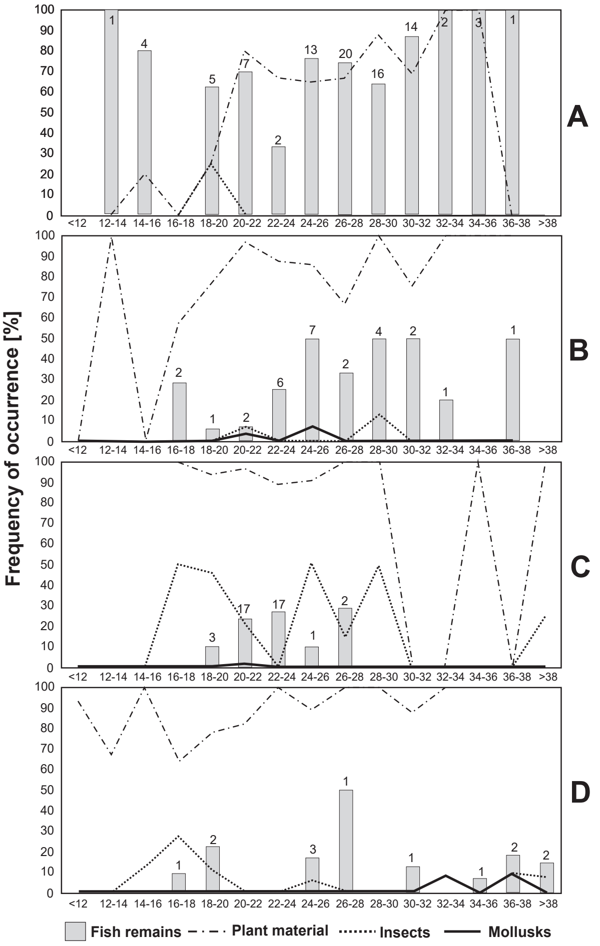

Figure 5. The frequency of occurrence of the main food types found in the gut contents of the different size classes of the *Scardinius acarnanicus* specimens taken from **A**: Lake Trichonis (with light), **B**: Lake Trichonis (without light), **C**: Lake Ozeros, and **D**: Lake Amvrakia. The numbers of *Scardinius acarnanicus* individuals with fish remains in their gut content are given.

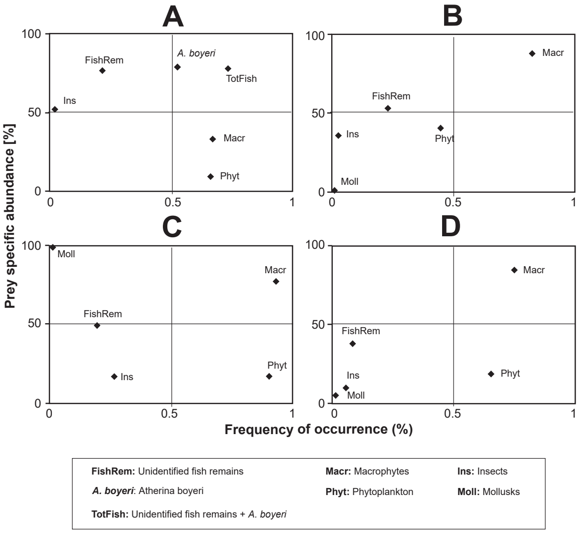

Figure 6. Graphical analysis of *Scardinius acarnanicus* feeding strategy using the modified Costello method in **A**: Lake Trichonis (with light), **B**: Lake Trichonis (without light), **C**: Lake Ozeros, and **D**: Lake Amvrakia.

for the fish remains were lower, with their maximum in autumn (50%). During spring, fish remains were absent from the gut content with the specimens presented a shift to other food types, such as plant material ($F = 95.2\%$) and the terrestrial insects ($F = 4.8\%$). In Lake Ozeros, the highest F value for the fish remains was observed during summer (26.0%), while no fishes had been preyed upon during autumn and winter. Finally, in Lake Amvrakia, the highest F value for the fish remains occurred during summer (20.0%), with no fish remains in the diet during autumn.

Considering the dietary differences in respect to the total length of *S. acarnanicus* (Fig. 5), it is observed that in the samples taken with light from Lake Trichonis, the F values of fish remains fluctuated between 44.4% and 100% with no obvious trend among size classes. Indeed, even the smaller specimens (<16 cm) were found to prey upon other fishes and especially *A. boyeri*. In contrast, no fish predation was recorded from the specimens being less than 16 cm in the other two lakes, as well as in the samples taken with gillnets from Lake Trichonis.

For the entire sampling period, *S. acarnanicus* demonstrated an opportunistic feeding strategy (Fig. 6). In the samples taken with the use of gillnets from the three lakes, the species showed a greater consumption of macrophytic vegetation and phytoplankton in their diet. Some individuals include occasionally other food types in their diet, such as terrestrial insects, bivalves and gastropod mollusks, and fish. On the contrary, in the samples taken by purse seining with light, *S. acarnanicus* present greater consumption upon *A. boyeri*. Macrophytes and phytoplankton had a general contribution to the diet, while there was specialized predation upon *A. boyeri.* The combination of *A. boyeri* and unidentified fish remains have as a result the high specialization of *S. acarnanicus* in the consumption of fishes (TotFish).

Discussion

Similar to what happens on land, the presence of artificial light in the water creates a strong focus of attraction for aquatic organisms that exhibit positive phototaxis. Although there is substantial literature on how aquatic organisms respond to artificial light, little is known about why they respond as they do (Nguyen and Winger 2019). In the most recent review article concerning the fish behavior in response to artificial light (Nguyen and Winger 2019), there was a thorough quotation of the negative ecological impacts of light ‘pollution’. It seems that artificial light affects the behavior of various species including turtles and seabirds, however, underwater lights like those used in fishery, impact fish foraging and schooling behavior, spatial distribution, predation risk, migration, and reproduction (Nightingale et al. 2006). Although some studies investigate how the feeding behavior of natural predators changes in the presence of artificial light (Becker et al. 2013), as predators achieve greater feeding success (Downing and Litvak 2001, Migaud et al. 2009, Sierra-Flores et al. 2016), few studies investigate how the presence of artificial light influences the feeding behavior of fish species other than the natural predators.

Among the objectives of this study was to confirm the suggestion that fishing lights can differentiate the feeding behavior of the approaching fishes towards predatory, even of those species that are not natural fish predators (Kehayias et al. 2018a, 2018 b). According to the presently reported findings, *S. acarnanicus* collected using light-assisted purse seine fishing in Lake Trichonis fed on other fish species, especially the commercially important *A. boyeri*. These *S. acarnanicus* fed on *A. boyeri* primarily in the summer, which is when the *A. boyeri* population peaks (Leonardos 2001). Interestingly, even young *S. acarnanicus* fed on *A. boyeri*. In contrast, *S. acarnanicus* that were collected using simple gillnet fishing were omnivorous, with a mainly plant-based diet that included only small numbers of terrestrial insects, mollusks, and fish remains. Very similar feeding patterns were found for *S. acarnanicus* in the two nearby lakes, where only gillnet fishing is practiced.

Although Lake Ozeros has a dense *A. boyeri* population, due to their natural introduction to the lake from the Acheloos River, *A. boyeri* are not targeted by local fishermen, who use gillnets that target larger species. The gut content analysis revealed that *S. acarnanicus* that were caught by gillnet fishing in both Lake Ozeros and Lake Trichonis had similar feeding patterns of preying upon fish. The high *F*-values of lakes Ozeros and Trichonis, in contrast to those of Lake Amvrakia, which is not inhabited by *A. boyeri*, suggest that *S. acarnanicus* likely benefits from the dense *A. boyeri* population in those lakes, resulting in its preying upon this species, although this hypothesis needs further examination.

Findings from this study indicate that the presence of night-time artificial light in Lake Trichonis promotes predatory instincts of *S. acarnanicus*, so that they take advantage of the rich *A. boyeri* food source. These findings are supported by those of Kehayias et al. (2018a, 2018b), who found that the normally zooplanktivorous *A. boyeri* showed elevated predation of the endangered endemic *Economidichthys trichonis* larvae in areas close to artificial fishing lights. More specifically, Kehayias et al. (2018b) found that, on average, and at certain times of the year, larger *A. boyeri* specimens had a nightly consumption of up to 14.5 *E. trichonis* larvae. This consumption placed an overall nightly predation pressure of 260 000 *E. trichonis* larvae when *A. boyeri* schooled around the lamp rafts of a single purse seine fishing boat (Kehayias et al. 2018b).

The presence of artificial night-time light sources in Lake Trichonis initiates a trophic food chain reaction. During the initial hours of illumination, numerous zooplankton and larval fish species start to gather around the lamp rafts (Kehayias et al. 2016, Kehayias personal observations). Gradually, zooplanktivorous species, in this case, *A. boyeri*, create large schools around the lamp rafts, and they start to prey heavily upon zooplankton and *E. trichonis* larvae. Larger species, such as *S. acarnanicus*, then start to gather around the lamp rafts and, in turn, they start to prey upon *A. boyeri* and other fish species. Our findings are supported by those of other studies from across the world that observe similar changes to fish feeding behavior in the presence of artificial night-time light (Ben-Yami 1976; Prinslow et al. 1980; Tabor et al. 2001; Nightingale et al. 2006; Bolton et al. 2017).

The presently reported study of *S. acarnanicus* diet in three lakes of western Greece showed that *S. acarnanicus* is an omnivorous species that can, in specific circumstances, become a fish predator. This is, to the best of our knowledge, the first report of fish predation by a member of the genus *Scardinius* in European inland waters. It is already known that the most widespread species, *Scardinius erythrophthalmus,* can be piscivorous in other geographical regions, where it is allochthonous (Kapuscinski et al. 2012; Guinan et al. 2015; Collier and Grainger 2015). *Scardinius acarnanicus* has many similar morphological and ecological characteristics to those of *S. erythrophthalmus*; indeed, only recently was a clear distinction made between the two species in Greece by Prof. Economidis in 1991 (Iliadou et al. 1996).

The only previous study of *S. acarnanicus* diet was conducted from 1977 to 1979, in the lake system of Trichonis–Lysimachia Lake (Iliadou 1991), and there have been no previous equivalent studies in either Lake Ozeros or Lake Amvrakia. The present results, concerning the composition of the plant material in the diet, are in general accordance with the reports of Iliadou (1991). However, they are in direct contrast to her reports according to which the species was strictly herbivorous, feeding exclusively on reeds, other aquatic vegetation, and phytoplankton. Considering similarities in the methodology used for gut content analysis both in previous studies and the presently reported study, and the large number of specimens collected from Lake Trichonis (289 and 360 specimens in the presently reported and previous studies, respectively), it is challenging to explain the apparent dietary shift of *S. acarnanicus* from herbivorous to omnivorous, and more specifically towards fish predation. This species showed a preference of feeding upon *A. boyeri*. Neither the existence, nor the dominance of *A. boyeri* is new for this ecosystem and, thus, it cannot be incriminated as the prime cause of a possible change of *S. acarnanicus* diet during the last 40 years. Instead, we suggest that changes to fishing practices might explain why the *S. acarnanicus* diet has changed. Until the early 1980s, *A. boyeri* were mostly caught using bottom trawling methods, while trammel nets and set gillnets were used to fish for other larger species; these methods were used to collect specimens in the study of Iliadou (1991). From the early 1990s, light-assisted purse seine fishing was introduced and quickly dominated when fishing for *A. boyeri*, mainly due to its efficiency and low environmental impact.

Furthermore, there is another factor, that may affect *S. acarnanicus*. When fishermen retrieve their purse seines, the catch includes large numbers of *S. acarnanicus* in addition to the targeted *A. boyeri*; the live *S. acarnanicus* are returned to the lake, due to low market demand. This practice of returning live *S. acarnanicus* to the lake, which has become more common in recent decades, may have inadvertently 'taught' the *S. acarnanicus* population that it is safe to prey on *A. boyeri* when there is artificial light at night. This 'educational hypothesis' may be further supported considering that *S. acarnanicus* can live for 10 years and that even the smaller members of its population can prey on *A. boyeri*. However, it cannot be verified with the presently reported data set, but it would be interesting to investigate it using a different approach (e.g., fish tagging). Although there are several other examples of how human behavior and practices may have conditioned animal behavior (Wong and Candolin 2015), to our best knowledge, there is no other similar case referring to the alteration of the feeding behavior of a wild fish.

The results of the presently reported study raise some interesting issues regarding the ecological consequences of light-assisted fishing, both locally and globally. Our results clearly demonstrate that, despite being mainly omnivorous, *S. acarnanicus* can also be a serious fish predator which prompts a question of whether *S. acarnanicus* was always strictly herbivorous, or whether its feeding habits have changed. The presently reported results would certainly be stronger if, also to the present method, we had the opportunity (and funding) to use approaches such as stable isotope and fatty acid analysis. Furthermore, we found that, in an enclosed lake environment, purse seine lights affect the behavior and feeding preferences of at least two fish species, *S. acarnanicus* and *A. boyeri*, while at the same time affecting the population of a third species, *E. trichonis*, being in the lower level of this particular feeding chain. It is suggested that the combination of specific fishing methods (light-assisted fishing) and improper catch handling (returning live *S. acarnanicus* to the lake), promotes population increases of *S. acarnanicus* relative to the *A. boyeri* population, which is the target species in Lake Trichonis. Although there are legal purse seine fishing boats, there are also many illegal boats, even outside of the legal fishing season and it is expected that this illegal fishing exasperates fish population changes. The proposed 'educational hypothesis' concerning the feeding behavior close to the light, is an interesting and broad ethological issue that warrants investigation in future studies. Finally, given that light-assisted fishing is a global fishing technique, more feeding studies on marine and freshwater species caught around light sources are needed to determine the real magnitude of the effect of artificial light on the ichthyofauna.

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Stocking density effect on survival and growth of early life stages of maraena whitefish, *Coregonus maraena* (Actinopterygii: Salmoniformes: Salmonidae)

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Abstract

The maraena whitefish, *Coregonus maraena* (Bloch, 1779), is often considered a suitable candidate for intensive aquaculture diversification in the EU. However, only a few such farms in Europe are in operation. Rearing this species in recirculating aquaculture systems is a recent innovation, and optimisation is necessary to standardise aspects of larviculture. This 30-day study investigated the effect of stocking densities of 25/L, 50/L, 100/L, and 200/L on the survival and growth of maraena whitefish larvae in a recirculating aquaculture system. The four groups of larvae (initial weight = 7.4 ± 0.1 mg; initial total length = 13.0 ± 0.1 mm) in three repetitions were reared in a recirculating system. Larvae were fed fresh live brine shrimp metanauplii every 3 h at a rate converted to larval stocking density. After the experiment, 10 larvae from each tank (30 of each density group) were weighed on a digital microbalance (ABJ 220-4M KERN, Germany, readout = 0.1 mg) and measured manually on images taken with Leica MZ16 A stereomicroscope and a digital colour camera with 5-megapixel resolution for Leica DFC420 Image Analysis. No significant differences in final body weight, total length, size heterogeneity, condition factor, or survival were found among treatments ($P > 0.05$). The highest non-significant survival rate and growth parameters were observed in larvae reared at 25/L. On the contrary, it is possible to rear maraena whitefish larvae at high stocking density without any subsequent negative consequences for growth and survival. As no significant differences in any evaluated parameter were observed between groups of larvae at the highest and lowest stocking density, we conclude that it is possible to rear maraena whitefish larvae at high stocking density (and 200/L) without any subsequent negative consequences for growth and survival.

Keywords

coregonids, fry, growth metrics, larviculture, recirculation systems

Introduction

The maraena whitefish, *Coregonus maraena* (Bloch, 1779), is a promising species for inland freshwater aquaculture throughout east-central Europe (Mukhachev and Gunin 1999), and northern Europe, especially Finland (Jobling et al. 2010) and Norway (Siikavuopio et al. 2011). Several decades ago, due to predation by the great cormorant *Phalacrocorax carbo*, the population dramatically declined (Suter 1997). Eutrophication has also contributed to the decrease

(Thomas and Eckmann 2007). At present, it is important that re-establishment of whitefish natural production be accompanied by the culture in intensive aqua systems. The recirculating aquaculture system is an important model in worldwide aquaculture, given its cost-effectiveness, low environmental impact, ease of regulating water quality, and final product quality control features (d'Orbcastel et al. 2009). The establishment of coregonid production in recirculating systems requires that optimal larviculture conditions, including stocking density as a crucial factor in the productivity of fish culture systems, be identified.

Excessively high density can produce a stress response, particularly increased plasma cortisol level (Li et al. 2012), impede thyroid hormone production (Herrera et al. 2016), and affect growth (Żarski et al. 2008) and survival (Molnár et al. 2004; Szkudlarek and Zakes 2007). High density can lead to fin erosion, gill damage, fish welfare impairment (Ellis et al. 2002), and promote cannibalism (Liao and Chang 2002). It can decrease food utilization (Sharma and Chakrabarti 1998) and alter metabolic rate (Tolussi et al. 2010) with respect to lipids (Mommsen et al. 1999), carbohydrates (Sangiao-Alvarellos et al. 2005), and proteins (Costas et al. 2008). Finally, high fish density can impair water quality (Montero et al. 1999), reducing oxygen levels and increasing ammonia concentration accompanied with parasitical incidence, moderate hyperplasia and absence of congestion (Azevedo et al. 2006) in commercial production systems. These negative aspects such as cannibalism or aggressive behaviour lead to mass mortality of larvae and cause economic loss (Smith and Reay 1991; Ruzzante 1994) as well as disrupt production stability (Rowland et al. 2006). On the other hand, low stocking density is associated with high production costs (Luz and Santos 2008). Stocking density has been shown to be a limiting factor in fish growth during early development (Webb et al. 2007), while its impact is mitigated in adult fish (Duarte et al. 2004).

In Europe, the initial rearing of coregonid larvae in intensive indoor tanks is usually practised with stocking densities ranging from 5 to 100 larvae/L (Dabrowski and Poczyczynski 1988; Beltran and Champigneulle 1992; Esmaeilzadeh-Leithner and Wanzenböck 2018). Moreover, larvae rearing of lake whitefish, *Coregonus clupeaformis* (Mitchill, 1818), as similar commercially important species from North America is usually done with low density (30 larvae per L) (Zitzow and Millard 1988, Harris and Hulsman 1991).

The optimal stocking density needs to be determined for each fish species and developmental/reproductive stage to facilitate survival and growth and enable efficient management to maximise production and profitability, as well as to provide proper conditions for fish. Information on stocking density effects on maraena whitefish larvae growth performance and survival is scarce. The goal of the presently reported study was to determine whether stocking density affects the survival and growth of maraena whitefish larvae reared in a recirculating aquaculture system (RAS).

Materials and methods

Eggs and larvae

Maraena whitefish were obtained from in the Szczecin Lagoon (the River Odra estuary), north-western Poland. The broodstock comprised 120 fish at a 1:1 sex ratio. Gametes of three-year-old 60 females (mean weight, 800.4 ± 80.1 g, mean ± SEM; mean total length, 30.2 ± 1.1 cm) and three-year-old 60 males (650.5 ± 49.7 g, mean ± SEM; mean total length, 26.4 ± 0.9 cm) were stripped manually (no hormone stimulation) by commercial fishermen in December 2016 shortly after fish capture and transported to local hatcheries for fertilization and incubation. Eggs (100 mg) were fertilized with 0.5 mL of milt mixed with 50 mL of hatchery water and incubated at the ambient water temperature of the river (2–3°C) with initial water inflow 3 L/min, oxygen saturation to 90%, and pH near 7.0. In February 2017, the eggs were taken to the Department of Lake and River Fisheries (Olsztyn, Poland) where they were distributed among five 8-L Zug jars (n = ~150 000 eggs/jar) in a recirculating system and incubated at 3.0–3.5°C with water inflow 3 L/min, oxygen saturation to 90%, and pH near 7.0. In total, ~750 000 eggs were incubated. After 60 days, eggs were transferred to the second set of 8-L Zug jars and incubated at 8–9°C to accelerate development and hatching. After 5 days, the temperature was increased to 10°C for mass hatching. Hatching success was estimated at 90%, and about 675 000 larvae were available for the experiment. Hatched larvae swam across to a tank (total volume 1 m^3) underlain with 0.2 mm mesh. After 24 h, larvae were transferred to tanks in the RAS.

Experimental system

Four groups of larvae in three replicates were transferred to the experimental aqua system consisting of twelve 2 L aquaria, 96 × 154 × 200 mm. The recirculating system (2300-L total water volume) included a series of filtration sections (total biofilter volume 1500-L), a settling tank (500-L water volume). Thirty fish were weighed and measured to obtain the initial values for weight and length. Maraena whitefish larvae (initial weight, 7.4 ± 0.1 mg, mean ± SEM; initial total length, 13.0 ± 0.1 mm) were placed into each aquarium at stocking density of 25/L (S25), 50/L (S50), 100/L (S100), and 200/L (S200). A biomass by litre (g/L) was 0.185 (S25), 0.370 (S50), 0.740 (S100), 1.480 (S200). A total of 2250 larvae were used in the experiment.

Rearing conditions

The oxygen level, water temperature, and pH were checked daily at 0800 and 1600 h. The pH range was monitored using an OxyGuard H04PP Handy pH meter (OxyGuard International, Denmark). The initial temperature without supplemental heat was 10°C. Water temperature ~19°C was regulated by a HAILEA HC-1000A cooler (China). The temperature was gradually elevated from 10°C to 19°C (3°C/day). Oxygenation was maintained using two SICCE Syncra 5.0 pumps (5000 L/h) (Italy). Ammonia, nitrate, and nitrite concentrations were analysed using HACH, LCK 304, LCK 339, LCK 341 (Germany) with a HACH DR5000 spectrophotometer (Germany). Disinfection used a 30 W UV MCT Transformatoren GmbH steriliser (Germany). NaCl was added at 1 g/L weekly to maintain a 16:1 chloride:nitrogen ratio. A constant inflow of 0.4 L/min was ensured. Dead larvae were removed and counted during daily cleaning. The level of organic matter remained low. A low CO_2 level was maintained via aeration and keeping alkalinity stable. During the 30-day trial, basic physico-chemical parameters were following: temperature = 19.1 ± 0.0°C, pH = 8.7 ± 0.0, O_2 saturation = 85.8 ± 0.9%, O_2 concentration = 7.9 ± 0.1 mg/L, NH_4^+ = 0.1 ± 0.0 mg/L, NO_2 = 0.8 ± 0.1 mg/L, NO_3 = 21.2 ± 5.4 mg/L.

Feeding

Larvae were fed fresh live metanauplii of brine shrimp, *Artemia salina* (Ocean nutrition, HE > 230 000 NPG, Belgium) (20–24 h old, 0.4–0.5 mm) four times daily at 3 h intervals during the light phase (0830 to 1730 h). The feeding level was fixed to the range of 500–700 *Artemia* sp. metanauplii per fish per day at a rate converted to larval stocking density (Table 1). The daily ratio was based on a previous experiment (unpublished data). Furthermore, this ration was in slight excess as some uneaten metanauplii were observed in tanks at the end of the day. The feeding level was adapted according to losses of larvae during the experiment and fish body weight (Fiogbé and Kestemont 2003) using the formula

$$R_{\mathrm{opt}} = 4.89W^{-0.27}$$

where R_{opt} = optimal daily feeding level, W = body weight [g].

Sampling and measurements

After the experiment, 10 larvae from each tank (30 of each density group) were weighed on a digital microbalance (ABJ 220-4M KERN, Germany, readout = 0.1 mg) and measured manually from images taken with Leica MZ16 A stereomicroscope and a digital colour camera with 5-megapixel resolution for Leica DFC420 Image Analysis.

Table 1. Concentration of brine shrimp (*Artemia salina*) fed to larvae of maraena whitefish, *Coregonus maraena* (Bloch, 1779) in a 30-day trial.

Group	Whitefish stocking density		*Artemia* feeding dose	
	[larvae/L]	[larvae/2L]	[mL/L]	[mL/2L]
S25	25	50	2.5	5
S50	50	100	5.0	10
S100	100	200	10.0	20
S200	200	400	20.0	40

Values for 2 L relate to the volume of 2-L aquarium.

A sample size of ten larvae per tank, 30 larvae per treatment, was used as in a number of studies (Kaiser et al. 2003, Mahmood et al. 2004, Fletcher et al. 2007, Celada et al. 2008, Nowosad et al. 2013, Palińska-Żarska et al. 2014, Laczynska et al. 2016).

The survival rate (SR), size heterogeneity (SH), and condition factor (K) and specific growth rate (SGR) were assessed as follows:

$$\mathrm{SR}\ (\%) = 100 \times (\mathrm{Nf}/\mathrm{Ni})$$

in which N_{i} and N_{f} = initial and final number of larvae, respectively;

$$\mathrm{SH}\ (\%) = 100 \times (\mathrm{SD}/W_{\mathrm{m}})$$

in which SH = size heterogeneity; SD = mean standard deviation of weight of 10 randomly selected larvae/tank; W_{m} = mean weight [mg] of 10 larvae/tank.

$$K = 100\,000 \times W \times (\mathrm{TL}^3)^{-1}$$

in which W = mean weight [g] of 10 larvae/tank; TL = mean total length [mm] of 10 larvae/tank

$$\mathrm{SGR}\ (\%) = 100 \times [(\ln W_{\mathrm{t}} - \ln W_0)/d]$$

in which W_{t} and W_0 are final and initial weight of larvae, respectively [g]; d = duration of the experiment [days].

Statistical analysis

Statistical analyses were performed using STATISTICA 12.0 (StatSoft, Praha, Czech Republic). Data are presented as mean ± SEM. The effects of stocking density on W, TL, SR, K, SH, and SGR were analysed by one-way ANOVA with stocking density as a fixed variable. Differences were considered significant at $P < 0.05$. Prior to ANOVA, SR, K, SH, and SGR were arcsine-transformed. All data were tested for homogeneity of variance using the Cochran, Hartley, and Bartlett test, and for normality with the Shapiro–Wilk normality test. The parametric Tukey test was used for assessing differences among groups in W, TL, SR, SH, K, and SGR (Table 2).

Results

At the conclusion of the trial, no significant (P>0.05) differences among treatments were observed in SR, W, TL, SH, K, or SGR (Table 2). The highest SR (92.7% ± 2.4%),

Table 2. One-way ANOVA results for the factor stocking density on total length (TL), body weight (*W*), size heterogeneity (SH), condition factor (*K*), survival rate (SR), and specific growth rate (SGR) of larvae of maraena whitefish, *Coregonus maraena* (Bloch, 1779).

Parameter	Source of variation	SS	DF	*F*	MS	*P*
TL	SD	0.9	3.0	0.3	2.3	0.2
W	SD	466.3	3.0	155.4	2.7	0.1
SH	SD	22.7	3.0	7.6	0.2	0.9
K	SD	0.0	3.0	0.0	2.2	0.2
SR	SD	3.6	3.0	1.2	0.2	0.9
SGR	SD	0.0001	3.0	2.1	0.00005	0.2

SD = stocking density; SS = sum of square; DF = degrees of freedom; *F* = distribution fitting; MS = mean square; *P* = probability.

Table 3. Effect of stocking density on growth and survival of larvae of maraena whitefish, *Coregonus maraena* (Bloch, 1779), in a 30-day growing trial.

Group	SR [%]	TL [mm]	*W* [mg]	SH [%]	*K*	SGR [%]
S25	92.7 ± 2.4	30.7 ± 0.3	147.9 ± 5.8	22.5 ± 4.3	0.51 ± 0.01	0.50 ± 0.003
S50	91.3 ± 1.5	30.4 ± 0.2	135.7 ± 1.6	20.3 ± 3.6	0.48 ± 0.01	0.49 ± 0.001
S100	91.33 ± 1.1	30.4 ± 0.1	135.1 ± 3.5	21.1 ± 4.9	0.48 ± 0.00	0.49 ± 0.003
S200	91.8 ± 1.0	30.0 ± 02	131.3 ± 5.2	18.7 ± 2.3	0.49 ± 0.01	0.49 ± 0.004

Groups represent stocking densities of 25, 50, 100, and 200 larvae/L, respectively; SR = survival rate, TL = total length, *W* = body weight, SH = size heterogeneity, *K* = condition factor, SGR = specific growth rate.

W (147.9 ± 6.3 mg), TL (30.7 ± 0.4 mm), SH (22.5% ± 1.1%), *K* (0.51 ± 0.01), and SGR (0.50 ± 0.003%) was observed at S25 (Table 3).

Discussion

The fact that growth–weight parameters did not differ significantly means that maraena whitefish growth was not influenced by stocking density at the tested levels. Slightly lower (non-significant) growth was found with increasing stocking density. It is important to sustain uniformity of fish size in aquaculture (Biswas et al. 2010). The effect of stocking density on larva size heterogeneity may be species-dependant. For instance, the relation of stocking density to size heterogeneity has been reported to be positive in red tilapia *Oreochromis niloticus* (Linnaeus, 1758) × *Oreochromis mossambicus* (Peters, 1852), when stocking density was 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 fry per litre (Huang and Chiu 1997), but negative in Arctic charr, *Salvelinus alpinus* (Linnaeus, 1758), with stocking density 10, 20, 28, 40, 60, 80, and 100 fry per litre (Wallace et al. 1988). We found size variation with respect to stocking density at the levels tested to be negligible with the only non-significant more uniform size in the S200 group and the least uniform in the S25 group. North et al. (2006) observed the same trend, with the highest size heterogeneity observed in rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), reared in low stocking density and vice versa.

Stocking density can influence mortality rate, with survival often negatively correlated with stocking density as shown for silver perch, *Bidyanus bidyanus* (Mitchell, 1838) (see Rowland et al. 2006). Fish species can be classified as density-independent or density-dependent. Tilapia larvae (Huang and Chiu 1997) were reported to be density-dependent. Survival was high and not significantly affected by stocking density in the presently reported study, thus maraena whitefish seem to be density-independent, and stocking density is not likely a limiting factor in their survival in intensive rearing. High survival in all groups indicates that high-density aquaculture may be suitable for the production of this species. This phenomenon was also seen in Kupren et al. (2011) for asp, *Leuciscus aspius* (Linnaeus, 1758); ide, *Leuciscus idus* (Linnaeus, 1758); and chub, *Squalius cephalus* (Linnaeus, 1758).

Stocking density has been reported to be an important factor in fish growth (Saoud et al. 2008) and is of particular concern in the welfare of intensively farmed fish (Ashley 2007, Wocher et al. 2011). Mortality (Ellis et al. 2012), as well as susceptibility to pathogen infections and fin damage (Turnbull et al. 1998, Jones et al. 2011), in farmed fish, are generally considered important indicators of welfare. Ashley (2007) suggests that unsuitable stocking density can result in damage or death of fish. Negative effects of high stocking density on fish growth and survival can be attributed to impaired water quality associated with accumulation of fish metabolites and carbon dioxide, with accompanying decline in pH level (Hosfeld et al. 2009). As no technical problems or disease occurred during the course of our study, we can conclude that water quality and stocking density effects were accurately evaluated. The high survival rate at all density levels and lack of observable damage to fins are evidence of appropriate rearing conditions with respect to fish welfare.

Conclusions

No significant differences in any evaluated parameter were observed between groups of larvae at the highest and lowest stocking density. It is possible to rear maraena whitefish larvae at high stocking density with no subsequent negative consequences for growth and survival. This study examined fry and early-stage larvae, but a further study, focusing on juvenile and adult maraena whitefish, is warranted. The effects of stocking density on stress hormone response, body composition, and haematological and biochemical parameters of maraena whitefish should be studied.

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Small-scale fisheries in the southern Black Sea: Which factors affect net profit?

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Abstract

Small-scale fisheries (SSF) is a local and community-based activity that can be traced back to ancient times, and thus, closely related to the history of humankind. However, large-scale fisheries have grown tremendously, approaching an industrial sector in the last century, due to their socio-economic and political properties, including both national and international aspects. This progress towards industrial-scale fisheries led to the involvement of scientific research, first aiming to improve production efficiency, and then, to protect ecosystems as resources exploited for fisheries activity, by mitigating their adverse impacts. During this evolutionary progress, SSF was usually neglected because of their limited production ability, and thus minimal economic contribution, until the later phase when the protection of ecosystem resources gained sufficient importance. As a result of this, many countries lack data on SSF, undermining efforts for the creation of proper policies for this type of fisheries. The aim of this study was to evaluate the productivity and the effects of some demographic characteristics, boat structures, and some cost (input) items on the net profit of SSF in the Black Sea. The eligible sample for this study consisted of 5575 small-scale fishing boats in the Black Sea. The number of fishers to be surveyed was determined as 315 using the "Simple Random Sampling" method, based on operators of boats < 12 m, i.e., boats in the SSF. Questionnaires were conducted face-to-face with fishers. In this study, it was tested if six parameters were investigated to determine whether they had a significant effect on net profit in SSF. These parameters were: (1) engine power; (2) number of fishing days; (3) boat length; (4) consumption of fuel in fishing; (5) education level of fishers; and (6) overall professional experience of fishers. To do so, Simple Linear Regression Analysis was performed to determine the effect of the data considered as independent variables when the net profit was set as the dependent variable. Atlantic bonito, *Sarda sarda* (Bloch, 1793); whiting, *Merlangius merlangus* (Linnaeus, 1758); rapa whelk, *Rapana venosa* (Valenciennes, 1846); and turbot, *Scophthalmus maximus* (Linnaeus, 1758) were the most important commercial fish species for small scale fishing. When catch per boat in SSF was evaluated, Kırklareli province ranked first with 97 007 kg, with Atlantic bonito (44 778 kg) being the most common species caught. Samsun had the second-largest catch per boat with 91 761 kg. The total net profit of 303 boats was calculated as €1 794 938 and the mean net profit per boat was €5924. The highest per boat mean net profit (€25 909) was in Kırklareli. According to the results of the study, the number of days at the sea, boat length, engine power, and fuel cost had a significant effect on the net profit while education level and professional experience were not important in productivity. The economically-fragile SSF sector may need some kind of supporting subsidy. It would be beneficial to provide support to the majority of fishers active in the SSF in terms of complementary alternative employment opportunities in the regions where they are located.

Keywords

Black Sea, net profit, productivity, revenue, small-scale fisheries, total cost

Introduction

Small-scale fisheries (SSF) is not considered as an economic sector. In their own right since SSF tend to be closely related to local communities, traditions, and values (Kolding et al. 2014). SSF catch is usually for direct consumption in the local community and trading is mainly dependent on irregular opportunities such as unexpectedly successful catch performance and the availability of proper logistics for market delivery. Many SSF, which may have important conservation values, are overseen by self-governing mechanisms, but they are under increasing pressure. For example, the Food and Agriculture Organization of the United Nations (FAO) estimated that around 90% of all the people who work in fishery sectors are active in the SSF around the world, which collectively contribute to almost half of global fish catches (FAO 2018) and this contribution increases by two-thirds when the proportion directly used for human consumption is considered. Given the importance of SSF in the human food supply, the attention of both scientists and decision-makers has been drawn to the challenges faced by SSF for sustainable fishery management. Studies have shown that SSF can make a significant contribution to nutrition, food security, sustainable livelihood, and poverty reduction, in addition to being more eco-friendly features than industrial or large-scale commercial fisheries (Berkes et al. 2001; Béné et al. 2007). According to the FAO (2020), about 90% of the 35 million people recorded worldwide as fishers are classified as small-scale and a further 20 million people are estimated to be involved in the small-scale post-harvest service sectors.

The amount of main SSF marine products used for human consumption, such as fish flesh, roe, fins, etc. is around 30 million tons/year (Jacquet and Pauly 2008). Other products of industrial fisheries, such as fish-meal and fish oil which are not produced for direct human consumption, yield about 35 million tons/year. Crowder and Murawski (1998) reported that approximately 40 million tons of fuel is consumed per year in industrial fishery, with 1 to 2 tons of target species and 8 to 10 tons of discarded bycatch species per 1 ton of fuel, although a global rate for bycatch rate is difficult to estimate. In the case of SSF, annual fuel consumption is around 5 million tons with a product: fuel ratio ranging between 4:1 and 8:1 and much lower discarded bycatch (Jacquet and Pauly 2008). These authors also estimated that 25 to 27 million dollars of subsidies are annually provided to industrial fisheries worldwide, whereas this value is around 5 to 7 million dollars for SSF. Such a comparison clearly demonstrates the level of importance of small-scale fishery in the overall fishery sector. However, not much is known about product-related processes, such as capture, preservation, value-adding, and marketing, which are highly variable, and difficult to track through an established value chain chart compared to a well-established organization (Staples et al. 2004). It has been emphasized that global SSF activities, which involve more than 12 million fishers for the subsistence of their households, have not been managed sustainably due to their lower visibility. The contribution of SSF to gross national domestic product (GNDP) has been underestimated because of the difficulty of following a product's value chain from catch to the market.

Due to the inherent variability, complexity, and uncertainty, it is difficult to create a consensus definition that would be applicable to all global SSFs. Therefore, sectoral-based definitions for SSF have to be general. SSF has been defined as a dynamic and developing sub-sector of fisheries, using labor-intensive catching, processing, and distribution technologies to take advantage of marine and inland fishing resources (Staples et al. 2004). Nonetheless, the general perception of SSF implies characteristics, namely that it is predominantly artisanal, local, coastal, traditional, small, subsistence, non-industrial, low-tech, and poor (Natale et al. 2015).

Fisheries, in general, is defined as an activity that involves the catching, preservation, processing, transporting, and marketing of the product. Additionally, there are bilateral related sectors, such as construction and repair/maintenance of fishing gear, boats, and engines. There is no doubt that SSF plays a prominent role in the value chain, in the fishery market as a product supplier, and in the industrial and service sectors as a dependent customer. All these associated sectors, together with fish production, create the total contribution of the fishery sector to national, regional, and local economies.

The aim of this study was to analyze the socio-economic status of the SSF in the Black Sea. A set of socio-economic indicators were investigated that enabled an estimate of their significance to net profit in the SSF so that these results can be exploited to enhance the management of fisheries in the region. The following SSF fleet composition will provide the required data and information.

Data from 2017 reported a total of 14 479 fishing boats operating in Turkey. Of these, 12 983 (89.7%) have a boat length less than 12 m and thus constitute the SSF fleet active throughout the coasts of Turkey. These proportions are similar in the Black Sea, with 5575 fishing boats, of which 5141 (88.6%) are shorter than 12 m (TURKSTAT 2017; GDAR 2018).

These numbers indicate that the majority of boats involved in the Black Sea fishing are actually part of the Black Sea SSF, both in terms of structure and function. Although the number of boats in the SSF fleet is known, data and information concerning the socio-economic properties are scarce and limited. As result, it is almost impossible to predict their potential socio-economic performance. Such predictions are essential for developing efficient management strategies and solutions, considering both the sustainability of SSF activities and ecosystem resources and thus achieving a more rational use of resources and sustainable levels of the fishery.

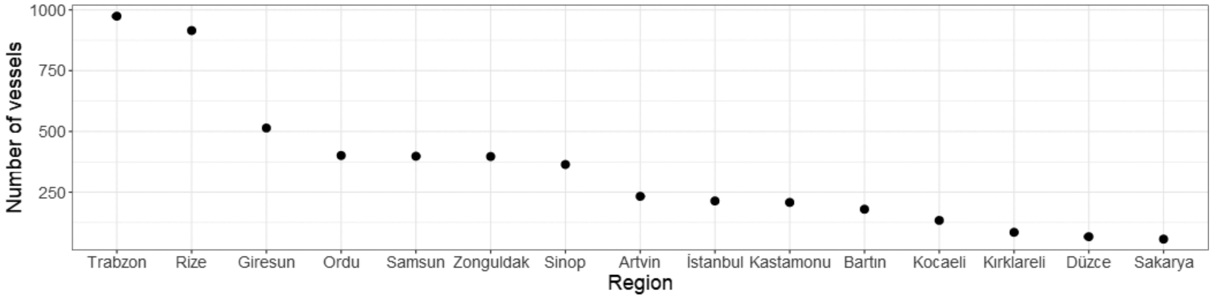

Figure 1. The number of SSF boats off the Turkish Black Sea coasts by region.

Material and methods

The sample was drawn from the SSF in the Black Sea. These numbered 5141 (88.6%) with a boat length < 12 m of a total of 5575 fishing boats operating in the Black Sea (TURKSTAT 2017; GDAR 2018). The majority of these smaller boats (59%) were located and operated in the eastern provinces of Ordu, Giresun, Trabzon, Rize, and Artvin. The coastal waters of these eastern provinces were closed for bottom-pelagic trawling and hydraulic dredge fishing in the 1980s, which may have resulted in such an SSF profile in the area (Fig. 1) (Mısır et al. 2020). Of the 5141 boats, 196 (3.8%) are C licensed (10–12 m) whilst the rest are D licensed (< 10 m). Regardless of their licenses, 447 of these boats (8.7%) were out of operation for various reasons, 1322 of them (25.7%) use only long lines, and the remaining 3372 boats (65.59%) used at least one or more extension gill nets or circular nets (Mısır et al. 2020).

The study was based on a field survey, to be conducted by face-to-face interview, in order to collect data via a specifically prepared questionnaire. The number of fishers to be surveyed was determined by using the simple random sampling method, using the following equation (Yamane 1967).

$$n = \frac{N(zC)^2}{Nd^2 + (zC)^2}$$

where, n is the number of boats surveyed; N refers to the total population of boats (5575); z is the standard normal distribution value corresponding to the desired confidence level (95%); C is the coefficient of variation; and d is the margin of error (±10%), accepted in the study.

In order to arrange the meetings with fishers, a series of informative meetings was first organized with the regional authorities, including the departments of Provincial Directorates of the Ministry of Agriculture and Forestry and Fisheries Cooperatives in order to prepare the work program schedule for each fishing port where the project personnel would meet with fishers who were boat owners in order to conduct the surveys.

The questionnaire forms were used to create the main data resource. The survey consists of three sections, each of which has a set of questions on: a) the social status of the boat owners; b) information on boats and fishing operations; and c) the economic features of their fishing operations.

Considering the possible variations due to demographic or technological differences which can influence the comparability of the analysis leading to bias, a set of parameters free from such bias were selected for evaluating the efficiency of SSFs in the Black Sea. Costs and revenue figures were converted to Euro equivalents (€) for standardization purposes at the exchange rate quoted by the Central Bank of the Republic of Turkey for 2015 (CBRT 2021).

There were six parameters selected to test for their influences on the net profit in SSF. These were: (1) engine power, (2) numbers of days at sea, (3) length of boat, (4) fuel costs, (5) education level, and (6) professional experience of fishers. Once these parameters were selected and the data associated was collected, collated, and compiled for analysis, a simple linear regression analysis (SLRA) was performed in SPSS, version 13.0 (IBM Inc., Armonk, NY, USA). This was done to determine the effect when data from the selected parameters were designated independent variables and the net profit was designated as the dependent variable. The SLRA is a statistical test that predicts the relations between the independent and the dependent variables and the nature of the relation (Nakip 2005). The calculation to determine revenue generated was performed prior to performing the SLRA. This calculation was made by multiplying the catch for each species by the mean sale price for that year. The unit price of fish caught by SSF is usually higher than that caught by industrial fishery, due mainly to the freshness of the product. After estimating the value of the catch, the net profit was calculated by subtracting the total costs including fuel, boat, and fishing gear maintenance and repair, clothing, documents, and registration expenses from this catch value (EC 2001).

Results and discussion

Even though the calculated number of fishers to be surveyed was 284, face-to-face interviews were carried out with 315 boat owners from 15 provinces including

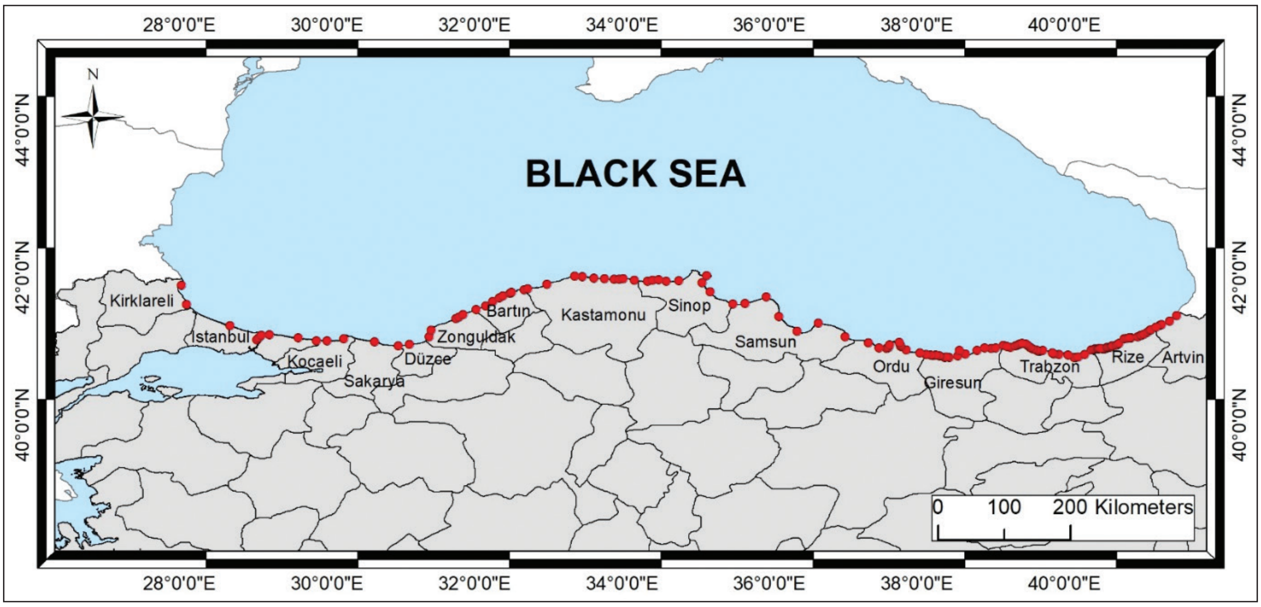

Figure 2. Areas with SSF's shelters in the Black Sea.

(eastern) Giresun, Ordu, Artvin, Rize, Trabzon, (central) Samsun, and (western) Bartın, Zonguldak, Kastamonu, Sinop, Sakarya, Kırklareli, İstanbul, Kocaeli, and Duzce (Fig. 2). However, the data collected from 12 participants were not suitable for analysis due to incomplete information and incorrect answers and was not included. Consequently, the total number of questionnaires used in the analysis was 303.

During the study, 32.3% of the respondents were in the age group aged 50–59 years with the mean value of 49.7 ± 11.4 years while the range was 21–78 years. Dağtekin (unpublished) in a study carried out in Trabzon province reported that 42% of the boat owners were in the 40–49 years age group. Similarly, Uzmanoğlu and Soylu (2006) stated that 35.7% of the fishers in Karasu were between the ages of 40–49 years. Çeliker et al. (2006) determined that the age of fishers in the Black Sea varied between 25 and 70 years and the mean age was 46.45 years. In a later report from the Aegean Region, Çeliker et al. (2008), found that the ages of the fishers varied between 19 and 73 years. Another study conducted along the Mediterranean coasts of Turkey (Taşdan et al. 2010), revealed that the mean age was 40 years while Güngör et al. (2012) stated that the largest age grouping of fishers in the Marmara Sea was between 41 and 50 years (34.2%).

The fishers' education level was predominantly primary school level (48.6%). This proportion for Turkish fishers showed a slight improvement on earlier studies. The proportion who were primary school graduates in earlier studies were: 58.44% in the Black Sea (Çeliker et al. 2006), 78.6% in Karasu, a coastal town in the western Black Sea (Uzmanoğlu and Soylu 2006), 70.1% for the Aegean Sea (Çeliker et al. 2008), 60.7% along the Mediterranean coasts of Turkey (Taşdan et al. 2010), 60% in Trabzon on the Black Sea coast (Dağtekin unpublished), and 64% for the Sea of Marmara (Güngör et al. 2012). Thus, it appears that the education level of the fishers operating in the Turkish SSF is mainly elementary school.

In the presently reported study, the mean number of years of experience of the fishers surveyed was 29.4 ± 12.7. The household population of fishers varied between 2 and 7 family members. The corresponding mean values were 3.38 in the Aegean region (Çeliker et al. 2008), 3.8 in the Mediterranean (Taşdan et al. 2010), and 3.68 in the Black Sea region (Çeliker et al. 2006). In a study conducted in the Marmara region, it was reported that 88.4% of fishers were married (Güngör et al. 2012) while another study determined that 81% of the fishers in the Eastern Black Sea were married (Özbek unpublished). The maximum number of children at home was 5, but the mean value was 2.14 (Table 1). Similar figures were reported in previous studies, including 2.2 for the Black Sea region (Çeliker et al. 2006), 1.7 for the Aegean Region (Çeliker et al. 2008), and 1.9 for the Mediterranean coasts of Turkey (Taşdan et al. 2010). The number of individuals living in households in the Black Sea Region was higher than the other fishing communities in Turkey. The majority (78.3%) of fishers have social security. The ratio of fishers who have a second revenue source was 43.9%. The number of children who also engaged in the fishing profession was 19%. Although 51.4% of the fishers reported being moderately satisfied with their job, the majority of (87.7%) of the interviewed fishers intended to continue their profession in the future. Despite the difficulties of fishing as a profession, their preference for continuing their job mainly stems from the lack of other job opportunities, experience relating only to fishing, and their passionate love for the profession. Nevertheless, 68.5% of fishers were not satisfied with the legal regulations, which are currently in the notification (Table 1).

The analyses of the distribution of catch by species, the net profit by province, and the relation between inputs

Table 1. Socio-demographic characteristics of fishers on the Turkish Black Sea coasts.

Category	Variable	[%]
Age (years)	20–29	4.2
	30–39	15.7
	40–49	26.2
	50–59	32.3
	60–69	18.5
	≥70	3.2
Marital status	Single	10.5
	Married	89.5
Number of children	0	13.2
	1	15.8
	2	39.2
	3	21.9
	4	8.0
	5	1.9
Level of education	Primary school	48.6
	Secondary School	21.1
	High school	25.9
	Associate degree	2.6
	University	1.9
Social security	With social security	78.3
	Without social security	21.7
Second revenue	Fishing only	56.1
	Having a second revenue	43.9
Fishing experience (years)	1–10	8.4
	11–20	25.0
	21–30	24.4
	31–40	26.6
	41–50	7.5
	>50	8.1
Number of children engaged in fishing profession	Yes	19.9
	No	80.1
Satisfaction	Satisfied	34.8
	No Satisfied	13.4
	Moderate level satisfied	51.8
Do you plan to continue the profession in the future?	Yes	87.7
	No	11.4
Are you satisfied with the legal regulations?	Yes	31.5
	No	68.5

and net profit of SSF were performed using data from fishing operations in one season along the Black Sea coastline of Turkey. Kırklareli, the westernmost province, was where the maximum yield (97 007 kg) of fish was caught. Atlantic bonito, *Sarda sarda* (Bloch, 1793), was more frequent and one of the most important species in the populations studied. The mean value of the catch of Atlantic bonito was 44 778 kg/boat in Kırklareli and 22 977 kg/boat in Samsun. Moreover, Atlantic bonito was also the top species caught by the boats in Kocaeli province (19 183 kg) whilst anchovy (10 500 kg) was the second species caught most often by boats (Table 2). Every year, Atlantic bonito migrates for reproduction and nursery from the Mediterranean Sea through the Turkish Strait System, consisting of the Straits of the Dardanelles, Sea of Marmara, and the Straits of the Bosphorus, to the Black Sea and return through the same path for feeding (Genç et al. 2019). Due to its migration pattern, the Atlantic bonito catch is greater in provinces close to the Bosphorus. Rapa whelk was also an important catch species for SSF. When the regions are compared with each other, the revenue obtained from rapa whelk in the Samsun region was higher than the other regions, in terms of higher landing volume [kg] and thus revenue. It should also be noted that prices for rapa whelk vary with the mean length and this is an effective factor for changing the revenue level in disparities between regions. Turbot, *Scophthalmus maximus* (Linnaeus, 1758), stocks in the Black Sea were overexploited (GFCM 2019). Therefore, the landing volume per boat was far lower than historical records show. The total number of gillnets and entangling nets in the boats comprised 107 331 panels in the Turkish Black Sea coasts. The number of gillnets and entangling nets for turbot, whiting, *Merlangius merlangus* (Linnaeus, 1758), Atlantic bonito, and red mullet, *Mullus barbatus* Linnaeus, 1758, constitute 91% of the total number of nets (Dağtekin et al. 2019).

The total and mean net profits of the 303 boats surveyed were, respectively, €1 794 938.00 and €5924.00. Total catch earnings of the 10 active boats in one year in Kırklareli amounted to €259 090. Fishers from Kırklareli reported both the highest revenue and the highest mean net profit among the provinces included in this study. However, many provinces had comparatively low net

Table 2. Landing volume by region mean per boats [kg].

Region	Target species													Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	
Artvin		2487	5015	1500	9029	6300	3378	500	1500	3437			3750	36 896
Bartın		4820	8465	250	200	3000	790	6250		750		17 050	300	41 875
Düzce		15 500	18 817	1602		3200	3000			24 000		10 000	2000	78 119
Giresun		1656	4642	200	21 552	2025	2640	2850	235	1500		10 000	2000	49 300
İstanbul		4332	9573	3831		3900	3875	4750	300	1917		26 000	18 112	76 590
Kastamonu		2252	13 732	3308	1150	5475	1588	683	325	993	250	10 200	3972	43 928
Kırklareli		19 857	44 778	10 643	750	3360	2950	700	100	1483		5486	6900	97 007
Kocaeli	10 050	1399	19 183	3439	8833	8950	5350	750	8000			6583	2333	74 870
Ordu		2225	13 905		34 911	6190	7291		1010	5360		4600	3000	78 492
Rize		2672	4638	1375	6000	839	1995	500	150	3451	3417		3500	28 537
Sakarya		1710	11 800	5540	1500		2733		550			8775	3300	35 908
Samsun		2592	22 977	4862	3100	11 312	4500	1687	6967	2718	3000	23 225	4821	91 761
Sinop		400	14 723	3933	9583	2887	4570	762	1208	2818	150	1717	1150	43 901
Trabzon	187	1910	8485	2934	11 572	3144	2998	700	1804	1325	200	16 900	2192	54 351
Zonguldak		1039	5962	2992	4267	16 667	7927	7877		500	2250	10 230	12 740	72 451

Abbreviations: (1) anchovy *Engraulis encrasicolus*, (2) horse mackerel (*Trachurus mediterraneus*), (3) Atlantic bonito (*Sarda sarda*), (4) bluefish (*Pomatomus saltatrix*), (5) whiting (*Merlangius merlangus*), (6) turbot (*Scophthalmus maximus*), (7) red mullet (*Mullus barbatus*), (8) scorpion fish (*Scorpena porcus*), (9) shad (*Alosa immaculata*), (10) grey mullet (*Liza aurata* and *Mullus cephalus*), (11) sea bass (*Dicentrarchus labrax*), (12) rapa whelk (*Rapana venosa*), (13) Others.

Table 3. Total and mean revenue, net profit, and total costs of boats by provinces.

Province	Number of total boats	Interviews number of boats	Total net profit [€]	Mean net profit [€]	Mean total revenue [€]	Mean total costs
Artvin	233	13	111 341	8565	11 596	3031
Bartın	180	12	36 766	3064	5458	2394
Düzce	67	7	78 271	11 182	15 618	4436
Giresun	514	27	58 988	2185	6184	3999
İstanbul	214	21	108 035	5145	8791	3646
Kastamonu	208	16	128 899	8056	10 928	2872
Kırklareli	85	10	259 090	25 909	30 703	4794
Kocaeli	134	19	189 101	9953	12 832	2879
Ordu	401	20	200 855	10 043	19 007	8964
Rize	915	39	43 835	1124	4573	3449
Sakarya	57	7	89 215	12 745	18 501	5756
Samsun	398	23	173 980	7564	11 850	4286
Sinop	364	23	103 244	4489	7938	3449
Trabzon	974	46	84 323	1833	6626	4793
Zonguldak	397	20	128 994	6450	10 605	4155
Total	5141	303	1 794 938	5924	10 108	4184

profit in one season: the mean were €1124 in Rize; €1833 in Trabzon, and €2185 in Giresun per boat (Table 3). The migration of pelagic fishes (Genç et al. 2019), which is important for SSF, was an effective factor in the differences between regions. The low number of active fishing boats in the region compared to other regions increased the catch weight per boat while the high number of boats in the eastern Black Sea decreased the mean revenue. Revenue and net profit were generally found to be low in studies conducted in various regions of the Turkish SSF (Çeliker et al. 2006; Çeliker et al. 2008; Taşdan et al. 2010; Ünal and Franquesa 2010; Zengin et al. 2017; Birkan and Öndes 2020).

The reported boat engine power was highly variable, ranging from only 2.98 kW to 261 kW, with a mean value of 45.54 kW. The number of days at sea also varied widely, from 10 to 310 days, with a mean of 159 days. The length of boats ranged between 4.4 to 12 m, with a mean of 7.47 m. Fuel cost varied, as expected by both engine power and the number of days of fishing, with a mean of €1325 (Table 4). In a study covering the EU's 54 fishing fleets from 2002 through 2008, it was stated that the proportion of total expenses made up of fuel costs varied between 17% and 29%. The mean annual fuel consumption of a boat in the EU fleet was reported to range between 1.570–1.055 tons annually according to the status of the fishing (Cheilari et al. 2013). The price of the fuel during the year and the number of days at sea were the main factors involved in the change of the rate in the total costs.

Table 4. Boat features, number of fishing days, and fuel costs.

Parameters	Min.	Max.	Mean	SD
Engine power [kW]	2.98	261.00	45.54	42.50
Number of days at sea	10.00	310.00	159.08	79.06
Length of boat [m]	4.40	12.00	7.47	1.63
Fuel costs [€]	66.00	9917.00	1482.50	1325.28
Age of boat	1.00	45.00	14.95	9.89

Each of the six selected parameters had some effect on net profit, with the exceptions of professional experience and education level (see Table 5). The variable with the strongest effect was the length of the boat (19%). A moderate effect was found for both fuel consumption while fishing (11%) and boat engine power (8%), whilst the weakest was the number of days at sea (3%).

The management of SSF is important not only for the protection of natural resources but also for the sustainable living standards of the citizens whose subsistence is dependent on this activity. We estimate that a total of 26 800 people are reliant on the Black Sea SSF made up of approximately 6700 crew, who are directly dependent on fishing, on the Black Sea coastline of Turkey on 3372 active boats, and including the number of people in their households. Thus, it would be reasonable to assume that the numbers reliant to some extent on the Black Sea SSF will exceed 100 000 when the sectors related to the fishery, such as wholesalers and retailers, equipment manufacturers of engines and the fishing gear are also considered. Nonetheless, the focus of this study was to analyze only the profitability of fishing performance of the boats smaller than 12 m, as they compose the core of the SSF fleets. A further aim was to raise awareness of the sustainable use of fishing resources as a social responsibility, which goes far beyond simple environmental issues

Table 5. Relations between engine power, number of fishing days, fuel consumption, boat size, and net profit.

Relation of variables with net profit	Model summary table			Coefficient table	
	R^2	F	Sig.	β	Sig.
Engine power [kW]	0.084	20.180	$P < 0.05$	547.520	$P < 0.05$
Number of days at sea	0.030	9.309	$P < 0.05$	322.161	$P < 0.05$
Length of boat [m]	0.196	73.371	$P < 0.05$	16.179	$P < 0.05$
Fuel costs [€]	0.109	36.832	$P < 0.05$	29.657	$P < 0.05$
Professional experience of fishers	0.010	0.290	$P > 0.05$		
Education level	0.010	0.198	$P > 0.05$		

Conclusion

The number of days that the boats of SSF fleet spend at sea is low. According to FAO criteria, the boat is considered active when it is at sea fishing even for one day. Therefore, when the mean revenue and catch per boat is reported, it is unlikely to reflect the status of the active boats throughout the year.

The mean age of fishers in this study was late middle age. As SSF is an active form of artisanal fishing, if this sector is to be encouraged then it will be necessary to provide support to attract younger people into the SSF, as has been reported previously (Çeliker et al. 2006, 2008; Taşdan et al. 2010; Dağtekin unpublished).

The majority of the fishers were primary school graduates. When the reasons for practicing SSF were examined, it was found that this job was sought around 20% of the time as either a hobby or as a post-retirement activity. This finding would also explain why some of the boats reported relatively few active days. The mean net profit was €5924 per fisher per season. This figure will have been influenced by the fifth of respondents who considered their activity in the SSF as part-time. However, it would be beneficial to provide support to the majority of fishers active in the SSF in terms of complementary alternative employment opportunities in the regions where they are located.

Almost all settlements along the Black Sea coast have traditional shipbuilders who build, repair the boats, and provide maintenance services. With an investor group of at least two people in a fishing port and shelter, a new employment opportunity can be created for the fishing sector, so that coastal fishers will be able to access services for boat maintenance, repair and construction works with less cost in their local settlements. There are some examples that have been applied and successfully managed around the Gulf of Gökova, in the Aegean Sea SSF (Ünal and Kızılkaya 2019). The SSF sector can also be supported by other means. For instance, stocks of turbot, sturgeon, etc. can be restored by releasing hatchery-produced juveniles within the frame of a well-designed and executed stock enhancement program (Charles et. al. 2003). Also, SSF boats can also be utilized for tourism purposes (Lai et al. 2016). Viable suggestions are continuously forwarded and promoted by FAO and EC via workshops and reports for strengthening of SSF (EC 2018; FAO 2020).

Within the value chain, different systems should be investigated to increase the fisher's revenue. With cooperation, all scenarios can be developed, including e-commerce, which has come to the fore during the recent pandemic. Moreover, any changes to the legal standards for fishing gear may cause an acute increase in overall operational costs for SSF fishers who are not able to adapt due to their vulnerable economic status. Thus, a subsidy program will be needed and recommended when such changes in the legal standard for fishing gear are proposed and implemented. The overall evaluation of the results suggests that the Black Sea SSF in Turkey is not sustainable under current circumstances, like many other countries.

Recently, marine cage culture systems have been increasing in the Black Sea. It is very likely to pose some potential conflicts with the capture fisheries sectors. These include over-exploitation of marine areas as well as supply to local markets. Nevertheless, a well-designed marine spatial planning program can result in a symbiotic existence for the SSF and aquaculture, large-scale fisheries, ports and shipping in the Black Sea, instead of destructive competition.

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Southward range extension of the goldeye rockfish, *Sebastes thompsoni* (Actinopterygii: Scorpaeniformes: Scorpaenidae), to northern Taiwan

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Abstract

The goldeye rockfish, *Sebastes thompsoni* (Jordan et Hubbs, 1925), is known as a typical cold-water species, occurring from southern Hokkaido to Kagoshima. In the presently reported study, a specimen was collected from the local fishery catch off Keelung, northern Taiwan, which represents the first specimen-based record of the genus in Taiwan. Moreover, the new record of *Sebastes thompsoni* in Taiwan represented the southernmost distribution of the cold-water genus *Sebastes* in the Northern Hemisphere.

Keywords

cold-water fish, DNA barcoding, neighbor-joining, new recorded genus, phylogeny, *Sebastes joyneri*

Introduction

The rockfish genus *Sebastes* Cuvier, 1829 is the most speciose group of the Scorpaenidae, which comprises about 110 species worldwide (Li et al. 2006; Hyde and Vetter 2007; Kai and Nakabo 2013; Nelson et al. 2016). Members of *Sebastes* generally occur in cold-temperate and boreal waters (Wourms 1991; Mecklenburg et al. 2002; Hyde and Vetter 2007), except for four species residing in the North Atlantic and two species being found in the Southern Hemisphere (Chen 1971; Rocha-Olivares et al. 1999; Garabana 2005; Hyde and Vetter 2007). In addition, rockfishes are economically important species, especially in temperate regions (Muto et al. 2018; Wang et al. 2020). The distributions of rockfishes in the Northern Hemisphere are generally present from northern Japan and the waters of Alaska to California (Robins et al. 1980; Wourms 1991).

On an occasional survey in a local fish market (25°7.77′N, 121°44.47′E), a mature female individual of *Sebastes thompsoni* (Jordan et Hubbs, 1925) was obtained in the local catches, which were caught off Keelung, northern Taiwan, by a bottom trawl. Based on the literature records, however, the goldeye rockfish, *S. thompsoni*, was previously known from southern Hokkaido southward to Kochi and Nagasaki at a depth of 80–160 m (Masuda et al. 1984; Nakabo and Kai 2013; Yu and Kim 2018) and from the Korean Peninsula (Kim et al. 2009). Iwatsubo et al. (2015) reported a single specimen of *S. thompsoni* captured by hook-and-line from Yaku-shima Island, Kagoshima Prefecture of Japan, which reveals the southernmost occurrence of the species. Thus, our specimen represents a southward range extension of *S. thompsoni*.

Members of the *Sebastes* are similar in external appearance between the closely related species (Chen 1985; Muto et al. 2018), hence we performed the molecular identification of the species using the DNA barcoding of the cytochrome c oxidase I (COI) gene, which is typically indicated as an effective genetic marker for identification of most fish species (Hebert et al. 2003; Ward et al. 2005; Ratnasingham and Hebert 2007; Ward et al. 2009). The identification of our specimen is confirmed as a new record from Taiwan and also the southernmost record for the genus *Sebastes* in the Northern Hemisphere. We herein describe the Taiwanese specimen in detail.

Methods

Counts and measurements generally followed Kai and Nakabo (2002) and Kai et al. (2011), including the definition of body depths 1 and 2. The last rays of the dorsal and anal fins are divided at their base and were counted as a single ray. Standard length is expressed as SL. Standardization of morphometric data is expressed as a percentage of SL. Measurements were taken by digital and common calipers, based on the length of the measured items, recorded to the nearest 0.1 mm. The terminology of the spines on the head followed Randall and Eschmeyer (2001). Osteological characters were determined by X-radiographs. The specimen was deposited in the Pisces Collection of the National Museum of Marine Biology and Aquarium (NMMB-P), Taiwan.

DNA was extracted from the muscle by the GeneMark Easy Tissue and Cell Genomic DNA Purification Kit, procedures following the manufacturer's protocol. PCR amplifications were used a 25 µL volume containing 3 µL of 10× Taq Buffer, 2 µL of dNTP mixture at 10 mM each, 1 µL each of forward and reverse primer at 5 µM, 0.125 µL of Pro Taq Plus DNA polymerase (Protech Technology Enterprise, Taiwan), 1 µL of template DNA and the remainder made up with ultrapure water. The portion of COI was amplified using combinations of universal COI primer pairs (Ward et al. 2005): FishF1 (5′-TCAACCAACCACAAAGAGATTGGCAC-3′) and FishR1 (5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′). The thermal cycle profiles consisted of an initial denaturation step at 95°C for 4 min, followed by 35 cycles of 94°C for 30 s, 53°C for 30 s and 72°C for 1 min, with a final step at 72°C for 10 min. PCR products were verified in 2% agarose gels and were purified using SAP-Exo Kit (Jena Bioscience). PCR products were sequenced in the forward and reverse directions by a biotechnology company (Genomics, Taiwan). *Sebastes atrovirens* (Jordan et Gilbert, 1880), *Sebastes inermis* Cuvier, 1829 and *S. thompsoni* were selected as the comparative sequences (Chen 1985) that were downloaded from BOLD (the Barcode of Life Data System). K2P (Kimura-2-parameter) model was used for describing the genetic variation of COI gene in the genetic distance and phylogenetic analysis. The phylogenetic relation was reconstructed with the neighbor-joining (NJ) method using MEGA ver. 10.1.1 (Kumar et al. 2018). The branch support value was assessed by the bootstrapping criterion with 1000 replicates.

Results

This study identified the specimen NMMB-P33709 (Figs 1 and 2) as *Sebastes thompsoni* by morphological and molecular approaches. The counts and proportional measurements of the specimen are listed in Table 1 and described below. Molecular analysis is shown in Fig. 3 and explained. The COI sequence of *Sebastes thompsoni* (NMMB-P33709) was submitted to GenBank (accession number MZ144755).

Table 1. Morphometric measurements of examined specimen of *Sebastes thompsoni* from Taiwan (NMMB-P33709).

Character	Value	
	[%SL]	[mm]
Standard length		170.9
Head length	34.2	
Snout length	8.1	
Orbit length	10.8	
Interorbital width	8.2	
Postorbital length	16.6	
Upper jaw length	14.6	
Body depth 1	31.2	
Body depth 2	35.5	
Body width	17.1	
Caudal peduncle depth	10.0	
Upper peduncle length	11.6	
Lower peduncle length	18.1	
Pectoral-fin length	29.2	
Pelvic-fin length	21.9	
Dorsal-fin base length	60.4	
Spinous dorsal-fin base length	32.2	
Soft dorsal-fin base length	24.8	
Preanal length	69.5	
Predorsal length	33.2	
Prepelvic length	40.8	
Anal-fin base length	19.7	
Pelvic-to-anal-fin length	30.6	
1st dorsal-fin spine length	7.4	
2nd dorsal-fin spine length	10.4	
3rd dorsal-fin spine length	14.1	
1st anal-fin spine length	6.9	
2nd anal-fin spine length	13.4	
3rd anal-fin spine length	13.3	
Pelvic-fin spine length	13.3	
Gill raker length	16.1	

Family Scorpaenidae Risso, 1827

Sebastes Cuvier, 1829

Sebastes thompsoni (Jordan et Hubbs, 1925)

[English name: goldeye rockfish]

Description of the Taiwanese specimen (NMMB-P33709). Dorsal-fin elements XIII, 14; anal-fin elements III, 7; all dorsal- and anal-fin rays branched; pectoral-fin rays 16, lowermost eight rays unbranched; pelvic-fin elements I, 5, all soft rays branched; total caudal-fin rays 37, principal caudal-fin rays 8 + 8 (upper + lower), procurrent rays 11 + 10 (upper + lower); pored lateral-line scales 54, not including 3 pored scales extending on to the caudal fin; gill rakers on first gill arch 11 + 27 (upper + lower), all rakers developed; pseudobranchial filaments ca. 40; branchiostegal rays 7; vertebrae 11 + 15 (precaudal + caudal) = 26 (total).

Figure 1. *Sebastes thompsoni*, NMMB-P33709, 170.9 mm SL, off northern Taiwan (fresh specimen).

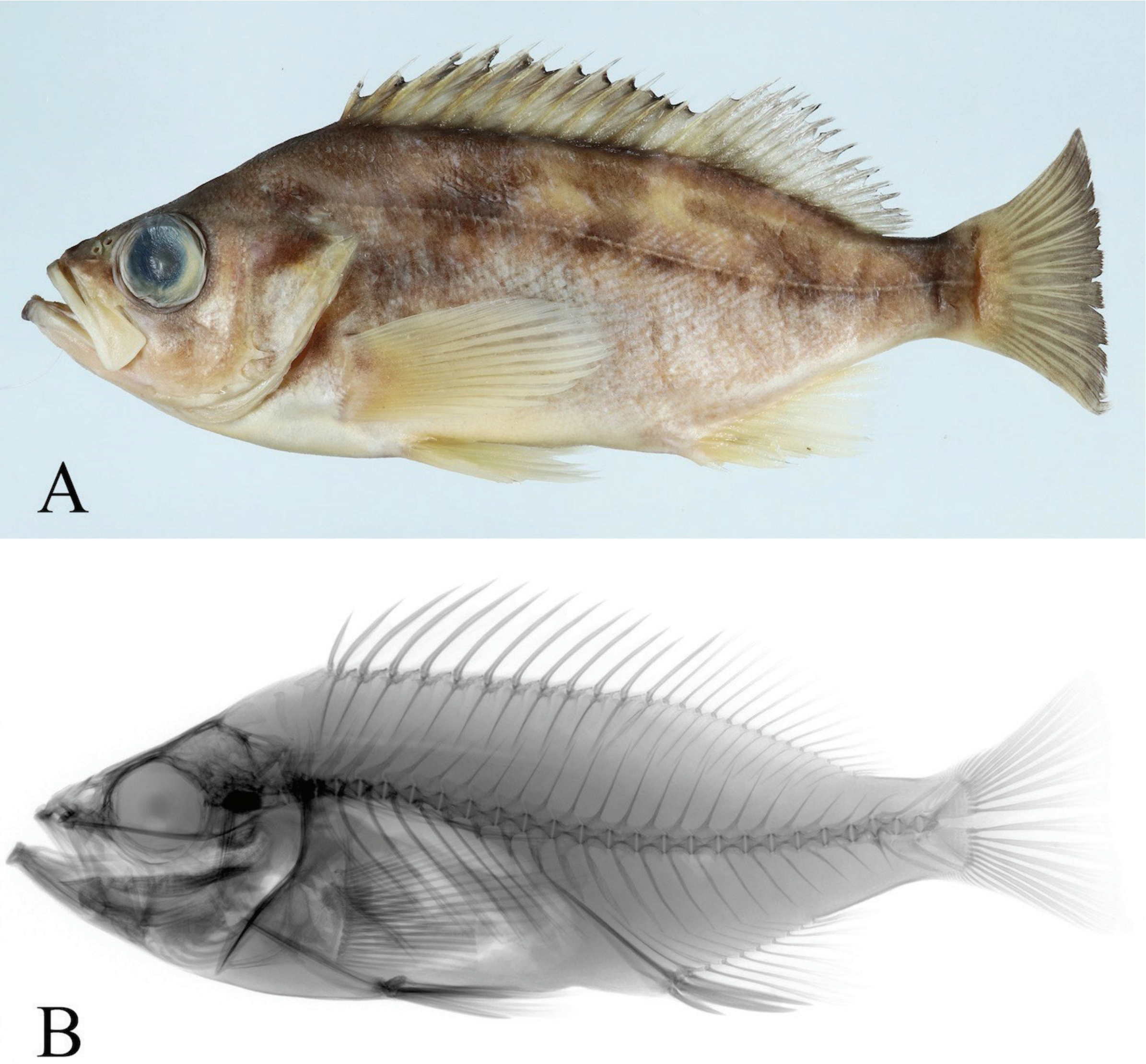

Figure 2. Preserved condition of *Sebastes thompsoni*, NMMB-P33709. **A:** After being preserved in 70% of ethanol, **B:** X-radiograph of the same specimen. It should be noted that the caudal-fin was folded artificially when preserved.

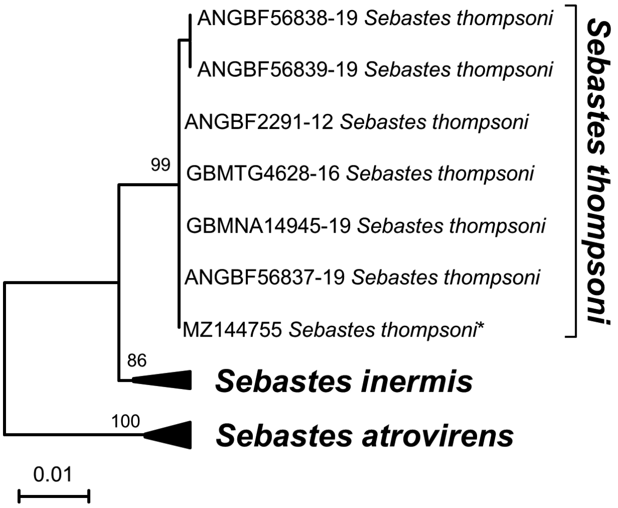

Figure 3. Neighbor-joining tree of COI gene was reconstructed with sequences of *Sebastes atrovirens*, *S. inermis*, and *S. thompsoni*. Bootstrap support values are shown at the nodes. Name with asterisk represents the sample from the presently reported study.

Body relatively deep and compressed. Mouth moderately large, slightly oblique, posterior margin of maxilla not reaching middle level of orbit. Dorsal fin continuous, fourth spine longest, length of remaining spines gradually shorter posteriorly, except last spine (13th) distinctly longer than penultimate spine; shape of soft portion of dorsal fin triangular, length of soft rays gradually shorter posteriorly. Anal fin with three spines, second and third spine long, subequal in length; shape of soft portion of anal fin triangular, length of soft rays gradually shorter posteriorly. Caudal fin emarginate.

Lower jaw with short symphyseal knob, pointed and protruding anteriorly; upper jaw with band of tiny conical teeth, outermost row larger; lower jaw with band of tiny conical teeth; both jaws without enlarged canines; vomer with V-shaped tooth patch, formed by tiny conical teeth; palatine with two long narrow tooth patches with tiny conical teeth.

Head spines relatively short, except spines on opercle and pre-opercle; lachrymal with two sharp, developed spines, posterior one slightly longer than anterior; nasal spines present, situated above each anterior nostrils; upper margin of orbits with pre-orbital and postorbital spines; pair parietal ridge, posterior end terminating with weak spine; single, short cleithral spine present, located above uppermost edge of opercle membrane; short supracleithral spine, located anterior to cleithral spine; opercle with two prominent spines, subequal in length; margin of pre-opercle with five robust and developed spines.

Body mostly covered with tiny ctenoid scales. Head fully scaled, including snout, cheek, lower jaw (dentary and angular surface) and maxilla; scales present on branchiostegal membrane. Dorsal and anal fins scaled; tiny ctenoid scales covering more than half of fin, some scales extending further along fin rays; pectoral fin base with ctenoid scales, scales gradually becoming smaller and extending to fin along fin rays; pelvic fin with scales present along spine and rays, scales becoming cycloid distally; caudal-fin fully scaled, its base covered with relatively larger ctenoid scales, remaining area generally covered with tiny ctenoid scales, distal end of fin covered with tiny cycloid scales.

Color. In fresh condition (Fig. 1), body pale red, duskier along dorsum; five dark-brown irregular transverse bands under base of dorsal fin and caudal peduncle. A faint brown blotch on upper part of opercle. Dorsal fin dusky red with two faint and elongated blotches at base of soft-rayed portion; anal, caudal, and pelvic fins dusky red; pectoral fin reddish. In preserved specimen (Fig. 2A), body, dorsal and caudal fin dusky. Anal and paired fins pale. Transverse bands along dorsum shown in fresh condition remain dark.

Genetics. In the molecular analysis, a total of 22 COI sequences of three species of *Sebastes* were obtained to reconstruct the neighbor-joining tree (Fig. 3) by the Kimura-2-parameter (K2P) model. The result showed the sequence determined here was placed in the cluster of previously determined sequences of *S. thompsoni* with a high bootstrap value (99%). The interspecific K2P distance ranged from 1.1% to 5.1% (mean 2.7%). Therefore, the identification of *S. thompsoni* was further confirmed by the molecular approach.

Discussion

According to several studies of biodiversity of fishes in Taiwan, 62 species of the Scorpaenidae (*sensu* Smith et al. 2018) were documented in Taiwanese waters (Chen 1981; Shao et al. 2008; Motomura et al. 2011; Shen and Wu 2011; Koeda and Ho 2019). The presently reported specimen (NMMB-P33709) cannot be keyed or classified into the ichthyofauna of Taiwan. Therefore, it represents a new recorded genus and species in Taiwan.

We identified this specimen as the genus *Sebastes*, based on a combination of characters: weak spines on head and opercular bones; dorsal-fin spines XIII; relatively large body size; and fins were not elongated. In addition, the genus *Sebastes* can easily be distinguished from most Scorpaenidae species by having a greater number of vertebrae (mostly more than 25), except for *Adelosebastes*, *Hozukius*, *Sebastolobus*, and *Trachyscorpia* (see Ishida 1994). In this study, the vertebrae count of the specimen (NMMB-P33709) was 26 (Fig. 2) which agreed with the range of *Sebastes*.

Based on the morphological and molecular evidence, we recognized the specimen (NMMB-P33709) as *Sebastes thompsoni* in the presently reported study. It was originally described, based on two type specimens collected from Iwate and Osaka in Japan, respectively (Jordan and Hubbs 1925). The presently described specimen (NMMB-P33709) agrees with the original description of *S. thompsoni*. It could be distinguished from the most related species, *Sebastes joyneri* Günther, 1878, by three characters: five dark brown transverse bands on lateral body in *S. thompsoni* (vs. round blotches in *S. joyneri*); a faint blotch on opercle (vs. without); 51–56 pored lateral-line scales (vs. 47–53) (Ida et al. 1982; Masuda et al. 1984; Nakabo and Kai 2013).

The genus *Sebastes* is generally limited to temperate and boreal regions (Chen 1971; Wourms 1991; Rocha-Olivares et al. 1999; Mecklenburg et al. 2002; Garabana 2005; Hyde and Vetter 2007). Hence, the objective record of *Sebastes thompsoni* in Taiwan implied that the distribution of the cold-water fish *Sebastes* extended to the southernmost part in the Northern Hemisphere. However, although *S. joyneri* was listed in Taiwan by Jin (2006), we considered this record as questionable as no voucher specimen is available. In addition, Masuda et al. (1984) indicated that *S. thompsoni* live in colder waters than *S. joyneri*. The potential dispersal mechanism of the southward extension of *S. thompsoni* is uncertain.

A similar case was reported in Koeda and Muto (2019) of another cold-water species, *Pholis fangi* (Wang et Wang, 1935), which was previously only known from the Bohai and Yellow seas. However, a single specimen of *Pholis fangi* was captured by a bottom trawl off Donggang, south-western Taiwan. It represents the first and unexpected case of cold-water species from the Northern Hemisphere with a southward distribution extension to Taiwan. Morphological examination and DNA barcoding analysis highly supports the identification of the specimen. The specimen of *Sebastes thompsoni*, obtained in the presently reported study, is highly similar to the reported case of Koeda and Muto (2019) and yet, it is still uncertain whether the presently described specimen represented a rare local population or a rare dispersal from the Bohai or Yellow Seas.

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PENSOFT.

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Age, growth, mortality, and exploitation rate of blueline snapper, *Lutjanus coeruleolineatus* (Actinopterygii: Perciformes: Lutjanidae), from Dhofar Governorate, Sultanate of Oman

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Abstract

The blueline snapper, *Lutjanus coeruleolineatus* (Rüppell, 1838), is a significant commercial fish species harvested from the traditional fishery in the Sultanate of Oman. Deficient data on this species, however, make the management strategies challenging, especially in fisheries ecology. A total of 978 specimens were obtained from Dhofar Governorate off the coast of the Arabian Sea during the period between February 2015 and March 2016. In total, 296 sectioned otoliths were analyzed and growth was estimated from non-seasonal growth by using von Bertalanffy method. The parameters of von Bertalanffy growth function, total mortality (Z), natural mortality (M), fishing mortality (F), and exploitation rate (E) were determined and compared with those for Lutjanidae fishes elsewhere. The age structure of male fishes was between 1 and 14 years for males while the age of females ranged from 1 to 18 years. Growth rate (K) was 0.21 y^{-1} and 0.16 y^{-1} for males and females, respectively. The hypothetical length of female (L_∞ = 46 cm) was relatively higher than that of male (L_∞ = 42 cm). The natural mortality (M) was 0.296, total mortality (Z) was 0.372, fishing mortality was 0.076, and exploitation rate (E) was estimated as 0.2. The presently reported study is conducted to examine this fish in relation to growth parameters by analyzing otolith structure. The results of the presently reported study will contribute towards planning the regional fishery management policies in Oman.

Keywords

Arabian Sea, blueline snapper, Dhofar, exploitation rate, mortality, otolith

Introduction

Fishery resources are one of the greatest marine resources in the Sultanate of Oman and have been extensively helping fishermen for their livelihood (Al-Marzuqi 2011). The total landing of artisanal, coastal, and industrial fishery, as well as its aquaculture in the Sultanate of Oman, raised from 211 000 t with a value of about US $ 166 million in 2014 to 580 000 t with a value of about US$ 795 million in 2019. Oman's coastal regions are surrounded by three bodies of water, including the Arabian Sea, the Sea of Oman and, the Persian Gulf. The country is rich in biodiversity of marine resources and possesses a 3165 km long coastal line (Al-Anbouri et al. 2013). The fisheries in the Sultanate of Oman constitute a variety of commercially important marine species that support the artisanal fisheries sector and industrial fleet. In addition, the seafood trade and related industries have investment opportunities that help to grow and sustain the income of the fishermen (Al-Abdessalaam 1995).

The blueline snapper, *Lutjanus coeruleolineatus* (Rüppell, 1838) (hereafter blueline snapper), is a member of the family Lutjanidae which contains 17 genera and about 113 species (Froese and Pauly 2019). There are five species of the genus *Lutjanus* characterized by yellowish body color with 4 to 8 blue longitudinal stripes (Barman et al. 2014). Such snappers include *Lutjanus bengalensis* (Bloch, 1790), *Lutjanus kasmira* (Forsskål, 1775), *Lutjanus notatus* (Cuvier, 1828), *Lutjanus quinquelineatus* (Bloch, 1790), and *Lutjanus viridis* (Valenciennes, 1846) (see Allen 1985). However, *L. coeruleolineatus* can be distinguishable from other species of the genus by yellow body, darker on back and whitish ventrally with distinguished blue elongated stripes (7–8) on the sides, a large black spot on the lateral line, and the fish head has blue spots and broken lines (Randall 1995). It is commonly known as snappers and it is also called 'Neissar' and/or 'Qalaya' in Oman. The species is properly common and is frequently found at a fish market and landing sites. It is mainly caught with handlines, traps, and gillnets (Al-Abdessalaam 1995).

The presently reported study was conducted to analyze the stock assessment parameters of blueline snapper, *Lutjanus coeruleolineatus*, using otolith microstructure. Furthermore, the presently reported study attempted to determine the growth parameters based on males and females of blueline snapper that can reveal signs of exploitation level. Such data clarify the state of the stock which can be then used by decision makers to manage and control the exploitation of the stock of blueline snapper for sustainability over time. Hence, an essential study on the age and growth of this species is required to understand their longevity and growth rate in the Arabian Sea.

Materials and methods

Samplings and laboratory works

In total, 978 specimens of blueline snapper were landed by gillnets and handlines from Salalah Fish Market (SFM) (17°01.2′N, 54°04.58′E) at Dhofar Governorate from March 2015 to February 2016. Among the samples collected, randomly selected 296 fish (158 females and 138 males) were used for age determinations using sagittal otolith. A pair of otoliths (Fig. 1) were removed from each specimen. The sagitta was mounted on a glass slide using epoxy resin (Brothers et al. 1983). The otolith was then grounded and Isomet low-speed diamond saw was used to make 200–300 µm thick transverse sections of sagittal otoliths containing the core of the otolith (Cowan et al. 1995). The sections were examined under a stereomicroscope using both reflected and transmitted lights. The fish ages were determined by counting annuli which consist of an opaque and translucent ring. The opaque zones were observed with reflected light while the translucent rings were observed in transmitted light. All counts were made by two readers, repeated at least two times, and then appropriate readings were selected. Sectioning otoliths were reading and determine the age based on counting the number of opaque rings along the dorsal edge of the sulcus acoustics (Allman et al. 2005). For instance, otolith with three completed annuli and a large translucent zone will be classified as 4 age.

Estimation of growth parameters

The growth parameters of blueline snapper were estimated as a relation between body size and age the non-seasonal von Bertalanffy growth function (Ricker 1975) using below the formula:

$$L_t = L_\infty \times (1 - e^{K(t - t0)})$$

where L_t is length of fish at age t, L_∞ is the hypothetical length, K is the instantaneous growth coefficient and t_0

Figure 1. A pair of sagittal otoliths for blueline snapper, *Lutjanus coeruleolineatus*.

is the hypothetical age at which length is equal to 0. The growth parameters of sex's pooled, male and female data were fitted using the Vonbitb program (Stamatopoulos and Caddy 1989) with solver parameters to reduce the residual sum of squares.

The longevity (life span) was estimated by (Pauly 1983) formula to estimate maximum size at age:

$$t_{\max} = 3K^{-1}$$

where $t_{\max}$ is the longevity and K is the instantaneous growth.

Age validation

In order to validate if the opaque and hyaline zone are laid annually, the marginal increment analysis of the otoliths was investigated following Lessa et al. (2006):

$$\mathrm{MIR} = (R_C - R_L) \times (R_L - R_{L-1})^{-1}$$

where MIR is the marginal increment ratio, R_C is the otolith radius from the primordium to the edge, R_L is the otolith radius from the primordium to the last annulus, and R_{L-1} is the otolith radius from the primordium to the annulus next to the last one.

The mean marginal increment ratio was calculated monthly and plotted to examine if the annuli of the otolith formed annually or not and to estimate the translucent ring growth (slow–fast) during the year.

Mortality and exploitation rate

The annual instantaneous rate of mortality (Z) was estimated using the length converted catch curve method (Pauly 1983) and Beverton–Holt method (Beverton and Holt 1957) employed by LFDA5 program (Kirkwood et al. 2003). The fishing mortality (F) was calculated following Pauly (1980):

$$F = Z - M$$

Natural mortality (M) was estimated from the empirical equation using temperature and growth parameter K (Pauly 1980) described by:

$$\log M = -0.0066 - 0.279 \log L_\infty + 0.6543 \\ \log K + 0.4634 \log T$$

where the T value was taken as 24°C as this value represented the mean bottom water temperature in the Arabian Sea of Oman (Thangaraja 1995). The natural mortality (M) also was measured based on longevity (Alagaraja 1984; Hoenig 1984; Hewitt and Hoenig 2005) and growth parameter K (Jensen 1996).

The exploitation rate can be defined as the proportion of harvestable-sized fishes that are removed from a population annually through different fishing activities (Meyer and Schill 2014). The exploitation rate (E) was calculated by using Sparre and Venema (1992) equation:

$$E = F \times Z^{-1}$$

where E is the exploitation rate, F is the fishing mortality, and Z is the annual instantaneous rate of mortality.

Results

Length frequency

Total lengths (TL) of all blueline snapper sampled ranged from 19.4 to 43.7 cm with a mean of 28.4 cm (±0.14). Among them, 529 female and 449 male (sex ratio of F:M = 1.18) specimens were determined, females ranged from 20.4 to 43.7 cm TL with a mean of 29.1 cm (±0.21), which was significantly longer than the mean TL of males (27.5 ± 0.16 cm, $t_{0.05,\, \mathrm{df} = 976} = -5.63$, $P < 0.05$).

Analysis of the length distribution for blueline snapper revealed that the majority of specimens (male and female) were concentrated at length classes between 26 cm TL and 36 cm TL. However, lengths classes less than 26 cm TL and bigger than 36 cm TL were recorded by few specimens (Fig. 2).

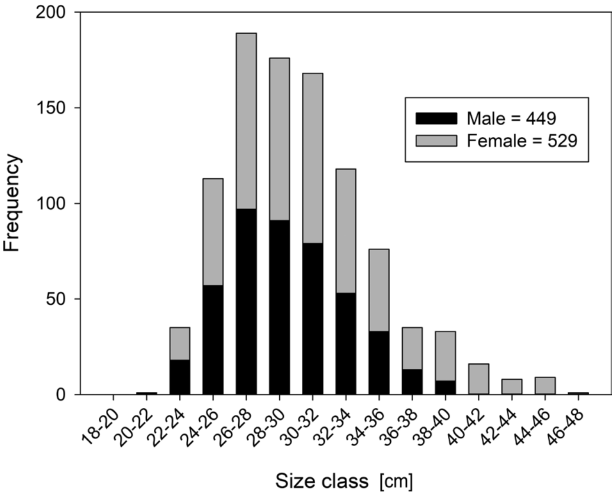

Figure 2. Length frequency distributions (cm, TL) for male and female blueline snappers, *Lutjanus coeruleolineatus* sampled from Dhofar Governorate.

Age and growth

The rings were clearly visible with a high level of readability (90%) and the age of the fish could be determined in the majority of them.

The size of blueline snapper to age data were fitted for each sex separately by using the non-seasonal von Bertalanffy growth function (VBGF) in order to estimate the growth pattern of this species (Fig. 3). The age structures

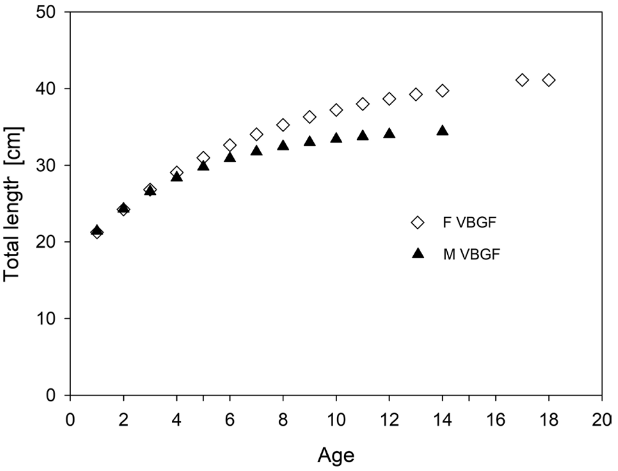

Figure 3. VBGF curves for male and female($n = 158$) and male ($n = 138$) blueline snapper, *Lutjanus coeruleolineatus*.

estimated by otolith annuli analysis of blueline snapper ranged from 1 to 14 years for males and 1 to 18 years for females. Age distributions at each length class for male, female, and all sex combined were illustrated in Tables 1, 2, and 3, respectively. Overall, the majority of the individuals were below age 15 (Only 3 individuals had the age upper 15 years, with one specimen showing the oldest age of 18 years; Table 3). The von Bertalanffy growth function (VBGF) suggests that the male fish had a higher growth rate ($K = 0.21\ y^{-1}$) than female. While, the hypothetical length of the female ($L_\infty = 46$) was significantly higher than that of male (27.6 ± 0.33 cm, $t_{0.05,\ \mathrm{df} = 296} = 3.01$, $P < 0.05$). The age estimation indicates the life spans ($t_{\max}$) are 14.2, 18.7, and 17.6 years for male, female, and combined sexes, respectively.

Table 1. Age–length key of *Lutjanus coeruleolineatus* males from Dhofar Governorate, Sultanate of Oman.

TL [cm]	Age [year]														Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
18–20															0
20–22	1	5													6
22–24	3	15	2												20
24–26	8	9	11		1										29
26–28		11	6	4	2										23
28–30		2	4	8	6	1		1							22
30–32		1	2	1	8	1	2	1							16
32–34			1		2	4	4	2		1	1			1	16
34–36						1				1		1	1	1	5
36–38								1							1
38–40															0
40–42															0
42–44															0
Total	12	43	26	13	19	7	6	5	0	2	1	1	1	2	138
Mean length [cm]	23	24	26	28	29	32	31	32		33	33	34	35	35	27

The (VBGF) which describes male, female and combined sex of blueline snapper at age can be expressed as:

$$\text{Male: TL [cm]} = 42\ [1 - e^{-0.21(t\ +\ 0.22)}],\ (r^2 = 0.70)$$

$$\text{Female: TL [cm]} = 46\ [1 - e^{-0.16(t\ +\ 0.15)}],\ (r^2 = 0.78)$$

$$\text{Combined sex: TL [cm]} = 44\ [1 - e^{-0.17(t\ +\ 0.18)}]\ (r^2 = 0.73)$$

Table 2. Age–length key of *Lutjanus coeruleolineatus* females from Dhofar Governorate, Sultanate of Oman.

TL [cm]	Age [year]																		Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
18–20																			0
20–22	3	2																	5
22–24	3	17	1																21
24–26	3	15	6																24
26–28	1	5	12	4															22
28–30		6	2	13	2														23
30–32		2	3	8	5		1	1											20
32–34					5	3	2	1											11
34–36						3	2	1	2	1									9
36–38					1	2	2	1	3				1	2					12
38–40									2	1		1	1						5
40–42											1	1	1				1	1	5
42–44																	1		1
Total	10	47	24	25	13	8	7	4	7	2	1	2	3	2	0	0	2	1	158
Mean length [cm]	23	25	26	29	31	34	34	34	34	36	36	40	39	39			41	43	29

Table 3. Age–length key of sex combined *Lutjanus coeruleolineatus* from Dhofar Governorate, Sultanate of Oman.

TL [cm]	Age [year]																		Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
18–20																			0
20–22	4	7																	11
22–24	6	32	3																41
24–26	11	24	17		1														53
26–28	1	16	18	8	2														45
28–30		8	6	21	8	1		1											45
30–32		3	5	9	13	1	3	2											36
32–34			1		7	7	6	3		1	1			1					27
34–36					0	4	2	1	2	2		1	1	1					14
36–38					1	2	2	2	3				1	2					13
38–40									2	1		1	1						5
40–42											1	1	1				1	1	5
42–44																	1		1
Total	22	90	50	38	32	15	13	9	7	4	2	3	4	4	0	0	2	1	296
Mean length [cm]	23	24	26	28	30	33	32	33	36	35	36	36	38	36			41	43	28

MIR Ratios

Mean monthly marginal increment ratios (MIR ± SE) in the otoliths of blueline snapper are shown in Fig. 4. The MIR fluctuated during the year ranging between 0.48 and 0.79 mm and confirmed that the growth of translucent ring was slow. The MIR values showed a low mean value (0.56) between February and July and a high mean value (0.72) between August and December reaching a peak value (0.79) in November. These monthly changes in MIR over a year figured that the recognized otolith annulus was deposited once a year.

Mortality and exploitation rate

The coefficient of total mortality (Z) of blueline snapper was estimated by the length converted catch curve method ($Z = 0.393$) for combined sex (Fig. 5). The Z value estimated through the Beverton-Holt method revealed as $Z = 0.350$. Therefore, the mean value of Z value from two methods was taken and hence the $Z = 0.372$ was used for the calculation of fishing mortality. The natural mortality

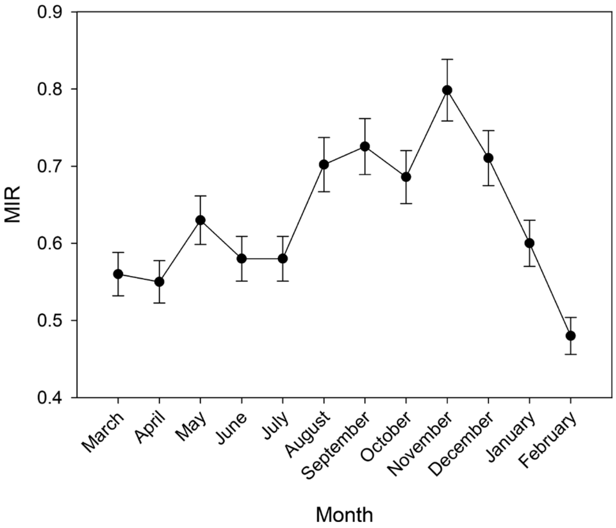

Figure 4. Monthly variations in the mean (±SE) marginal otolith increment (MIR) [mm] of blueline snappers *Lutjanus coeruleolineatus* in the Dhofar Governorate coast of the Arabian Sea from March 2015 to February 2016.

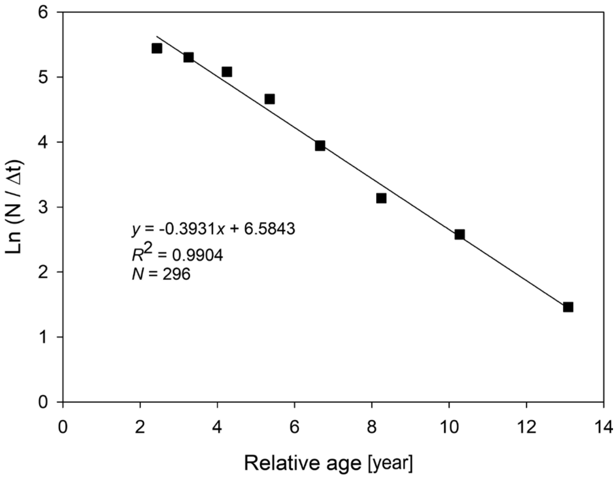

Figure 5. Length converted catch curve of blueline snapper, *Lutjanus coeruleolineatus*.

(M) calculated from the empirical equation using temperature and growth parameter (K), longevity, and growth parameter (K) was 0.47, 0.26, 0.24, 0.25, and 0.26 respectively. Thus, the mean values of natural mortality (M) from all methods were taken and equal to 0.296. Therefore, fishing mortality (F) was equal to 0.076 by subtracting the natural mortality (M) from the total mortality (Z). The exploitation rate (E) is estimated as 0.2.

Discussion

The basic biological studies such as morphometric, age and growth parameters, and reproductive characteristics are important in understanding the life history of the fishes. The maximum fish length of the presently reported species was 43.7 cm TL while previous estimation (Randall 1995) yielded 40 cm TL. We found a significant difference in the mean total length between males and females, with females having bigger body sizes than males. This observation coincides with other Lutjanidae fishes including *Lutjanus fulviflamma* (Forsskål, 1775) from southern Arabian Gulf (Grandcourt et al. 2006) and Mafia Island in Tanzania (Kamukuru et al. 2005), and *Lutjanus campechanus* (Poey, 1860) from the Atlantic waters of the southeastern USA (White and Palmer 2004). These results may be related to differences in metabolism between sexes, such as differences in the level of surplus energy between reproduction and somatic growth (Rljnsdorp and Ibelings 1989).

In the presently reported study, sectioned otoliths were used to estimate growth parameters. Various methods have been used for estimating growth parameters of *Lutjanus* species, but reading otolith sections are one of the best application for annuli determination due to its accuracy and reliability (Newman et al. 1996). Many previous listings suggested that snapper species generally have single annual ring formation (Cappo et al. 2000; Newman et al. 2000a, b; Burton 2002), corresponding to the majority of tropical fishes (e.g., Pilling et al. 2000). In addition, many previous studies have considered Lutjanidae fishes growing faster especially during their first year of their life history (Newman et al. 1996; Newman et al. 2000a; Kritzer 2004; Grandcourt et al. 2006).

Age–length frequency distributions for the presently reported blueline snapper revealed that only 1% of the samples were older than age 15. Although the sample size in this study was not enough for validating population-level age structure. Male's age ranged from 1 to 14 years, while females were between 1 and 18 years showing toward higher age distribution. The age structures were variable between sexes, with the female having the highest longevity at 18.7 years. This estimation of longevity is similar to those attained by *Lutjanus carponotatus* (Richardson, 1842) sampled within the Great Barrier Reef (Newman et al. 2000b; Kritzer 2004). On other hand, males blueline snapper had less life span (14.2 years) than females (18.7 years). Such results were also reported by Heupel et al. (2009) with male *Lutjanus gibbus* (Forsskål, 1775), *Aprion virescens* Valenciennes, 1830, and *Lutjanus fulviflamma* displaying shorter life spans of maximum ages of 12, 16, and 17 years, respectively. Shorter longevities in male snappers were also recorded in *Lutjanus vitta* (Quoy et Gaimard, 1824) (see Newman et al. 2000b), *Lutjanus guttatus* (Forsskål, 1775) (see Amezcua et al. 2006), and *L. fulviflamma* (see Grandcourt et al. 2006).

The asymptotic length (L_∞) of blueline snapper estimated was 44 cm TL for combined sex. The growth rate of males ($K = 0.21$) was higher than females ($K = 0.16$). Such finding is relatively in agreement with other Lutjanidae fishes (Newman et al. 2000b; Kamukuru et al. 2005; Heupel et al. 2010; Shimose and Nanami 2014). Additionally, Newman et al. (2000b) have clarified that the variations in the growth rate of *L. carponotatus* were probably linked to gonad maturation and sexual maturity. Moreover, the growth rate observed for blueline snapper was rapid during 3–4 years of age attaining over

25.0 cm TL for combined sexes and then became slower. Similar to this result, a growth study of blacktail snapper, *Lutjanus fulvus* (Forster, 1801), from the Yaeyama Islands, Okinawa, Japan revealed that such rapid growth is probably due to their maturation (Shimose and Nanami 2014). Shimose and Tachihara (2005) also suggested that reproductive status is often considered to be the main reason for inhibiting the growth of small *Lutjanus* species.

Some *Lutjanus* snappers have a relatively long life span and larger maximum size (≥50.0 cm TL), often exceed 40 years such as *Lutjanus bohar* (Forsskål, 1775) nearly close to 56 years (Marriott and Mapstone 2006), *L. campechanus* to 45 years (White and Palmer 2004), *Lutjanus malabaricus* (Bloch et Schneider, 1801) to 48 years, *Lutjanus erythropterus* Bloch, 1790 to 42 years (Fry et al. 2009), and *Lutjanus fulviflamma* (Forsskål, 1775) to 40 years (Shimose and Tachihara 2005). In contrast, some smaller *Lutjanus* snappers had a short life span; for example, the age of *Lutjanus ehrenbergii* (Peters, 1869) reaches to around 12 years (Grandcourt et al. 2011), *Lutjanus synagris* (Linnaeus, 1758) to 19 years (Luckhurst et al. 2000), *Lutjanus adetii* (Castelnau, 1873) to 12 years (Newman et al. 1996), and *Lutjanus sebae* (Cuvier, 1816) to 22 years (Newman et al. 2000a).

Estimates of natural mortality (M) for the presently reported species was 0.296 which is similar to those for other Lutjanidae fishes. Generally, many *Lutjanus* species are long-lived fishes and have a low value of the instantaneous rate of natural mortality; for instance, natural mortality of *Lutjanus analis* ranged from 0.28 and 0.49 (Burton 2002) and *L. campechanus* was 0.11 (Topping and Szedlmayer 2013). Newman et al. (2000b) also investigated the life histories of two species (*L. carponotatus* and *L. vitta*) and found the natural mortalities of those species were between 0.20–0.21 and between 0.34–0.35, respectively. Moreover, the natural mortality of *L. griseus* was between 0.15 and 0.50 (Fischer et al. 2005), *L. fulviflamma* was around 0.29 (Grandcourt et al. 2006) and *L. carponotatus* was between 0.18 and 0.30 (Heupel et al. 2010). While, Pauly (1980) estimated the natural mortality of fishes via Von Bertalanffy growth parameters (L_∞ or W_∞, and K) and mean environmental temperature (T), and assumed that there is a relation between fish size and natural mortality. Environmental factors such as seawater temperature possibly affect animal life; e.g., warm water will have higher mortality rates than an equivalent animal living in cooler water (Pauly 1980). Whereas estimation of natural mortality for the presently reported species that lives in warm waters have a similar range of that species that inhabiting colder waters.

The presently reported study estimation of the annual instantaneous rate of mortality (Z) for the blueline snapper in the Dhofar Governorate was 0.372 and the fishing mortality (F) was 0.076. Total mortality calculations based on the length converted catch curve method has been recorded high value (0.393) than the Beverton–Holt method (0.350). Estimates were greatly lower than that found by Burton (2002) ($Z = 0.49$) and higher than Mason and Manooch (1985) ($Z = 0.33$) for *L. analis*. The optimum exploitation rate (E) is assumed to be close to 0.5 (Gulland 1971). We compared the exploitation status of blueline snapper (presently-reported study) with results from previous studies on different families for demersal fish in Oman. The exploitation rate (E) of blueline snapper was 0.2 showing the stock at a lower optimum level of exploitation. Similar to these results, Al-Mamry et al. (2011) stated that the exploitation status of sea bream species, *Argyrops spinifer* (Forsskål, 1775) was optimal and the stock was in a healthy status. On the contrary, Abd El Barr (2016) specified that overexploitation of emperors, groupers, sea bream, and rabbit fish due to the rise number of boats and increase fishing activities on those species. Few studies on exploitation status of demersal fish caught from the artisanal fishery in Oman. Therefore, further studies are required to compare the exploitation status between demersal species.

The study was competent to investigate age structure, growth rate, and the mortality of blueline snapper, *L. coeruleolineatus* in Omani waters (Arabian Sea). The length and weight distributions between females and males were comparable to those reported in *L. fulviflamma* from Southern Arabian Gulf, Mafia Island in Tanzania, and *Lutjanus campechanus* from the northern Gulf of Mexico suggesting females having bigger and heavier body than males (Wilson and Nieland 2001; Kamukuru and Mgaya 2004). Estimation of growth rate was rapid during the first 3–4 years of age attaining over 25.0 cm TL and then became slower. This study provides basic biological data that is useful in sustainable fishery management of this species in the Sultanate of Oman.

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Cross-species amplification of microsatellites and identification of polyploid hybrids by allele dosage effects in *Cobitis hankugensis* × *Iksookimia longicorpa* hybrid complex (Actinopterygii: Cypriniformes: Cobitidae)

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Abstract

During the course of evolution, numerous taxa abandoned canonical sex and reproduced asexually. Examination of the *Cobitis hankugensis* × *Iksookimia longicorpa* asexual complex already revealed important evolutionary discoveries tackling phenomena like interspecific hybridization, non-Mendelian inheritance, polyploidy, and asexuality. Yet, as in other similar cases, the investigation is hampered by the lack of easily accessible molecular tools for efficient differentiation among genomotypes. Here, we tested the cross-species amplification of 23 microsatellite markers derived from distantly related species and investigated the extent to which such markers can facilitate the genome identification in the non-model hybrid complex. We found that 21 out of 23 microsatellite markers were amplified in all genomotypes. Five of them could be used for easy diagnostics of parental species and their hybrids due to species-specific amplification profiles. We also noted that three markers, i.e., IC654 and IC783 derived from *Cobitis choii* Kim et Son, 1984 and Iko_TTA01 from *Iksookimia koreensis* (Kim, 1975), had dosage-sensitive amplification efficiencies of species-specific alleles. This could be further used for reliable differentiation of genome composition in polyploids. The presently reported study introduces a noninvasive method applicable for the diagnosis of ploidy and genome composition of hybrids, which are not clearly distinguished morphologically. We showed that very detailed information may be obtained even from markers developed in distantly related taxa. Hybridization is being increasingly recognized as a driving force in evolution. Yet, proper detection of hybrids and their ploidy is particularly challenging, especially in non-model organisms. The present paper evaluates the power of microsatellite cross-amplification not only in the identification of hybrid forms but also in estimating their genome dosage on an example of a fish taxon that involves asexuality, hybridization as well as ploidy variation. It thus demonstrates the wide applicability of such cheap and non-invasive tools.

Keywords

Microsatellites, cross-amplification, *Iksookimia longicorpa* × *Cobitis hankugensis* complex, hybridization, asexual reproduction, polyploidy

Introduction

Although initially neglected in zoological literature, hybridization and polyploidy are attracting considerable research interest as mighty evolutionary mechanisms. Both phenomena are further linked with aberrant reproductive modes leading to the so-called asexual lineages with more or less severe deviations from canonical Mendelian reproduction (Janko et al. 2018). The order Cypriniformes represents a diverse group of primarily freshwater fish where incidences of hybridization, polyploidy, and asexuality are relatively frequent and new cases are still being discovered in recent times (Li et al. 2014). The group divides into several lineages of which one, the suborder Cyprinoidei, is heavily exploited and explored for both scientific and commercial purposes. Unlike Cyprinoidei, its sister lineage Cobitoidea remains largely understudied although it represents a very speciose group. Only recently has the proper taxonomy of this group been investigated, which lead to discoveries of new species (Janko et al. 2005) and even entirely new families (Bohlen and Šlechtová 2009). The group Cobitiodei is adapted to almost every water habitat ranging from standing anoxic waters to high mountain streams and contains several extravagant cases of independently evolved hybrid complexes (including the intergeneric gene exchange (Šlechtová et al. 2008), polyploidizations and asexually reproducing lineages (Kim and Lee 2000; Janko et al. 2007). The reason why this group remains relatively poorly studied lays in its benthic lifestyle promoting conservativeness of body shape and the consequent presence of many cryptic species, which may be diagnosed only by molecular markers (e.g., Janko et al. 2005). This study focuses on a South Korean member of the suborder, the so-called *Cobitis hankugensis* × *Iksookimia longicorpa* hybrid complex, which contains two hybridizing species and a wide array of asexual diploid and polyploid hybrid lineages, which will also be referred to as genomotypes in the subsequent text. In particular, we describe new molecular tools for the proper determination of those forms, which will streamline future research of this model taxon.

The hybridization between *Cobitis hankugensis* and its confamilial relative *Iksookimia longicorpa* was first reported at the Nakdong River tributaries by Kim and Lee (1990) who documented the existence of di- and triploid all-female hybrid population. Later, Lee (unpublished thesis) reported that ecological and morphological traits of hybrids appear intermediate between their parental species. Based on the results of chromosome analysis, Lee (unpublished thesis) revealed that hybrids consist of two genomotypes, namely diploid *hankugensis* × *longicorpa* (hereafter HL, where the letter L stands for *longicorpa* genome whereas the letter H stands for *hankugensis* genome) and triploid with two *hankugensis* and one *longicorpa* genomes (hereafter HHL). Additionally, Lee (1995) reported another triploid form with one *hankugensis* and two *longicorpa* genomes (hereafter HLL). Kim and Lee (2000) and Ko (2009) documented an exceptional way of reproduction in these hybrids where diploid females produce unreduced eggs, which accept the sperm of males from one of the parental species and give rise to one or the other type of triploids. Triploid females exclude the entire genome of the parental species contributing to the haploid chromosomal set and subsequently upon meiotic divisions of the remaining two chromosomal sets produce a haploid egg, which, if fertilized by a male of the former species may give rise to diploid clone again. Such diploid and triploid generation alternation has never been described in any other asexual fish to date, making this taxon an outstanding model for the research of aberrant reproductive modes. The summary of known reproductive interactions is provided in Fig. 1.

Although studies of this complex may bring discoveries of general importance, they are complicated by non-trivial morphological identification of the three types of hybrids. Cytological and molecular biological approaches are therefore required for their accurate discrimination. However, while diploid and triploid forms may be easily discriminated through the measurement of erythrocyte cell size or by flow cytometry, the two types of triploids may not be discriminated by the flow cytometry due to the absence of significant differences in DNA content between the parental species. Chromosomal counting thus remains the most reliable differentiation method to date, but it has a fatal disadvantage of being extremely timely and invasive. For these reasons, a new approach is needed for further study of the hybrid complex.

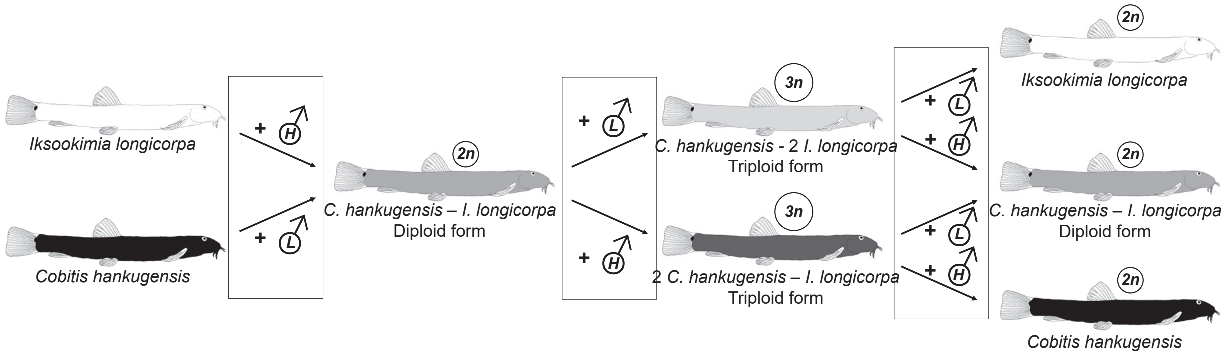

Figure 1. The summary of known reproductive interactions between parental species and hybrid forms of the *Cobitis hankugensis* × *Iksookimia longicorpa* hybrid complex.

Microsatellite loci analysis, one of the most widely used molecular biology research methods, is an accurate tool for verifying genealogy and identification of relatives, as well as demonstrating genetic diversity by separating and analyzing markers that are inherent in each chromosome (McConnell et al. 1995; Nelson et al. 1998; Smith et al. 1998; Goldstein and Schlötterer 1999; Beacham et al. 2000; Sunnucks 2000; Tian et al. 2017). To date, the microsatellite markers have been developed for several species of the family Cobitidae, including *Cobitis taenia* Linnaeus, 1758 (see de Gelas et al. 2008), *C. choii* (see Bang et al. 2009), *Iksookimia koreensis* (see Yu et al. 2014), and *Koreocobitis naktongensis* Kim, Park et Nalbant, 2000 (see Anonymous 2011a). However, the development of microsatellite markers targeting *C. hankugensis*, *I. longicorpa*, and *C. hankugensis* × *I. longicorpa* hybrid has not yet been reported. While the *de novo* development of microsatellite markers is labor and cost intensive (Zane et al. 2002; Squirrell et al. 2003; Thiel et al. 2003; Gonzalez-Martinez et al. 2004; Senan et al. 2014), the cross-amplification using the already identified markers is relatively easily accessible. Therefore, in this study, we apply the cross-amplification of markers previously developed microsatellite for related *Cobitis* species on the *C. hankugensis* × *I. longicorpa* hybrid complex with the special aim to discriminate among all hybrid genomotypes including the two types of triploids.

Materials and methods

Sample collection and identification

Sampled fish were treated according to the "Ethical justification for the use and treatment of fishes in research" (Anonymous 2006). This study was carried out in strict accordance with the recommendations in the Guide for the care and use of laboratory animals of the National Institutes of Health (Anonymous 2011b). The fish dissection was performed under MS-222 anesthesia, and all efforts were made to minimize the pain. The collection of *I. longicorpa*, *C. hankugensis*, and their hybrids was carried out in three areas with several localities (Fig. 2, Table 2), i.e., in the Seomjin River basin, Imsil, Jeollabuk-do, where *I. longicorpa* occurs, in the Nakdong River basin, Hapcheon, Gyeongsangnam-do, where *C. hankugensis* occurs and in the Nakdong River basin, Namwon, Jeollabuk-do.

The identification of collected specimens was based on previously published methods. In particular, we examined each specimen by morphology, which is known to consistently distinguish both parental species from each other as well as their hybrids (albeit, we stress that morphological analysis may not reliably distinguish among different genomotypes of hybrids) (Kim and Park 2002). The ploidy was evaluated by erythrocyte size measurement (Ko 2009). To cross-validate our determination and to precisely evaluate the genomic composition, we employed karyotype analysis to a subset of specimens. This method provides reliable determination of sexual or hybrid genomotype given that both sexual species differ by chromosomal numbers (*I. longicorpa* 2n = 50; *C. hankugensis* 2n = 48), therefore allowing easy determination of both parental species (hereafter also labeled as LL and HH, respectively) from their diploid (HL, 2n = 49) and triploid (HHL 3n = 73 or HLL 3n = 74) hybrid forms (Fig. 3).

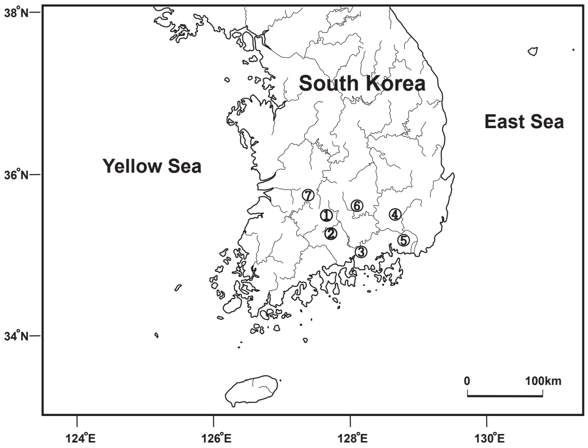

Figure 2. Sampling localities of *Cobitis hankugensis* × *Iksookimia longicorpa* hybrid complex used in this study.

Finally, to obtain comparative material with known origin and genome composition, we have also performed 4 experimental crosses of parental species to obtain strict F1 HL hybrids, and we also crossed natural diploid HL hybrid females with either LL (3 families) or HH (3 families) males to obtain triploid HHL and HLL hybrids, altogether yielding a total of 133 experimental progeny of verified origin for microsatellite genotyping.

Microsatellite marker selection

For the cross-species amplification analysis, the aforementioned fish samples were scrutinized for previously published microsatellite markers developed for related species of the Cobitidae family. The list of loci is shown in Table 1.

DNA Amplification and Genotyping

For DNA analysis a piece of pectoral fin was dissected from each specimen and stored in 100% ethyl alcohol. Total DNA was purified with the genomic DNA Prep Kit for blood and tissue (QIAGEN Co., USA). PCR reactions were completed in a total volume of 50 μL, consisting of 2 μL of genomic DNA, 1 μL of the 10 uM forward (fluorescently labeled) and reverse primer solutions, 24 μL of Premix Taq (Takara, Japan), and 22 μL of distilled water (Takara, Japan). Polymerase chain reactions for all specimens were executed in GeneAtlas G-02 thermocycler (Astec, Japan) with the initial denaturing step at 95°C for 5 min and 35 cycles of 30 s at 94°C, 30 s at 55°C, and

Table 1. Details of 23 microsatellite markers used for *Cobitis hankugensis* × *Iksookimia longicorpa* hybrid detection. Cross-amplification results are indicated for both *Iksookimia longicorpa*, *Cobitis hankugensis* species. Five markers with suited properties for genomotype identification and dosage effects are highlighted in bold.

Locus	Primer sequence (5′ → 3′)	Repeat motif	Reference species	Accession No.	Reference species		*Iksookimia longicorpa*		*Cobitis hankugensis*	
					N_A	Allele size [bp]	N_A*	Allele size [bp]	N_A	Allele size [bp]
Cota_006	F: HEX-GCAGGTACAGAACCCCGACATGG R: AGTACGGCCCTATGGGGTTTGAC	TTG/CTAT	*Cobitis taenia* (see de Gelas et al. 2008)	EU276579	11	336–374	2	163–165	2	163–165
Cota_025	F: 6-FAM-TGCGTTTACAAGATTCGTGTGGAC R: GCTGCATATGAGTAACATGTCTG	CACG		EU276580	3	144–160	2	42–52	2	42–52
Cota_032	F: 6-FAM-TGGTCATGACTGGCACACCGTC R: AGGAGGTTTGAAGAAGGGCAAG	TCTT		EU276582	2	232–236	2	271–290	4	271–301
Cota_033	F: HEX-TTTCTGAATCAAGAGCCCAGCAGT R: AGATATGACATCCAATCACACGCT	AGAC		EU276583	3	211–235	2	203–207	1	207
Cota_037	F: 6-FAM-GCACTCGAGTCGATTCGGTGGCGC R: GTAATCAATCAGTCCAAAGCACTT	GA		EU276584	3	272–276	6	275–304	4	280–298
Cota_093	F: 6-FAM-CCCTGGGAGTTCTCAGCAGGACTG R: ATAATGCACATTGTTGGGCTGC	AC		EU276586	4	341–357	1	304	1	304
IC248	F: HEX-CACTCTGAGGCGAAACTGGAG R: TCAAATCATATAGTGCAGCCAAGC	CA	***Cobitis choii*** **(see Bang et al. 2009)**	EU252088	24	123–187	6	117–148	4	107–119
IC252	F: HEX-AATGAGACGGGTAACTTGTGTATG R: GCTGATCTATGATTGGTTGTGTC	CA		EU252089	12	188–218	1	155	1	155
IC276	F: 6-FAM-GTAACTCCGGGCGTGTAACTCTG R: CACTGTAGAACCCAGCCAAAACC	GT		EU252090	14	82–114	1	70	1	70
IC372	F: 6-FAM-ACACGCACACCTATTACAACCTA R: GATTTGCCAGTGTGCTAATTG	AC		EU252091	33	77–169	2	86–90	2	86–90
IC434	F: 6-FAM-TCCACCATGACCATTTTTACATA R: GGTGTCTGGATCTCATCTTGAA	AC		EU252092	23	83–165	1	78	1	78
IC645	F: 6-FAM-CTCTGAGACAACTCGGTAGTCCC R: CACATACATGGCCTGCAACAT	CA		EU252095	19	161–225	1	189	1	189
IC654	**F: HEX-TGAGCCGACACTAGAAACAGAGC** **R: GACAAAGTGCAGGCACAGAATG**	**CA**		**EU252096**	**14**	**158–208**	**1**	**130**	**1**	**138**
IC783	**F: HEX-GGAGAAGATGTGATGGAGATG** **R: ATATTATGATGGGAAGACACGAC**	**AC**		**EU252098**	**22**	**146–196**	**2**	**120–123**	**1**	**127**
IC839	F: 6-FAM-TTGTTCCCCTCTGAAACCCAATC R: GTGTTAGCCCGTGTGCCAAAG	CA		EU252100	13	99–125	5	92–110	5	82–94
IC875	**F: HEX-AGCGGTGTGGATGTGAATGCTAA** **R: CTTGTCAGGCTCTGGCACTCG**	**CA**		**EU252101**	**22**	**132–182**		**–**	**9**	**134–158**
Iko_AAT08	**F: 6-FAM-GTGATGCAAATGTCTTCTGTGT** **R: CAAATCTTTCCTTTGTCTTTGG**	**ATT**	***Iksookimia koreensis*** **(see Yu et al. 2014)**	**KJ588473**	**5**	**147–163**	**2**	**125–135**		**–**
Iko_TTA01	**F: 6-FAM-ACATTAGTGGGGTAAGATGTGC** **R: AAGGAAGGAATAGGGTAAGCTG**	**TTA**		**KJ588474**	**8**	**180–238**	**1**	**321**	**1**	**330**
KN03	F: HEX-TTTGAGAATTGACAAAATCACTGC R: TGATATCATCGGTGTAAATGTTTAAGA	CA	*Koreocobitis naktongenesis* (see Anonymous 2011)	JN203057	8	134–156	1	116	1	116
KN16	F: HEX-CGACGTAGAGTCAAAAGTGCG R: TGGAGATCAGGTTACGGGTG	CA		JN203058	10	135–157	1	126	1	126
KN20	F: HEX-TTGTGCTGATAACACATCCTGC R: GATTGAATCATCCGCAGAGC	CA		JN203059	10	144–172	1	137	1	137
KN25	F: 6-FAM-CGTTCCCCTCAGGTCTCAAT R: CCTGCAGTTTTCAGCCAAGA	CA		JN203060	9	275–295	4	293–313	4	307–313
KN34	F: 6-FAM-CCAGTGGACATCTGCAACAAC R: GCCCTGCTAGTGAGGAACAA	TG		JN203062	9	273–289	1	286	1	286

* N_A = number of alleles.

1 min at 72°C. A final extension step at 72°C for 5 min. The PCR amplicons were visualized on a 2% agarose gel stained with LoadingStar (Dyne, Korea) together with negative controls and Takara 1 KB molecular size ladder for preliminary size determination. The final PCR products were run on an ABI-3730XL sequencer (Applied Biosystems, USA) with the size standard at 350 ROX. The resulting electropherograms were analyzed in Peak Scanner v1.0 (Applied Biosystems, USA).

To evaluate whether particular markers bear consistent information about the allelic dosage in diploid and triploid hybrids, we used the Gene scan peak analysis with the Peak Scanner v1.0 (Applied Biosystems, USA) to analyze and compare the relative intensities of alleles in analyzed individuals.

Results and discussion

Determination of experimental animals with classical markers

Altogether, based on the classical determination methods including karyotype analysis we selected for marker vali-

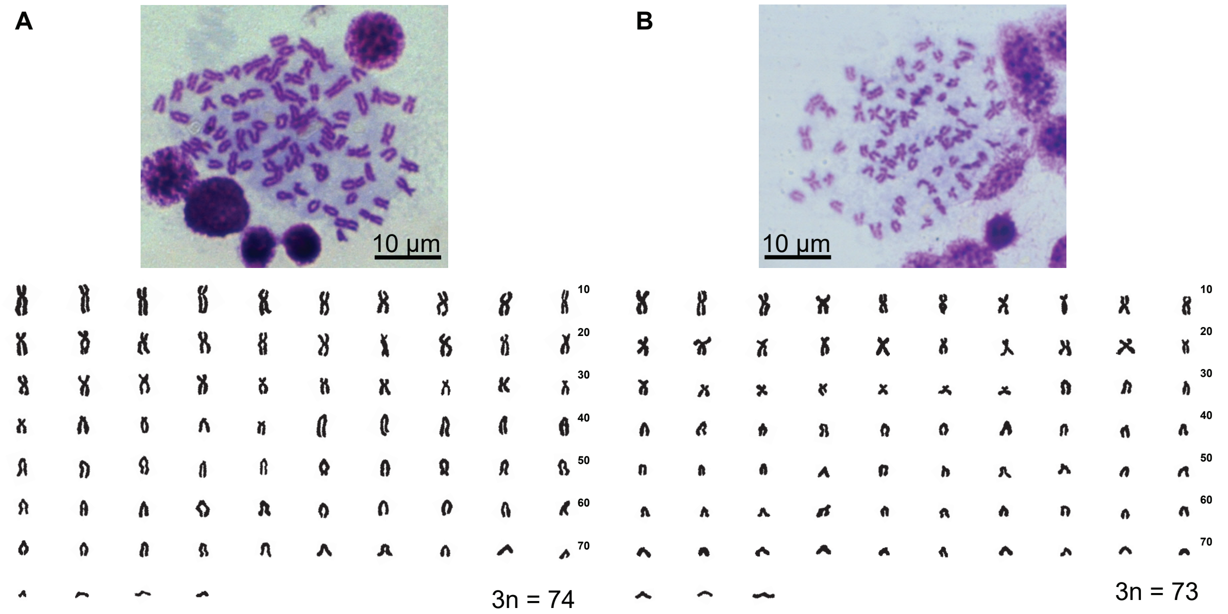

Figure 3. Metaphase plates and karyotypes of two triploid *Cobitis hankugensis* × *Iksookimia longicorpa* hybrids. A: genotype HLL; B: genotype HHL.

Table 2. Study localities of *Cobitis hankugensis* × *Iksookimia longicorpa* hybrid complex used in this study.

No.	River basin	Locality	Coordinates
1	Ram Stream	Inwol-myeon, Namwon-si, Jeollabuk-do	35°27′27.2″N, 127°36′25.6″E
2	Nam River	Saengcho-myeon, Sancheong-gun, Gyeongsangnam-do	35°28′46.9″N, 127°50′56.9″E
3	Banseong Stream	Ibanseong-myeon, Jinju-si, Gyeongsangnam-do	35°9′51.8″N, 128°17′44.8″E
4	Cheongdo Stream	Punggak-myeon, Cheongdo-gun, Gyeongsangbuk-do	35°38′37.2″N, 128°37′25.8″E
5	Unjeong Stream	Muan-myeon, Miryang-si, Gyeongsangnam-do	35°29′37.6″N, 128°40′11.1″E
6	Hwanggye Stream	Yongju-myeon, Hapcheon-gun, Gyeongsangnam-do	35°30′20.3″N, 128°6′17.2″E
7	Oknyeodong Stream	Unam-myeon, Imsil-gun, Jeollabuk-do	35°39′35.3″N, 127°9′20.0″E

dation 25 individuals of *I. longicorpa*, 25 of *C. hankugensis*, and also 5 HL, 5 HLL, and 5 HHL hybrid individuals. We further included into the analysis 59 natural hybrids (without karyotype analysis) sampled at five sites in the Nakdong River basin, and we also scrutinized 133 progenies generated by artificial crossing experiments with known origin and genomic composition.

Cross-species amplification results and species diagnostics

The cross-amplification of 23 published markers showed that 19 loci were amplified in all genomotypes of the hybrid complex. Moreover, we further noticed that the IC875 marker did not amplify with *I. longicorpa* but it did amplify in the *C. hankugensis* and hybrids while the Iko_AAT08 marker did not amplify at *C. hankugensis*, but it did amplify in the *I. longicorpa* and in hybrids (Table 1). We observed that 50.0% of tested loci were monomorphic in *I. longicorpa*, 59.1% in *C. hankugensis*, and 43.5% in all three types of hybrids. The number of alleles per locus, except for monomorphic ones, ranged from 2–6 (mean 3.2) in *I. longicorpa*, 2–9 (mean 4.0) in *C. hankugensis*, 2–5 (mean 2.5) in hybrid (HL type), 2–4 (mean 2.9) in hybrid (HLL type), and 2–5 (mean 2.7) in hybrid (HHL type), respectively.

We note that in each locus, the numbers of detected alleles were always lower than those reported in the reference species for which given microsatellite marker has been developed and where 2–33 alleles per locus per species (mean 12.2) have been reported in the original publications. When compared to the reference species, analyzed hybrids had the highest numbers of alleles in markers taken from *C. taenia* (mean value 2.3 alleles per locus), the second-highest numbers of alleles in loci taken from *C. choii* (mean value 2.2), while the markers taken from different genera showed lowest numbers of alleles, i.e., mean value 1.5 in *K. naktongensis* markers and 1.4 in *I. koreensis* markers, respectively. This is in line with the general expectation that the efficiency of cross-species amplification tends to decrease with increasing phylogenetic distance between the reference species and the target species (Moore et al. 1991; Peakall et al. 1998). This study used the cross-amplification between species of the same genus or family and showed relatively high amplification efficiency. However, nearly half of essayed loci appeared as monomorphic and many of other loci shared the same alleles between species, making them of limited use for species diagnosis. Future studies of the genetic diversity of *I. longicorpa* and *C. hankugensis* and their hybrids would certainly profit from the direct development of microsatellite loci from their DNA.

Nevertheless, we discovered three loci, which seem very useful for fast and efficient identification of genomotypes from the studied hybrid complex because they possess non-overlapping allelic size ranges between species. Specifically, the loci IC654, IC783, and Iko_TTA01 always distinguished between the specimens identified as pure *I. longicorpa* and *C. hankugensis*, respectively, while they always provided amplification products of both species in the hybrid individuals. Furthermore, we found two additional loci with selective amplification (IC875, Iko_AAT08), where one sexual species was characterized by absence of amplification, while the other species and all hybrid individuals provided specific amplification product (Table 1).

This altogether suggests that tested cross-amplification identified three markers with species-specific allelic variants and two loci with species-selective amplification that may be used as haploid detection markers for *C. hankugensis* and *I. longicorpa*, respectively. In addition, some other loci also appear as useful for subsequent population genetic studies given they possess a moderate number of alleles per species, which may allow for frequency-based analyses.

Hybrid detection and allele dosage effects

Given that the scope of this paper was to find a fast and efficient method to discern parental species and hybrid genomotypes, we will describe in the following text the properties of three markers that we propose for such a purpose given their ability to diagnose both species as well as the ploidy of hybrid individuals. The IC654 and IC783 markers derived from *C. choii* and the Iko_TTA01 marker derived from *I. koreensis* were of particular interest for us as they were fixed for different alleles in both parental species and showed the consistent presence of both species-specific amplification products in hybrids with different relative peak intensities depending on the genotypes (Fig. 3).

The patterns were straightforward in IC654 and Iko_TTA01 markers (Fig. 4A–B) since in diploid hybrid HL genomotype with one genome of *I. longicorpa* and the other of *C. hankugensis*, the heights of the amplified peaks were similar to each other. In triploid hybrid HLL genomotype with two genomes of *I. longicorpa* and one of *C. hankugensis*, the allele peak intensity of *I. longicorpa*—

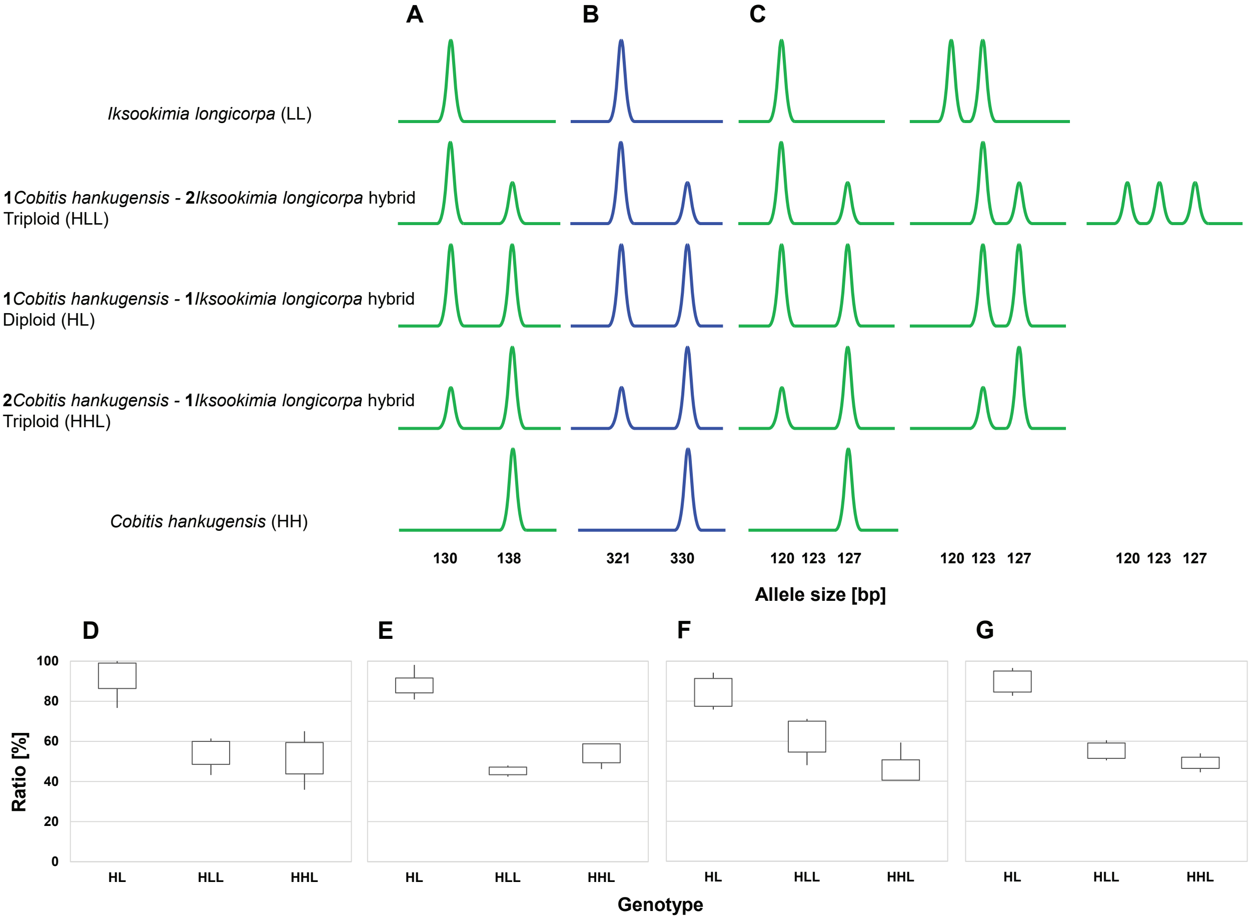

Figure 4. Demonstration of Microsatellite analysis of IC654 (**A**), Iko_TTA01 (**B**) and IC783 (**C**) loci in *Cobitis hankugensis* × *Iksookimia longicorpa* hybrid complex. Upper panels **A**–**C** show the electropherograms of the three loci in all biotypes. Boxplots in the lower panel depict for each locus the relative intensity (%) of the minor peak to the major one (**D**–**F**) and the mean relative values of the total of three markers for each genomotype (**G**).

specific allele was approximately twice as strong as that of *C. hankugensis* – specific allele, while in triploid hybrid HHL genomotype the situation was opposite with approximately double intensity of the *C. hankugensis* allele compared to *I. longicorpa* allele. The patterns in IC783 were more complicated by the presence of two alleles in *I. longicorpa*. Consequently, the diploid HL form always possessed one allele diagnostic for *I. longicorpa* and the other for *C. hankugensis*, HHL triploids also possessed two alleles with clear dosage pattern, but HLL triploids either possessed two alleles with the apparent dosage pattern or three alleles, each with similar intensity (Fig. 4C).

To verify the possibility of applying the three selected markers (IC783, IC654, and Iko_TTA01) for the identification of unknown hybrid genomotype, we demonstrate the relative size ratio of the minor peak to the major one (Fig. 4 D–F). As a result, three hybrid genomotypes were clearly distinguished by the ratio of the peaks. Diploid HL genomotype had relatively similar intensities of the less intense allele, i.e., 92.7% (IC654), 84.4% (IC783), and 87.9% (Iko_TTA01), while in triploid HLL genomotype, the ratios were 54.3%, 62.3%, and 45.3%, respectively, and in HHL genomotype, we found 51.6%, 45.6%, and 54.1%, respectively (Fig. 4, D–F). Finally, we also plotted the mean relative values of the total of three markers for each genomotype, (Fig. 4, G), where the mean value in HL type was 89.8%, in HLL type was 55.3%, HHL type was 49.2%. This result strongly supported that the microsatellite marker can be used to the correct method of discrimination of known genomotypes of the *C. hankugensis* × *I. longicorpa* complex.

To date, the identification of *C. hankugensis* × *I. longicorpa* hybrid complex had a fatal disadvantage in that it requires complex processing and fish sacrifice. In this study, we provided a reliable identification method of the *C. hankugensis* × *I. longicorpa* hybrid complex using microsatellite markers through a single Genescan analysis using only a small piece of fin tissues. This has the great advantage that the fish are kept alive and can be used for additional hybridization experiments by reducing the stress.

Microsatellite markers have indeed been previously used to identify hybrid groups of fish, including the family Cobitidae, (e.g., You et al. 2007; de Gelas et al. 2008) and these markers have also been applied to polyploid hybrids, (e.g., Janko et al. 2012; Mishina et al. 2014), when triploids were typically inferred by the possession of three peaks in at least one locus. However, the identification method proposed in this study suggests that microsatellite markers can be used as a powerful method to determine triploids even in cases when hybrids possess no more than two alleles, relying on the relative amplification intensities of species-specific alleles.

In a summary, the cross-species amplification of microsatellite markers can be used as an easy and fast identification method in studies of reproductive modes of investigated hybrids.

Acknowledgments

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Short-term change in fish assemblages after the passage of a typhoon in a temperate, coastal bay

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Abstract

To study the effects of a typhoon on a temperate, coastal bay community, the species composition, catch amount, and diversity of epipelagic fish assemblages were investigated. Fish samples were taken from catches of a purse seine fishery in Tachibana Bay, Japan between May and July 2011, covering before and after the passage of a typhoon in the area. Although major changes in total catch amount were not observed before and after the passage of the typhoon, the abundance of the Japanese anchovy, *Engraulis japonicus* Temminck et Schlegel, 1846, markedly decreased and bycatch of species increased, accompanied by increasing levels of diversity of the fish assemblage. Multivariate analysis showed that community differences before and after the passage were quantitative rather than qualitative. Comparisons in total length frequencies between the two periods indicated that specimens of the species compared were bigger in size for *Trachurus japonicus* (Temminck et Schlegel, 1844) and smaller for *E. japonicus* in the “after” period. These results suggest that the passage of the typhoon triggered not only interspecific faunal change but also intraspecific recruitment shifts in and around the bay.

Keywords

bycatch, diversity, fish assemblage, migration, purse seine fishery, typhoon

Introduction

The effects of hurricanes, typhoons (also termed tropical cyclones), and tropical storms, are major sources of physical disturbance to shallow water communities, affecting geological features (Kahn 1984), hermatypic corals (Woodley et al. 1981; Harmelin-Vivien and Laboute 1986), and other reef organisms (Yoshioka and Yoshioka 1987; Moran and Reaka-Kudla 1991). Although much information on the impact of hurricanes and tropical cyclones on fish assemblages is available in tropical, coral reef areas, the results of these studies are inconsistent (Kaufman 1983; Lassig 1983; Fenner 1991; Letourneur et al. 1993; Cheal et al. 2002; Guillemot et al. 2010; Kawabata et al. 2010; Foster et al. 2011). Breder (1962) noted the loss of the most abundant fish species, the pinfish, *Lagodon rhomboides* (Linnaeus, 1766), and proposed that a faunal shift had occurred. High post-hurricane mortality among metamorphosed damselfishes (Beecher 1973) and juveniles of other fishes (Lassig 1983; Bouchon et al. 1994) suggests that susceptibility to hurricane-induced stress may be related to the life-stage. In some cases, post-hurricane increases in fish abundance have been observed (Woodley et al. 1981; Turpin and Bortone 2002) while in other cases minimal or no effects on fish assemblages were found when compared with pre- and after-passage of the hurricane (Springer and McErlean 1962; Fenner 1991).

It has been suggested that global warming may increase the frequency of strong typhoons (Emanuel 2005; Webster et al. 2005), and consequently temperate, coastal communities would increasingly be exposed to such severe disturbances.

However, only a few studies refer to the effects of typhoons on fishery activities in subtropical and temperate, coastal bays (Kawabata et al. 2010; Yu et al. 2013, 2014; Chang et al. 2014). Additionally, as far as I knew, there is no information about the impact of a typhoon on changes in species composition obtained from purse seine fishing. This type of information is important in order to consider if the change in catch amount and/or composition originates from either human or natural factors for fishery management and to estimate how communities and coastal marine ecosystems are impacted by typhoons. For these objectives, I considered that monitoring the species composition of purse seine catch, including bycatch (i.e., the fraction of the catch that consists of nontarget species that are encircled by the fishing gear and are unable to escape (Romanov 2002) is an effective method to determine the structure and spatial organization of multispecies aggregations of coastal communities (Hall 1996; Erzini et al. 2002).

On 26 June 2011, the typhoon MEARI (international number: 1105) passed approximately 500 km offshore of the west coast of Kyushu, Japan (Fig. 1). Maximum wind velocities near the storm center were 30 m•s^{-1} (Table 1). The presently reported study examined the effect of this typhoon by comparing catch amount, species composition, and diversity obtained from purse seine catches before and after the passage of the typhoon, to test the hypothesis that such meteorological phenomena change the fish assemblage community structure and diversity in a temperate, coastal bay.

Materials and methods

Study area

Tachibana Bay is located in the southern part of Nagasaki Prefecture, Kyushu, Japan. The northern and southern sides of this bay are enclosed by the Nagasaki and the Shimabara peninsulas, and the bay is connected to the Ariake Sound in the east, and exposed to the East China Sea in the west (Fig. 1). In Tachibana Bay, purse seine fishing has been operated since the early 1950s and has become the main fishery for epipelagic fish species. Although the purse seine fishery in Tachibana Bay mainly targets Japanese anchovy *Engraulis japonicus* Temminck et Schlegel, 1846, bycatch occurs. Our sampling of the purse seine fishing catch takes place within an area at a mean depth of 36 m (hatched area, Fig. 1).

Sampling design

Six random sampling operations were carried out opportunistically before and after the passage of the typhoon MEARI from May to July in 2011. Each sample of approximately 3 kg was taken from the purse seine catches prior to the sorting procedure at the Kyodomari Port of Tachibana Bay. The fishing gear was approximately 380 m in length, 30 m in depth, and 6 mm in minimal stretched mesh size. Total catch amount, the number of hauls, and sea surface temperature of the fishing grounds on sampling days were collected from the fishing master of this fishery.

In the laboratory, fishes from each sample were counted and identified to the lowest possible taxonomic level. The total weight of each taxon was also recorded in 0.1 g order and the total length frequency for each species, except for Japanese anchovy, was measured from randomly chosen 100 individuals.

Data analysis

The total catch amount and abundance of the samples were expressed as the weight of fish per haul. To analyze

Table 1. Best track data of MEARI (1105) in 2011 from the Regional Specialized Meteorological Center (RSMC), Tokyo, Japan.

Date (June)	Time	Center position (DDM) Latitude and Longitude	Central pressure [hPa]	Maximum wind speed near the center [m•s^{-1}]	Radius of area of winds [km]	
					25 m•s^{-1}	15 m•s^{-1}
	09	13°12′N, 129°18′E	998	18	–	NE: 440 SW: 280
	15	14°00′N, 128°54′E	998	18	–	NE: 440 SW: 280
	21	14°48′N, 128°42′E	994	20	–	E: 700 W: 370
23	03	15°36′N, 128°24′E	990	23	–	E: 700 W: 370
	09	16°36′N, 127°54′E	985	25	–	SE: 750 NW: 370
	15	17°24′N, 127°24′E	985	25	–	SE: 750 NW: 370
	21	18°12′N, 126°54′E	985	25	–	SE: 750 NW: 370
24	03	19°18′N, 126°30′E	985	25	–	SE: 750 NW: 370
	09	20°48′N, 126°00′E	980	30	190	SE: 750 NW: 440
	15	22°48′N, 125°18′E	980	30	190	SE: 750 NW: 440
	18	23°36′N, 125°00′E	975	30	E: 220 W: 190	SE: 750 NW: 440
	21	24°24′N, 124°30′E	975	30	E: 220 W: 190	SE: 750 NW: 440
25	00	24°54′N, 124°06′E	975	30	E: 220 W: 190	SE: 750 NW: 440
	03	25°30′N, 123°48′E	975	30	E: 220 W: 190	SE: 750 NW: 440
	06	26°00′N, 123°36′E	975	30	E: 220 W: 190	SE: 750 NW: 440
	09	26°36′N, 123°18′E	975	30	E: 220 W: 190	SE: 750 NW: 440
	15	27°42′N, 123°18′E	975	30	E: 220 W: 190	SE: 750 NW: 370
	21	29°12′N, 124°06′E	980	30	150	SE: 700 NW: 370
26	03	32°06′N, 124°42′E	980	30	150	SE: 700 NW: 370
	09	35°06′N, 124°24′E	980	30	150	SE: 700 NW: 370
	15	36°48′N, 123°00′E	980	30	130	SE: 650 NW: 370
	21	37°06′N, 122°48′E	980	30	130	E: 600 W: 370
27	03	37°30′N, 123°00′E	985	25	–	E: 560 W: 370
	09	38°30′N, 124°18′E	990	23	–	330

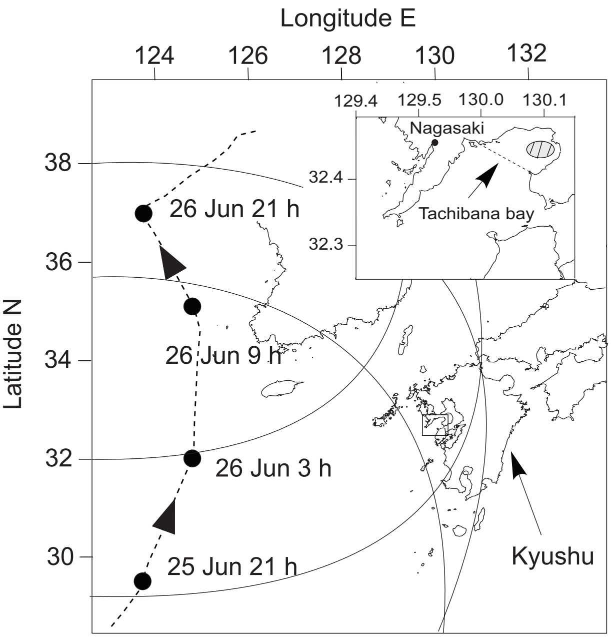

Figure 1. Map showing the trajectory of the tropical cyclone MEARI (dashed line) and the fishing ground (inset, shaded area) of the purse seine fishing in Tachibana Bay in 2011. Radii of circles show the areas affected by winds of 15 m•s^{-1}. Best track data of MEARI is available in Table 1.

the species composition per haul before and after the typhoon, the following indices were calculated: number of species, abundance (i.e., weight of fish) of *E. japonicus* and of bycatch species, Simpson's reciprocal index ($1 \cdot D^{-1}$), Shannon–Wiener species diversity (H'). Simpson's reciprocal index $1 \cdot D^{-1}$ was calculated as:

$$D = \sum_{i=1}^{S} p_i^2$$

where S is the number of species and p_i is the ratio of abundance of occurrences of the ith species to that of total species in the sample. Similarly, H' was derived by:

$$H' = -\sum_{i=1}^{S} p_i \log_2 p_i$$

where H' is the Shannon–Wiener function, S is the number of species and p_i is the ratio of abundance of occurrences of the ith species to that of total species in the sample. Both indices $1 \cdot D^{-1}$ and H' reflect not only species richness but also provide an index of the evenness of a community (Krebs 1989). The index $1 \cdot D^{-1}$ is more sensitive to changes in abundant species, whereas H' is more sensitive to changes in rare to intermediate species in a community (Peet 1974; Krebs 1989). *K*-dominance curves (Clarke and Warwick 1994; Machias et al. 2004) were also plotted for each sampling period. Multivariate analysis of fish community data involved the use of the Bray–Curtis similarity index on double square-root transformed and on presence-absence data.

The mean values of the pooled data from the 3 purse seine fishing samples carried out 'before' and in the 3 samples 'after' the passage of the typhoon of total catch amount, abundance, and diversity for the community were compared by student *t*-test. Mann–Whitney U test was used to compare the total lengths of three species, *Engraulis japonicus*, *Trachurus japonicus* (Temminck et Schlegel, 1844), and *Sarda orientalis* (Temminck et Schlegel, 1844) (for which the data greater than two individuals were recorded in both 'before' and 'after' the typhoon) between the two sampling periods ($P < 0.05$).

Results

The distribution of sea surface temperature data in Tachibana Bay, Kyushu, Japan is presented in Fig. 2. Changes in surface water temperature and total catch amount per haul during the experimental period are presented in Fig. 3. The sea surface temperature of the purse seine fishing grounds in Tachibana Bay ranged between 18.3 and 25.7°C (Fig. 3A). After the passage of the typhoon, ranges between the maximum and minimum sea surface temperature in the fishing ground became narrower. There is no significant difference in the mean total catch amount per haul between 'before' and 'after' ($t = 1.14$, $P = 0.16$) (Fig. 3B, C).

Ratios of the number of individuals and of the abundance of bycatch species such as *T. japonicus* and *S. orientalis* abruptly increased just after the passage of the typhoon, and *E. japonicus* decreased (Fig. 4). *Engraulis japonicus* accounted for more than 90% of abundance in the period 'before' passage while this ratio decreased to 57% in the 'after' period (Fig. 4). A total of 16 species were caught in total, in 'before' and 'after' the passage of the typhoon. Seven species were found in the samples of 'before' and 16 species in the 'after' typhoon. All species caught 'before' were also found in 'after' with the exception of one species *Stephanolepis cirrhifer* (Temminck et Schlegel, 1850). In the 'after' samples an additional nine species, *Sardinops sagax* (Jenyns, 1842), *Decapterus maruadsi* (Temminck et Schlegel, 1843), *Seriola quinqueradiata* Temminck et Schlegel, 1845, *Nuchequula nuchalis* (Temminck et Schlegel, 1845), *Spratelloides gracilis* (Temminck et Schlegel, 1846), *Trichiurus lepturus* Linnaeus, 1758, *Auxis rochei* (Risso, 1810), *Scomber australasicus* Cuvier, 1832, and *Lagocephalus wheeleri* Abe, Tabeta et Kitahama, 1984, were found that were not caught in the 'before' samples (Fig. 4).

Several descriptors of the fish community are shown in Fig. 5. The mean number of species increased from 1.0 to 4.4, but this is not a significant change ($t = 0.99$, $p = 0.19$) (Fig. 5A). The mean abundance of *E. japonicus* significantly decreased ($t = 3.98$, $p = 0.0082$) (Fig. 5B), thus the abundance of bycatch species increased in the period after the passage of the typhoon. Conventional diversity indices such as those of Shannon and Simpson showed significant increases indicating that the distribution of individuals among species was more even in the period

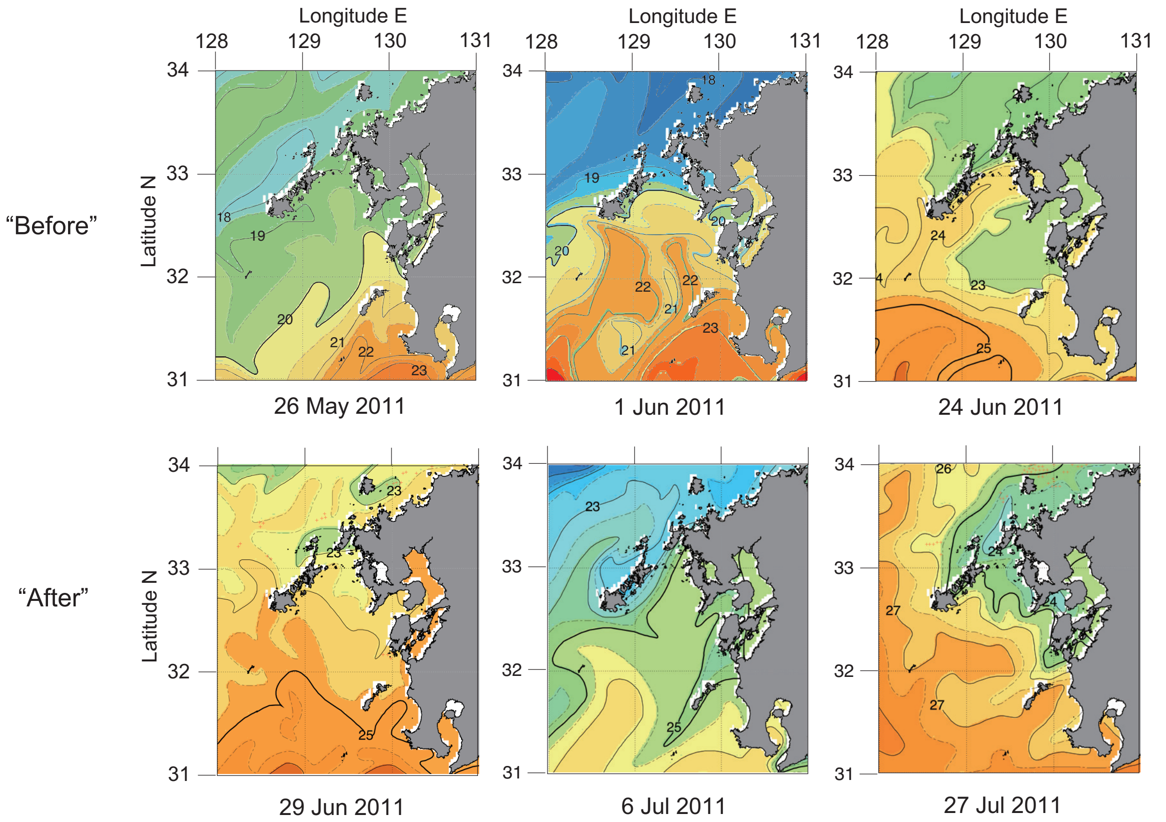

Figure 2. The distribution of sea surface temperature data in the sea around Tachibana Bay, Kyushu, Japan collected by the Japan Fisheries Information Service Center (JAFIC), Tokyo, Japan. The contour intervals are 1.0°C (solid lines) and 0.5°C (dashed lines).

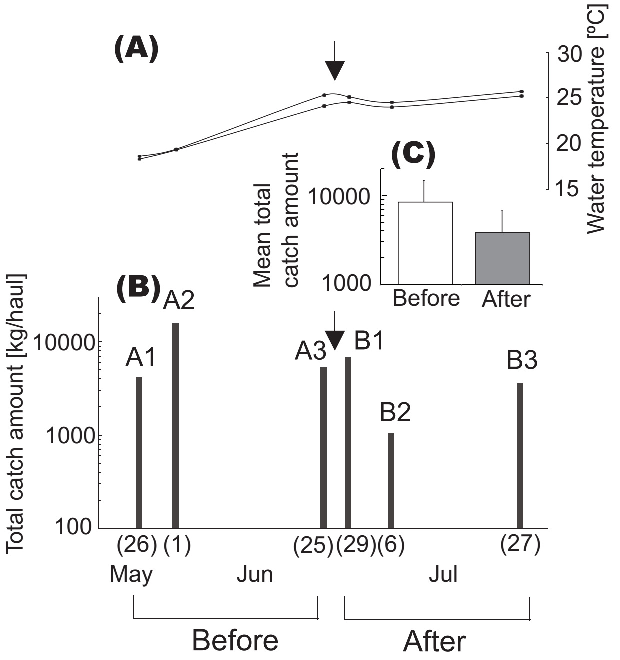

Figure 3. Changes in sea surface temperature and total catch amount during the experimental period in Tachibana Bay, Kyushu, Japan in 2011. **A:** Observed maximum and minimum sea surface temperature in the fishing ground, **B:** Total catch amount per haul, **C:** Mean total catch amount between 'before' and 'after' the passage of the typhoon. Data are means ± S.D. Arrows indicate the passage of the typhoon, A1~B3 indicate sample numbers and numbers in parentheses indicate dates.

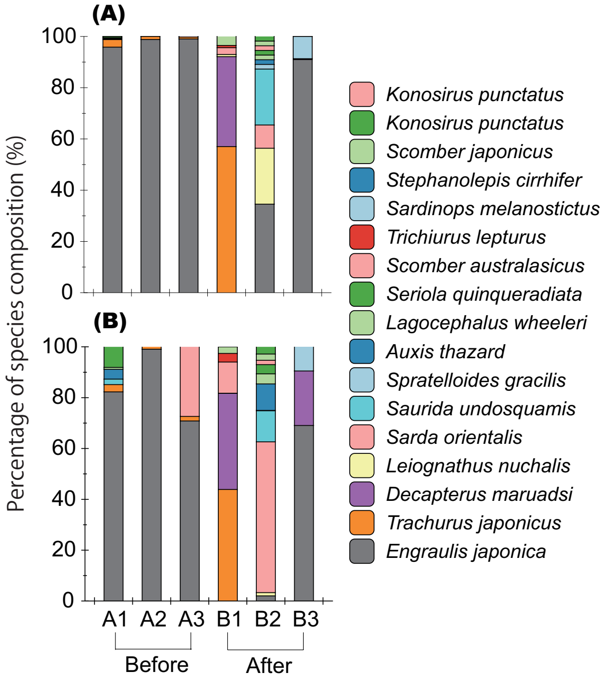

Figure 4. Changes in species composition of the fish community during the period before (A1, A2, and A3) and after (B1, B2, and B3) the passage of the typhoon in Tachibana Bay, Kyushu, Japan. Percentages of the Japanese anchovy *Engraulis japonicus* (grey) and bycatch species, based on **A:** The number of individuals and **B:** Total wet weight of the species sampled (abundance) data.

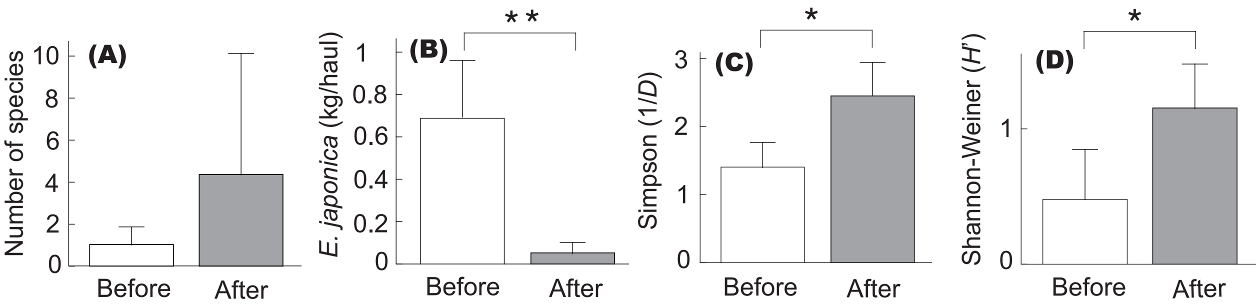

Figure 5. Comparisons of several descriptors of the fish community between the two sampling periods 'before' and 'after' the passage of the tropical cyclone (*$P < 0.05$, **$P < 0.01$, student-t test). **A:** Number of species, **B:** Abundance (wet weight) of the Japanese anchovy *Engraulis japonicus*, **C:** Simpson's reciprocal index $1 \cdot D^{-1}$, **D:** Shannon–Weiner species diversity H' (log 2). Data are means ± SD.

than before ($t = 2.98$, $P = 0.020$ for $1 \cdot D^{-1}$ and $t = 2.38$, $P = 0.038$ for H': Fig. 5C, D). The same is also true of the *k*-dominance curves (Fig. 6).

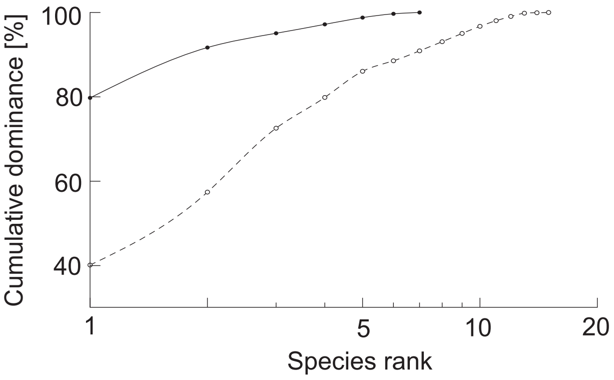

Figure 6. *K*-dominance curves on mean abundance of the period 'before' (solid line) and 'after' (dashed line) the passage of the typhoon.

The classification dendrogram showed two defined clusters corresponding to the 'before' and 'after' periods, except for one sample (B3), at similarity levels of 52% and 74%, respectively (Fig. 7A). By contrast, the classification based on presence–absence data did not reveal any difference between 'before' and 'after' (Fig. 7B), indicating that the overall changes in community structure between the two periods are quantitative rather than qualitative.

Comparisons in total length frequencies between the two periods indicated that specimens of the species compared were not significantly different in size for *S. orientalis* ($P = 0.68$; Fig. 8A) but were bigger for *T. japonicus* ($P = 2.4 \times 10^{-7}$: Fig. 8B) and smaller for *E. japonicus* ($P = 2.5 \times 10^{-75}$: Fig. 8C) in the 'after' period.

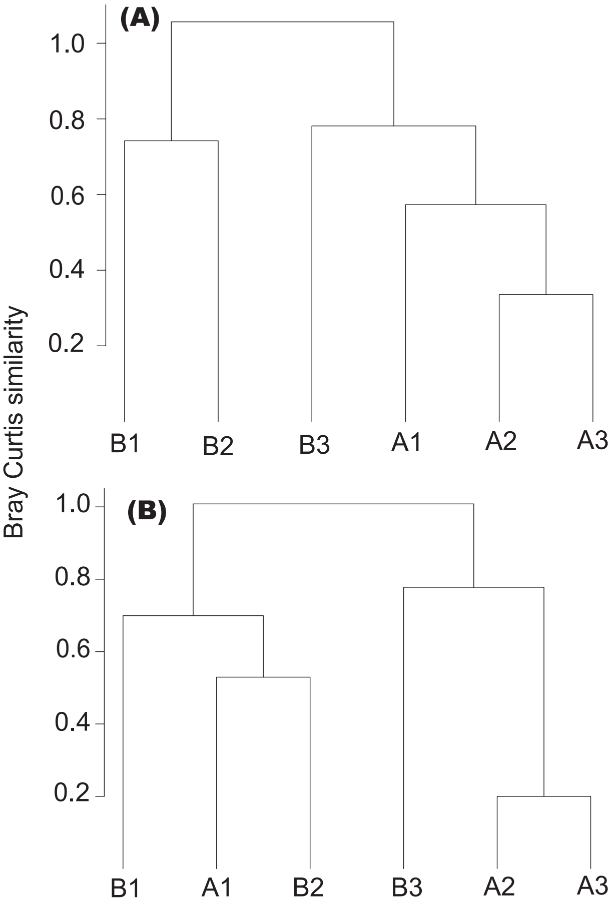

Figure 7. Cluster analysis dendrograms on data obtained during the period before (A1, A2, and A3) and after (B1, B2, and B3) the passage of the typhoon, based on **A:** 4th root-transformed species' total wet weight (abundance) data and **B:** Presence/absence data.

Discussion

In the presently reported study, although major changes in the total catch amount per haul obtained from purse seine catches were not observed before and after the passage of the typhoon, the abundance of *E. japonicus* markedly decreased and that of bycatch species such as *T. japonicus* and *S. orientalis* increased in the 'after' period, accompanied by increasing levels of diversity in the fish assemblage. Furthermore, changes in total length frequencies of two fish species (*T. japonicus* and *E. japonicus*) occurred, respectively. These results support the hypothesis that the typhoon was likely to have changed the structure and

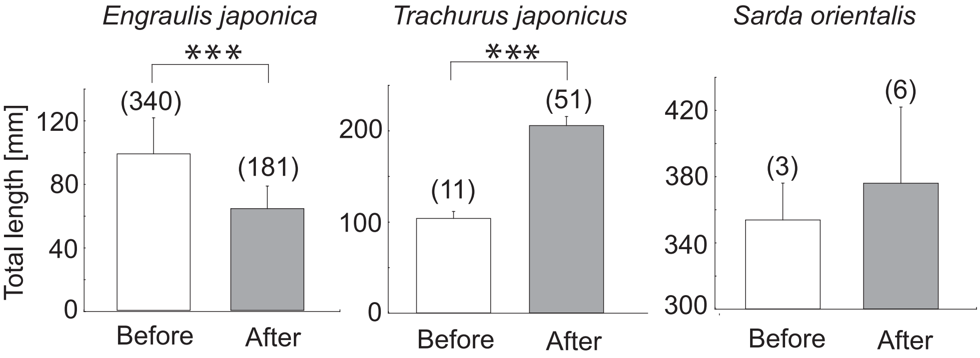

Figure 8. Comparisons of total length of the fish species between the two sampling periods 'before' and 'after' the passage of the tropical cyclone (***$P < 0.001$, Mann–Whitney U test).

spatial organization of multispecies aggregations of the fish community in the temperate, coastal bay.

An explanation for changes in fish assemblages could be that typhoon-induced forces affected the fish distribution. Typhoons have both direct and indirect effects on the distribution of fish species (Kaufman 1983; Halford et al. 2004; Wantiez et al. 2006; Kawabata et al. 2010; Yu et al. 2013, 2014; Chang et al. 2014). Direct effects are considered as the result of severe oceanic conditions, such as wind-associated strong currents that sweep fishes from their habitats (Lassig 1983). However, in this study, changes in fish distribution might be caused by indirect effects of the typhoon, because changes in oceanic conditions in Tachibana Bay did not notably occur.

Indirect effects could be caused by changes in abiotic factors, such as temperature, salinity, and turbidity of seawater. The main parameter known to affect the spatial organization of coastal communities is the temperature (Peterson and Ross 1991; Jaureguizar et al. 2004). Satellite observations and observational data obtained from moored instruments have shown cooling at the sea surface as well as in the mixed layer after a typhoon has passed (Price 1981; Cornillon et al. 1987; You et al. 2011). I also observed that ranges between the maximum and minimum sea surface temperature in the fishing ground became narrow just after the passage of the typhoon, suggesting that mixing in the bay had occurred (Fig. 3). This mixing of the water column in the fishing ground was thought to distribute heat deeper, where epipelagic fish species are present. Each species has an optimal temperature range within which it can be active, grow, reproduce, and metabolize. For example, the optimal temperature range is 15–17°C for *E. japonicus* (see Mitani 1981), but 20–23°C for *T. japonicus* (Ochiai et al. 1983) and 14–23°C for *S. orientalis* (Collette and Nauen 1983). Considering these differences in optimal temperature, *T. japonicus* and *S. orientalis* rather than *E. japonicus* prefer warmer temperatures. This indeed would comply with (i) the respective temperature ranges of each species given above and (ii) the trend for heat being distributed deeper after the passage of the typhoon as mentioned above. These fish species regulate body temperature by moving between areas or depths of different water temperatures (Pough et al. 2009). Thus, the water temperature could explain the change in their habitat use and consequently would affect species composition and abundance of purse seine catches. In fact, it was reported that temperature has an influence on species composition and abundance determined by trawl sampling (Jaureguizar et al. 2004). Similar observations were also reported using some other fishing methods, such as set net (Yoshida and Akimoto 2000), two-boat seine (Tomiyama and Yanagibashi 2004), beach seine (Abookire et al. 2002), and purse seine (Lehodey et al. 1997). To fully clarify our speculation, detailed studies on the multiple measurements of water monitoring before and after the passage of typhoons and behavioral responses of fish species to such typhoon-induced environmental change are necessary.

Although multivariate analysis showed that the overall changes in community structure between the two periods are quantitative rather than qualitative, changes in total length frequencies between the two periods of two fish species may be thought of as qualitative. In this study, the mean total length for *E. japonicus* decreased from 98 mm (sub-adults) to 64 mm (juveniles) in the period after the passage of the typhoon. *E. japonicus* generally spawn from spring to autumn, resulting in the occurrence of several seasonal cohorts (Funamoto et al. 2004). In Tachibana Bay, *E. japonicus* can be caught almost all seasons except the summer season. From late spring to early summer in Tachibana Bay, *E. japonicus* targeted by purse seine fishery were mainly the juveniles (total length < 70 mm) (spring population), which are considered to have been spawned in open waters of the Amakusa Sea and/or the Goto Sea, in early spring (Shimomura et al. 1970; Tanaka et al. 2010). On the other hand, *E. japonicus* fished from autumn to winter mainly consisted of sub-adults. This autumn population is considered to migrate from the inner bay of Ariake Sound and then moves to Tachibana Bay (Shimomura et al. 1970; Tanaka et al. 2010). Although the mechanism(s) for a delay in spring population's move in 2010 are currently unknown, I considered that replacement from autumn population to spring population occurred after the passage of the typhoon. In addition, the mean total length for *T. japonicus* increased from 100 mm to 200 mm in the 'after' period. In Tachibana Bay, frequencies of total length for *T. japonicus* gradually increased from spring to summer with growth. The observed abrupt increase in total length may be considered as an effect of the migration from open waters into the bay after the passage of the typhoon. These results suggest that passage of the typhoon may trigger intraspecific recruitment shifts in and around the bay area. On average, approximately 11 typhoons per year approach Japan within 300 km. Because typhoons have occurred with such frequency over the evolutionary history of the region's ecosystems, these disturbances may play an important role in shaping the seasonal migration patterns of the indigenous species (Pimm et al. 1994; Turpin and Bortone 2002).

Finally, before–after comparisons have an advantage over those involving impact since it is easier to eliminate the factor of spatial variability. However, this type of comparison might be sensitive to seasonal and inter-annual variability (Underwood 1997; Machias et al. 2004). Previous studies on the effects of typhoons on fish assemblages can be categorized according to temporal effects: short-term versus long-term (Turpin and Bortone 2002). Long-term (more than 1 year) effects of typhoons may be difficult to determine because the effects of other factors such as food supply, competition, and/or predator-prey interaction will become more important as the time elapsed after the event increases. Yu et al. (2013) showed short-term effects of typhoons on fishery catches to provide evidence of CPUE increase after the typhoon. The presently reported study has also tried to eliminate the factor of seasonal and inter-annual variability by carrying out the series of samples in a short period (2 months). However, the purse seine fishery is characterized by a moon-dependent cycle, with high catches during the new moon and low catches around the full moon (Dudley and Tampubolon 1986; Pet et al. 1997). Although I did not sample during a number of days around the full moon, lunar variability may have potential effects on the catches. Thus, further evaluations determined by other fishing methods as well as fish tagging and marking techniques are required to better clarify the impacts of a typhoon on fish communities in a temperate, coastal bay.

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Mistaken by dots: Revealing the misidentification of *Saurida lessepsianus* (Actinopterygii: Aulopiformes: Synodontidae) along the west coast of India (eastern Arabian Sea)

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Abstract

The presently reported study identified *Saurida lessepsianus* Russell, Golani et Tikochinski, 2015 from the west coast of India, which was earlier diagnosed as *Saurida undosquamis* (Richardson, 1848) due to their morphological similarities. *Saurida lessepsianus* is characterized by 48–51 lateral line scales, 44–47 vertebrae, 3–6 rows of teeth on tongue and anterior part of stomach black. The identity of this species was also confirmed by molecular examination (phylogenetic analysis of the COI sequences) and the study revealed a genetic divergence value of 0.08 between *S. lessepsianus* and *S. undosquamis*. Our study confirms the extended distribution of *S. lessepsianus* along the west coast of India (eastern Arabian Sea) from the Red Sea-Mediterranean Sea.

Keywords

DNA barcoding, Indian coast, Lessepsian lizardfish, Misidentification

Introduction

Species of the family Synodontidae, commonly known as Lizardfishes, are commercially important marine/estuarine demersal fishes. The family is represented by 83 valid species under 4 genera: namely *Harpadon* Lesueur, 1825; *Saurida* Valenciennes, 1850; *Synodus* Scopoli, 1777, and *Trachinocephalus* Gill, 1861. Species of genus *Saurida* are widely distributed in the Indo-West Pacific region and presently, 24 valid species are reported (Fricke et al. 2020). The taxonomy of this genus, however, is confusing because of overlapping morphological characters and there has been a widespread misidentification of species in the published literature.

In India, nine species of *Saurida*, namely *Saurida gracilis* (Quoy et Gaimard, 1824); *Saurida isarankurai* Shindo et Yamada, 1972; *Saurida longimanus* Norman, 1939; *Saurida micropectoralis* Shindo et Yamada, 1972; *Saurida nebulosa* Valenciennes, 1850; *Saurida pseudotumbil* Dutt et Sagar, 1981; *Saurida tumbil* (Bloch, 1795); *Saurida undosquamis* (Richardson, 1848), and *Saurida wanieso* Shindo et Yamada, 1972 have been reported (Chandra et al. 2020). The identification of *S. undosquamis*, however, is problematic. Inoue and Nakabo (2006) reported *S. undosquamis* to comprise a complex of four species which they called the "*S. undosquamis* group", covering *Saurida umeyoshii* Inoue et Nakabo, 2006; *Saurida macrolepis* Tanaka, 1917; *S. undosquamis*, and *S. longimanus*, based on the following shared morphological characters: black dots on upper margin of caudal fin, pectoral fin extending beyond origin of pelvic fin, anterior rays of dorsal fin neither elongate nor filamentous, pre-dorsal length greater than distance between dorsal-fin and adipose-fin origins, larger scales on body and 46–55 pored lateral-line scales. Russell et al. (2015), however, showed that the species, previously reported as *S. undosquamis* from the Red Sea and the Mediterranean, and reidentified as *S. macrolepis* by Inoue and Nakabo (2006), in fact, was a new species, *Saurida lessepsianus* Russell, Golani et Tikochinski 2015, that was genetically distinct from specimens of *S. undosquamis* and *S. macrolepis* (see Tikochinski et al. 2016). Thus, the presently reported study was undertaken to validate *S. undosquamis* inhabiting the west coast of India, using morphological and molecular markers. The results showed that the species reported as *S. undosquamis* from the west coast of India (Raje et al. 2012, Chhandaprajnadarsini et al. 2018, 2019) is indeed *S. lessepsianus* and confirms its extended distribution in the western Indian Ocean from the Red Sea–Mediterranean Sea.

Material and methods

A total of 40 individuals of *S. lessepsianus* were collected from the Versova landing center (19°08′N, 72°48′E) and New Ferry Wharf fishing harbor (18°57′N, 72°51′E) of the Mumbai coast (north-western coast of India) and Neendakara fishing harbor (8°56′N, 76°32′E) of the Kerala coast (south-western coast of India) during November–December 2019 (Fig. 1). The specimens were caught by commercial bottom trawls, operating in the region at depths of 150–200 m. Morphometric characters were measured following Russell et al. (2015). Measurements were taken by digital Vernier caliper to the nearest 0.1 mm. The number of vertebrae was counted using X-ray radiographs. Morphometric traits were expressed in percentage of standard length (**SL**) and head length (**HL**).

For molecular work, total genomic DNA was extracted by the salting out method (Miller et al. 1988). Partial Mitochondrial COI regions were amplified using reported primers as described by Ward et al. (2005). The PCR was carried out in a 25 µL reaction with 10 × Taq buffer, 200 µM of each dNTPs, 1 unit of Taq-DNA polymerase (Promega, USA), 1 × Taq buffer containing 1.5 mM $MgCl_2$, 10 picomoles of each primer, and 100 ng of template DNA. The PCR products were purified and sequenced in both directions using primers. The thermal conditions for amplifying COI region were set as an initial denaturation of 5 min at 95°C, followed by 35 cycles of 30 s at 94°C for denaturation, 30 s at 54°C for annealing, 60 s at 72°C for extension, with a final extension at

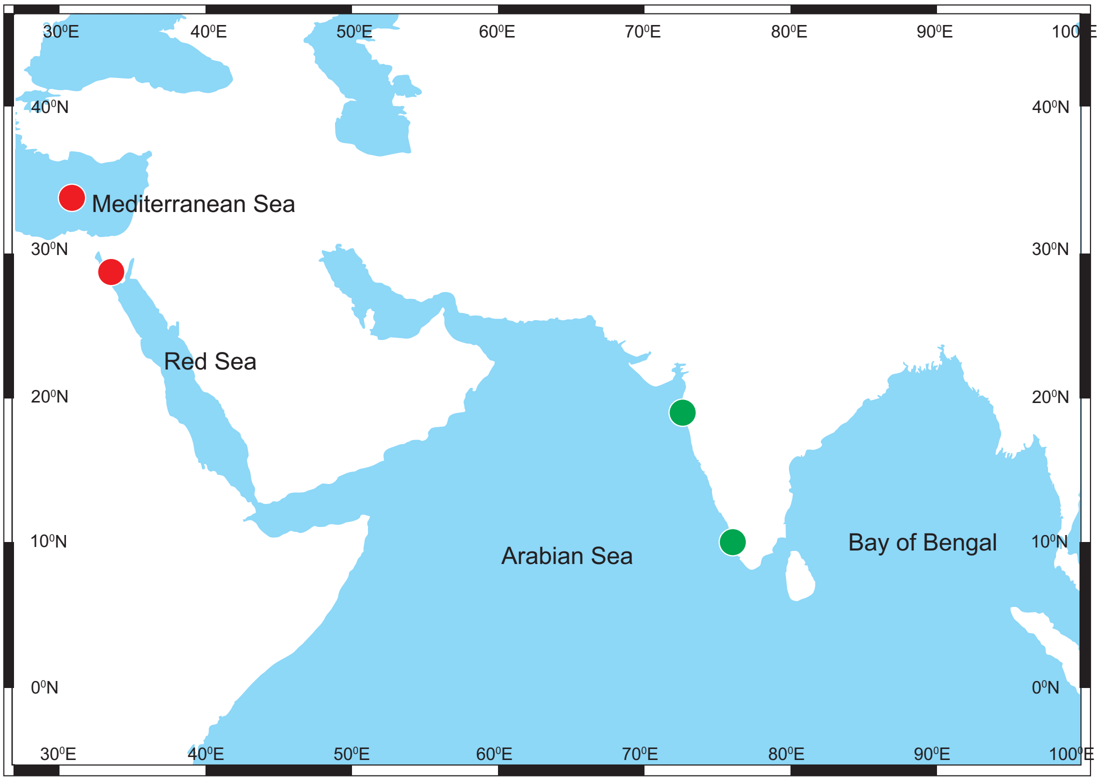

Figure 1. Distribution map of *Saurida lessepsianus*. The red circle indicates previous distribution and green circle indicates present distribution.

72°C for 10 min. The amplicons were purified and sequenced in both directions using PCR primers.

In addition, the reported COI sequences of *S. undosquamis* (*n* = 17) and *S. lessepsianus* (*n* = 3) and other related species were downloaded from the GenBank to estimate the genetic distance values. The sequences were aligned to their homologous position using the Clustal W program implemented in MEGA7 (Kumar et al. 2016). Nucleotide composition, intra-, and inter-specific genetic divergence values were calculated by Kimura two parameter (K2P) model. A neighbor-joining tree was constructed using K2P distances to represent divergence pattern between species (Saitou and Nei 1987). All the molecular analysis was carried out in Molecular Evolutionary Genetic Analyses (MEGA7) software (Kumar et al. 2016).

Results

Genus Saurida Valenciennes, 1850

Saurida lessepsianus Russell, Golani et Tikochinski, 2015

Figs 2–4; Table 1

Lessepsian lizardfish

Description of specimens from west coast of India. Body elongated and slightly cylindrical; body depth 17.2% of SL; depth of caudal peduncle 6.5% of SL (Table 1); pectoral fins moderately long, reaching or just extending beyond base of pelvic fins; axillary scale long

Figure 2. *Saurida lessepsianus* collected from Arabian Sea, Mumbai coast, India (131.28 mm SL).

Figure 3. Stomach of *Saurida lessepsianus*.

Figure 4. X-Ray radiograph of *Saurida lessepsianus*.

Table 1. Morphometric parameters of *Saurida lessepsianus* collected during the presently reported study compared with previous studies.

Morphometric trait	This study ($n = 40$)		*S. undosquamis* ($n = 8$) (*fide* Inoue and Nakabo 2006)		*S. lessepsianus* ($n = 37$) (Russell et al. 2015)	
	Range	Mean ± SD	Range	Mean	Range	Mean ± SD
Standard length [mm]	112.07–236.58		112.1–358.0		108.0–282.2	
Pre-dorsal length [%SL]	42.61–46.61	44.22 ± 0.38	41.1–43.1	42.2	40.3–45.0	42.6 ± 1.2
Pre-adipose length [%SL]	53.50–84.69	79.50 ± 2.93	79.5–81.8	80.6	76.9–83.2	80.9 ± 1.4
Pre-anal length [%SL]	48.67–76.77	71.74 ± 2.60	70.5–73.4	71.8	67.2–77.1	71.9 ± 1.9
Pre-anal fin length [%SL]	75.11–79.45	77.22 ± 0.51	72.9–76.1	74.8	65.0–79.3	74.8 ± 2.5
Pre pectoral length [%SL]	25.65–27.98	26.68 ± 0.20	24.1–26.7	25.5	23.4–28.7	25.7 ± 1.2
Pre pelvic length [%SL]	36.05–41.71	39.49 ± 0.58	35.8–38.9	37.4	35.4–42.6	38.8 ± 1.6
Head length [%SL]	19.21–26.05	24.40 ± 0.61	23.2–25.5	24.8	22.5–26.5	24.4 ± 1.0
Body depth [%SL]	14.42–20.65	17.19 ± 0.53	11.7–13.5	12.5	10.4–17.2	13.3 ± 1.5
Body width [%SL]	11.67–15.05	13.72 ± 0.33	11.4–14.6	13.2	10.9–15.6	13.0 ± 1.3
Inter-pelvic width [%SL]	5.06–8.68	7.58 ± 0.34	7.6–8.6	8.2	7.6–9.5	8.4 ± 0.5
Pectoral fin length [%SL]	12.77–17.63	15.04 ± 0.43	13.4–14.5	13.9	11.5–17.0	14.2 ± 1.3
Pelvic fin length [%SL]	14.81–18.11	16.77 ± 0.30	16.7–20.0	18.1	14.2–18.6	16.6 ± 0.8
Length of 2nd dorsal ray [%SL]	15.91–23.17	20.14 ± 0.74	16.5–20.3	18.7	16.0–21.5	19.8 ± 1.1
Length of last dorsal ray [%SL]	4.50–6.35	5.40 ± 0.22	4.6–6.3	5.4	4.9–6.9	5.8 ± 0.5
Length dorsal fin base [%SL]	12.83–14.54	14.03 ± 0.17	11.7–13.2	12.6	12.5–16.6	14.4 ± 1.0
Length of 2nd anal ray [%SL]	7.10–10.75	9.64 ± 0.32	8.3–10.4	9.5	7.7–11.1	9.5 ± 0.9
Length of last anal ray [%SL]	4.05–6.96	5.18 ± 0.29	5.1–7.8	6.1	3.8–10.5	5.8 ± 1.1
Length of anal-fin base [%SL]	5.94–10.96	9.58 ± 0.43	8.6–10.3	9.6	5.5–12.4	10.0 ± 1.2
Depth of caudal peduncle [%SL]	5.57–7.45	6.51 ± 0.20	5.9–7.2	6.4	5.7–7.1	6.4 ± 0.3
Eye diameter [%HL]	14.21–25.34	18.58 ± 0.92	15.3–22.9	19.6	18.4–25.6	21.4 ± 1.9
Pre-orbital length [%HL]	16.22–29.93	19.54 ± 1.28	—		—	
Inter-orbital width [%HL]	15.38–25.79	20.12 ± 0.96	16.3–24.8	20.6	14.5–21.3	18.2 ± 1.5
Post orbital length [%HL]	57.92–82.96	62.49 ± 2.31	54.5–63.2	58.2	54.7–63.5	59.4 ± 1.7
Upper jaw length [%HL]	59.58–89.06	67.67 ± 2.51	66.6–72.0	70.0	65.3–73.4	69.1 ± 1.8

and pointed; dorsal-fin base length comparatively longer than anal-fin base.

In fresh condition, body dark dorsally and light colored below the lateral line with a series of 9 small blotches along the lateral line; 6–10 indistinct black spots on the first two dorsal rays; 6–8 distinct black spots on the dorsal margin of the caudal fin; adipose fin whitish with black blotches anterodorsally; pelvic and anal fin whitish; lower lobe of the caudal fin and upper part of the pectoral fin blackish (Fig. 2); stomach black anteriorly and white posteriorly, with white intestine (Fig. 3).

Counts and proportional measurements of the specimens are provided in Table 1. The mode and range of meristic traits of *S. lessepsianus*: Dorsal-fin rays 11 (11–12); pelvic-fin rays 9; pectoral-fin rays 14 (13–15); anal-fin rays 11 (11–12); lateral-line scales 49 (48–51); transverse scales above and below lateral line 4½ and 5½, respectively; pre-dorsal scales 15 (15–19); pre-adipose scales 14 (14–16); vertebrae 47 (44–47) (Fig. 4); two rows of palatine teeth; no teeth on vomer; 4–6 tooth rows on tongue.

Around 650 bp of COI gene were amplified and sequenced using primers. The sequence quality was verified by observing the Phred score of each nucleotide using Finchtv software. The sequences were submitted to the GenBank with accession numbers of MN853856 and MT139594–MT139596 (COI). The ends of the COI sequences were trimmed to include all reported sequences which resulted in a sequence length of 544 bp. The genetic distance values found in the presently reported study and reported sequences from India were 0; accordingly, sequences were clustered into a single clade. The mean genetic distance values between *S. lessepsianus* of the Mediterranean/Red Sea and the Arabian Sea was 0.008 ± 0.002. These sequences formed separate branches of the clade. The mean genetic divergence value between *S. lessepsianus* and *S. undosquamis* of Australian waters was 0.079 ± 0.012 (Table 2). The sequences of *S. lessepsianus* were clustered distinctly from *S. undosquamis* reported from Australia (Fig. 5).

Discussion

Saurida lessepsianus is a recently described species of the genus *Saurida*, which was misidentified as *S. undosquamis* in the Red Sea and the Mediterranean Sea. Russell et al. (2015) described the species with the following combination of characters: elongated, cylindrical body with a depressed head; caudal peduncle slightly compressed; mouth very large with 2 rows of teeth on outer palatines; 0–2 rows of teeth on vomer; pectoral fin long, reaching line between origin of pelvic fins and dorsal fin; vertebrae

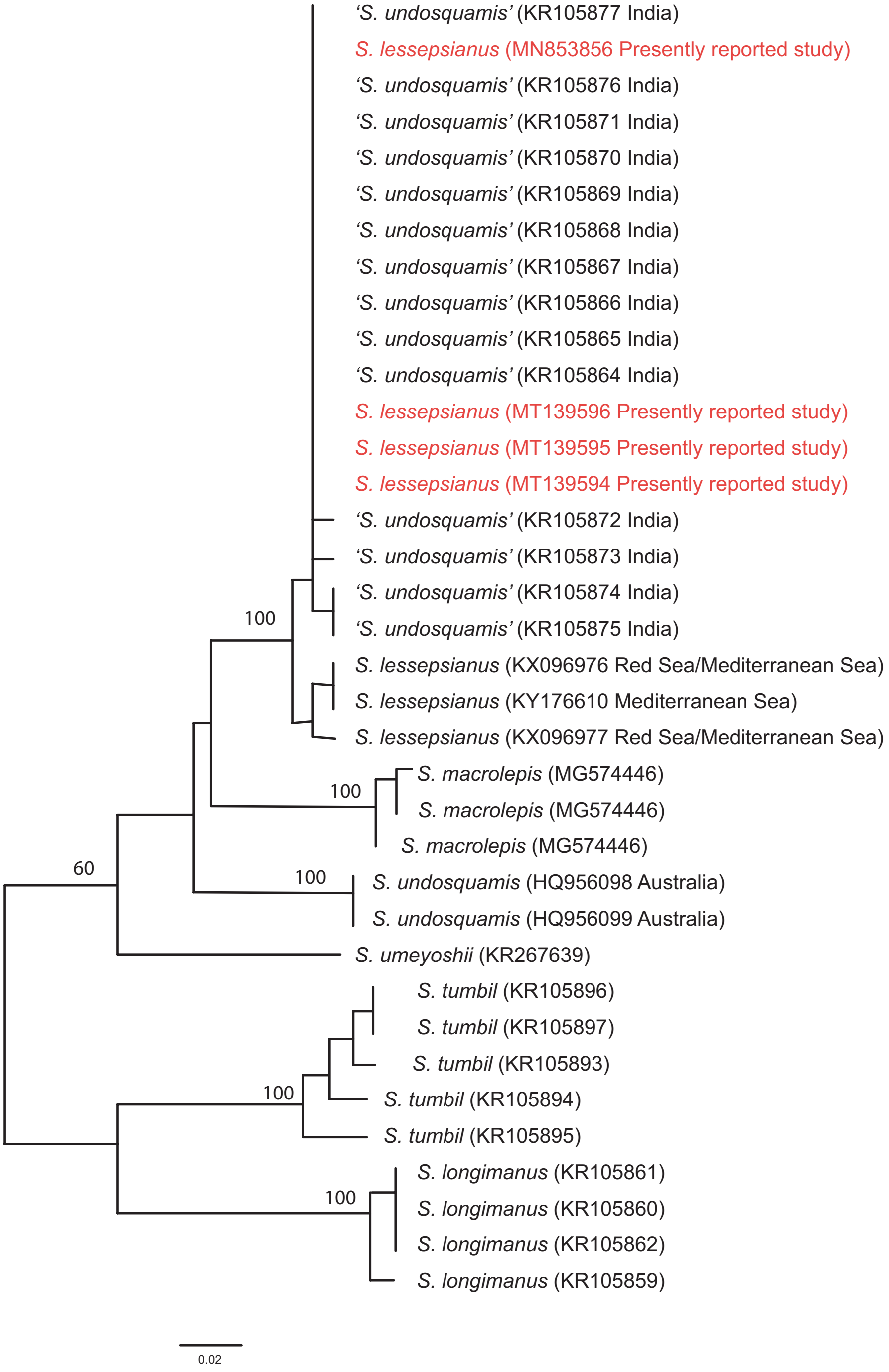

Figure 5. Neighbour-joining tree of the genus *Saurida* constructed using COI gene.

Table 2. Genetic distance values of *Saurida* species.

Species	PS	Su1	SLRS	SUAU	St	Sm	Sl	Su
S. lessepsianus (PS)	**0.000**							
S. 'undosquamis' (India: Su1)	0.000	**0.000**						
S. lessepsianus (Red Sea: SLRS)	0.008	0.008	**0.004**					
S. undosquamis (Australia: SUAU)	0.081	0.081	0.075	**0.000**				
S. tumbil (St)	0.206	0.206	0.205	0.199	**0.003**			
S. macrolepis (Sm)	0.088	0.091	0.098	0.093	0.215	**0.002**		
S. longimanus (Sl)	0.216	0.216	0.213	0.205	0.167	0.206	**0.001**	
S. umeyoshii (Su)*	0.119	0.119	0.119	0.107	0.188	0.124	0.193	

(Below the diagonal (values in bold): genetic distance values; PS: presently reported study samples; Su1: Reported sequences of *Saurida 'undosquamis'* from India; SLRS: Reported *S. lessepsianus* from Red Sea / Mediterranean Sea; SUAU: Reported *S. undosquamis* from Australia).
*Only one sequence used for analysis.

47–51; pored lateral-line scales 44–47; stomach grey to black anteriorly and white posteriorly.

During the presently reported study, the specimens of *Saurida* species examined from the west coast of India, differed from *S. undosquamis* (Table 3) by having 47–51 pored lateral-line scales (vs. 54–58), 44–47 vertebrae (vs. 52), 3–6 rows of teeth across the tongue (vs. 7–12), and stomach greyish or black anteriorly with intestine pale whitish (vs. stomach and intestine both pale whitish). The recorded morphological characteristics of the specimens collected along the west coast of India match with that of *S. lessepsianus* described from the Red Sea and Mediterranean Sea by Russell et al. (2015) (Table 3). Some morphological characters like dots on the dorsal fin and caudal fin were found to overlap between the *S. undosquamis* and *S. lessepsianus*. These characters are similar and are a major cause of misidentification.

DNA barcoding has been successful in resolving taxonomic ambiguity and validating many fish species (Pavan-Kumar et al. 2018, Mary et al. 2019). This method delimits the species based on the degree of COI sequence similarity or divergence value among individuals (Hebert et al. 2003). Accordingly, conspecific individuals show lower divergence values than congeneric individuals, irrespective of their geographic locations. In the presently reported study, COI sequences showed low divergence values with reported sequences of *S. lessepsianus* from the Red Sea/Mediterranean Sea and the Arabian Sea. Thus, the specimens from India, previously reported in the literature and in public sequence databases as *S. undosquamis*, are misidentifications of *S. lessepsianus*. Furthermore, the growing problem of incorrect species identification in public sequence databases such as GenBank, highlight the necessity for taxonomic verification of published sequence data by referencing voucher specimens.

Table 3. Comparison between two species *Saurida lessepsianus* and *Saurida undosquamis*.

Characters	***Saurida lessepsianus* (This study)**	***Saurida undosquamis* (Russell et al. 2015)**
Alimentary tract	Stomach greyish or black anteriorly; intestine pale whitish	Stomach and intestine pale whitish
Number of spots on upper margin of caudal fin	6–8	5–12
Pored lateral-line scales	47–51	54–58
Vertebra	44–47	52
Rows of teeth on tongue	3–6	7–12

The presently reported study reports the extension of the known distribution range of *S. lessepsianus* from the Mediterranean/Red Sea region to the west coast of India, eastern Arabian Sea. Most likely, *S. lessepsianus* is widespread in the western Indian Ocean and extended into the Red Sea, subsequently disconnected from its original population and then further migrated into the Mediterranean Sea through the Suez Canal. The connection between the Red Sea and the Indian Ocean is a shallow strait (Bab-el-Mandab) and the turbid waters of the southern Red Sea, combined with the cold nutrient-rich waters of the Gulf of Aden, act as a potential barrier for gene flow (Roberts et al. 1992, DiBattista et al. 2013), which may explain the intraspecific divergence in the populations of *S. lessepsianus* between these regions.

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Investigating otolith mass asymmetry in six benthic and pelagic fish species (Actinopterygii) from the Gulf of Tunis

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Abstract

Otolith mass asymmetry can significantly affect the vestibular system functionalities; usually, the X values of mass asymmetry vary between −0.2 and + 0.2 ($-0.2 < X < +0.2$). These values can change during a fish life and therefore they are not related to the fish total length. We collected a total of 404 fish specimens from the Gulf of Tunis, including three pelagic species: *Sardina pilchardus* (Walbaum, 1792) (74 otolith pairs), *Trachurus mediterraneus* (Steindachner, 1868) (66 otolith pairs), and *Chelon auratus* (Risso, 1810) (60 otolith pairs) and three benthic species: *Gobius niger* Linnaeus, 1758 (77 otolith pairs), *Mullus barbatus* Linnaeus, 1758 (60 otolith pairs), and *Trachinus draco* Linnaeus, 1758 (67 otolith pairs). The relation between the total length and the otolith mass asymmetry was first calculated and compared, and then was evaluated. The comparison of the otolith mass asymmetry between benthic and pelagic species showed a significant difference ($P < 0.05$), where the absolute mean value of X does not exceed the critical value (0.2) for all the studied species. No relation has been found between the magnitude of the otolith mass asymmetry and the length in both benthic and pelagic specimens. Environmental factors have an indirect effect on somatic growth and otolith accretion. The significant difference found in this study can be due to the difference between the benthic and pelagic environments.

Keywords

benthic, Gulf of Tunis, Otolith, otolith mass asymmetry, pelagic

Introduction

Otoliths are calcified structures found in the inner ears of teleosts, in the vestibular system. Every fish has three otolith pairs mostly composed of calcium and carbonate layers precipitated in an organic matrix (Carlström 1963; Panfili et al. 2002; Pracheil et al. 2019). These paired structures are involved in acoustic and balance systems of teleosts (Paxton 2000). These calcified structures are metabolically inert, they grow by accretion and they cannot be used by the organism as a source of calcium (Campana and Neilson 1985). Thanks to these properties, otolith pairs and especially the sagittas are used to rebuild fish life cycle and their interactions with the environment (Vignon and Morat 2010).

Several authors have used otoliths simply to study fish age status, sexual dimorphism, and migration (Walther and Limburg 2012; Fatnassi et al. 2017). Also, otolith weight has been used as a surrogate method to estimate the age of fish (Francis and Campana 2004; Nazir and Khan 2019).

For many years, otolith shape has been routinely used to differentiate between fish stocks (Keating et al. 2014; Bailey et al. 2015; Ider et al. 2017; Mejri et al. 2018b; Nazir and Khan 2019; Avigliano et al. 2020). Otolith microchemistry has also been used to explore the environmental and food effects on fish metabolism (Mille et al. 2015; Perry et al. 2015). Furthermore, many presently cited studies indicate that the otolith mass asymmetry is also an important parameter because otoliths play a major role in acoustic functionalities.

The inconsistency between the right and left otolith's movements inside the inner ear can cause balance problems and sound perception difficulties (Lychakov and Rebane 2005; Lychakov et al. 2006).

Several studies on otolith mass asymmetry have shown that the majority of the symmetric fish species have X values within the range of $-0.2 < X < +0.2$ (Lychakov and Rebane 2004, 2005; Jawad 2013). Thus, in theory, only an absolute value of X that exceeds 0.2 can alter the acoustic functionality of a fish (Lychakov et al. 2006). Moreover, the relation between otolith mass asymmetry and fish total length has been explored (Mejri et al. 2018a). Lychakov and Rebane (2004) have shown a relation between saccular otolith mass asymmetry and fish length exists only in littoral and bottom populations and not in the pelagic species. Also, some studies have shown that there is no relation between otolith mass asymmetry and fish total length (Jawad 2013; Yedier et al. 2018). The presently reported study aimed to compare the otolith mass asymmetry between pelagic and benthic species and to assess the relation between mass asymmetry and the fish total length. The following species were studied: three pelagic species: *Sardina pilchardus* (Walbaum, 1792), *Trachurus mediterraneus* (Steindachner, 1868), and *Chelon auratus* (Risso, 1810) and three benthic species: *Gobius niger* Linnaeus, 1758, *Mullus barbatus* Linnaeus, 1758, and *Trachinus draco* Linnaeus, 1758.

Materials and methods

Sample collection

A total of 204 benthic fish species and 200 pelagic fish samples were collected from the Gulf of Tunis, in the north of Tunisia (36°49′09″N, 10°18′22″E) from March to May 2017 (Fig. 1). In this study, we only used adult fishes to compare the otolith mass asymmetry between benthic and pelagic species without considering gender. Standard and total length (L_s, L_t) were measured for each specimen to the nearest mm. The mean total length of the benthic species ranged from 150.8 ± 14.34 for *Mullus barbatus* to 238.322 ± 16.59 for *Gobius niger* and from 150.375 ± 9.69 to 235.14 ± 11.62 for pelagic species (*Sardina pilchardus* and *Chelon auratus*, respectively) (Table 1).

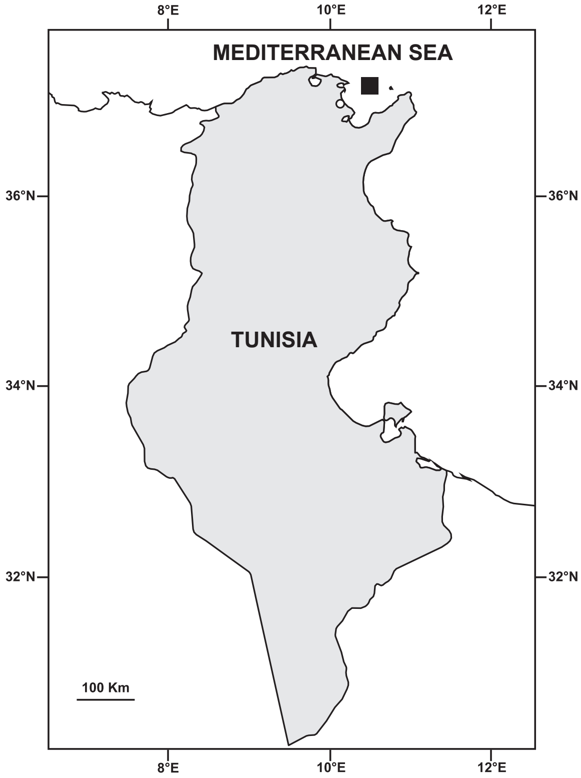

Figure 1. Sampling sites of the studied species in the Gulf of Tunis.

Otolith extraction

Sagittal otoliths pairs were manually removed by the dissection of the auditory capsules, washed with distilled water, and air-dried at room temperature. The weight of the right and left paired otoliths was also determined using a precision electronic balance (Mettler Toledo AL204) to an accuracy of 0.1 mg (Table 1).

Table 1. Descriptive statistics of the total length of benthic and pelagic fish species from the Gulf of Tunis.

Species	Domain	*n*	Mean ± SE [mm]
Mullus barbatus	Benthic	60	150.80 ± 14.34
Trachinus draco	Benthic	67	202.57 ± 17.99
Gobius niger	Benthic	77	238.32 ± 16.59
Chelon auratus	Pelagic	60	235.14 ± 11.62
Sardina pilchardus	Pelagic	74	150.38 ± 9.69
Trachurus mediterraneus	Pelagic	66	187.15 ± 17.32

Data calculation

The otolith mass asymmetry (X) was computed using the following formula:

$$X = (M_R - M_L)\, M_M^{-1}$$

where M_R and M_L are the otolith masses of the right and left paired otoliths, and M_M is the mean mass of the right and left paired otoliths.

Theoretically, X values vary from −2 to +2. These limit values indicate maximal asymmetry while the ‘0’ value refers to the absence of asymmetry between right and left otoliths of the same fish. A negative value of X means that the left otolith is heavier than the right one ($M_L > M_R$), whereas a positive value of X means the opposite.

The relation between absolute otolith mass asymmetry ($|X|$) and the total fish length was calculated using the following formula:

$$|X| = a \cdot L_t + b$$

where a is the coefficient characterizing the growth rate of the otolith and b is a constant for a given species.

Statistical analyses

The comparison of the otolith mass asymmetry between benthic and pelagic specimens was performed using Student’s t-test. To assess the existence of a relation between the total length and the absolute otolith mass asymmetry, a regression analysis was used to calculate correlation coefficients and regression equations for each species. XLSTAT (2007) software was used for all statistical analyses.

Results

It is known that the absolute symmetry between left and right otolith ($X = 0$) is rare, usually, X values fluctuate around 0. In this study, the percentage of asymmetry exceeded 50% in all the studied fish species. *Trachinus draco*, which is a benthic species, has shown an asymmetry rate equal to 100%. This means that all the otolith pairs of the 67 used fish samples are asymmetric in terms of weight, unlike the results observed in *Sardina pilchardus* population which have shown the lowest asymmetry rate with only 53%.

The otolith mass asymmetry was within the range of $-0.496 \leq X \leq 0.3379$ for all the studied species. It varied between −0.0513 and 0.1531 for benthic species and between −0.496 and 0.3379 for pelagic species (Table 2). The mean values of $|X|$ were calculated for all investigated species. The results have shown a mean value of $|X|$ equal to 0.0251 ± 0.0021 for benthic species and 0.0383 ± 0.0046 for pelagic species. Moreover, a significant difference was found between benthic and pelagic fish species ($P < 0.05$) (Table 3).

Table 2. Descriptive statistics results of otolith mass asymmetry (X) and absolute otolith mass asymmetry ($|X|$) for benthic (*Mullus barbatus*, *Trachinus draco* and *Gobius niger*) and pelagic fish species (*Chelon auratus*, *Sardina pilchardus* and *Trachurus mediterraneus*) from the Gulf of Tunis.

Species	$\lvert X \rvert$ Mean ± SD	X Minimum	X Maximum	Asymmetry rate [%]
Mullus barbatus	0.0304 ± 0.0424	−0.0258	0.0952	91
Trachinus draco	0.0399 ± 0.0648	−0.0347	0.0771	100
Gobius niger	0.0264 ± 0.0265	−0.0513	0.1531	54
Chelon auratus	0.0264 ± 0.0632	−0.4960	0.0508	53
Sardina pilchardus	0.0493 ± 0.0683	−0.3636	0.1538	93
Trachurus mediterraneus	0.0186 ± 0.0151	−0.3665	0.3379	89

Table 3. Descriptive statistics of absolute otolith mass asymmetry ($|X|$) for benthic (*Mullus barbatus*, *Trachinus draco*, *Gobius niger*) and pelagic fish species (*Chelon auratus*, *Sardina pilchardus*, *Trachurus mediterraneus*) from the Gulf of Tunis.

Parameter	Species	
	Benthic	Pelagic
n	204	200
Mean	0.0251	0.0383
Minimum	0.0000	0.0000
Maximum	0.2588	0.4962
SD	0.0021	0.0046
t-values	1.9660	
P-values	0.0102	

The correlation coefficients R^2 and regression equations were calculated for all of the six studied species each apart. The results reject the hypothesis since no significant relation between absolute otolith mass asymmetry and the total fish length was found ($0.0008 \leq R^2 \leq 0.0356$) (Table 4).

Table 4. Correlation coefficient and P-value of benthic (*Mullus barbatus*, *Trachinus draco*, *Gobius niger*) and pelagic fish species (*Chelon auratus*, *Sardina pilchardus*, *Trachurus mediterraneus*) from the G ulf of Tunis.

Species	R^2	P-value
Gobius niger	0.0008	0.993
Chelon auratus	0.0302	0.184
Mullus barbatus	0.0014	0.777
Sardina pilchardus	0.0040	0.958
Trachurus mediterraneus	0.0048	0.579
Trachinus draco	0.0356	0.138

Discussion

In the presently reported study, all benthic and pelagic species had a mean value of otolith mass asymmetry varying from −0.2 to 0.2 similar to previous studies (Lychakov et al. 2008; Jawad 2013; Jawad and Sadighzadeh 2013). Only pelagic species showed X values exceeding the critical limits, in particular, *T. mediterraneus* with an otolith mass asymmetry $-0.3665 < X < 0.3379$. This is probably related to the physiological state of this species, its habitat, and environmental factors (abiotic and biotic) as previously reported by Grønkjær (2016) and (Izzo et al. 2018) since the fish studied survived at different latitudes and longitudes. In the same context, the variations of environmental factors as well as anthropogenic ones have remarkable effects on the development of otoliths (Munday et al. 2011). In fact, in their study on the physicochemical parameters of the Gulf of Tunis, Ben Lamine et al. (2011) showed that this area suffers from several problems, such as metal pollution and urban discharges. Likewise, other studies have shown that pollution can affect the growth of otoliths (Elsdon and Gillanders 2002; Munday et al. 2011; Perry et al. 2015). It has been shown that metals impact otolith growth when fish inhabiting affected areas are likely to accumulate metals, including anthropogenic metals from surrounding environments, and transfer / transport them to the upper links in the food chain (Wang 2002). This is reflected in the continued incorporation of the metals into the crystal matrix of the otolith since they are metabolically inert and their increments can undergo resorption. Our results are consistent with other studies conducted on the effect of metal accumulation on otolith growth (Vrdoljak et al. 2020).

Morphological variability of sagittae is impacted by a dual regulation: genetic and environmental factors (L'Abée-Lund 1988; Lombarte et al. 2010; Vignon and Morat 2010; Annabi et al. 2013). Also, the increase or decrease in otolith mass asymmetry can negatively affect other factors that are necessary for the life of the fish, especially the sense of hearing and balance. The otolith mass asymmetry has been used as a bioindicator to test the condition in different aquatic habitats (Grønkjær 2016) and to test different environmental effects on fish populations. In our study, we have shown that the relative size of sagittae is larger in benthic species than in pelagic ones, which could be related to the different ecological niches of the studied fish (Lombarte and Cruz 2007).

The relation between the otolith mass asymmetry and the total length has been investigated in several studies (Mille et al. 2015; Yedier et al. 2018). We used the absolute value of X to prove whether there is any relation between the absolute otolith mass asymmetry and fish total length. According to the presently reported results, the absolute value of the otolith mass asymmetry does not depend on the total length in any studied species. Our results agree with the previous findings which investigated roundfish and flatfish (Jawad 2013; Yedier et al. 2018).

The otolith mass and shape asymmetry was explored in many studies all over the world. In Tunisia, otolith research is limited to fish stock identification and the assessment of sexual dimorphism. We need more research studying the impact of the environment on otolith mass asymmetry and on fish behavior. Therefore, it is important to use a large number of specimens and a wide range of body sizes in future studies to fully understand the relation between the asymmetry in otolith mass and the fish length.

Conclusion

Environmental factors have an indirect effect on somatic growth and otolith accretion. The significant difference found in this study can be related to the difference between the benthic and pelagic environments. Food and genetic variability can also be used to explain the presently reported results.

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Using otoliths for fish stock discrimination: status and challenges

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Abstract

Otoliths are calcified structures and the information contained within their chemistry or shape can be used to infer life history events, migration patterns, and stock structure of a fish population. Understanding how otolith chemistry is affected by temperature, salinity, interactive effects of abiotic factors, ontogeny, physiology, etc. is essential for the reconstruction of the environment that affected the fish. Otolith shape is also affected by environmental conditions in addition to the genotype. The applications of otolith chemistry and shape for stock discrimination have increased in recent years because of the advancements in analytical methods and the related software. The stock identification methods sometimes provide variable results but if we use complementary approach the information generated could be more reliable which can be used to prepare effective management and conservation strategies. It appears warranted to generate more information on the factors influencing otolith chemistry and shape especially when two or more factors exert synergetic influence. Therefore, the objectives of this review paper were to provide comprehensive information on various factors influencing the otolith chemistry and shape, and the utility of otolith chemistry and shape for fish stock discrimination with an emphasis towards the research areas needing additional studies.

Keywords

element, fisheries management, otolith microchemistry, otolith shape, salinity, stock identification, temperature

Introduction

Otoliths are calcified biominerals that are mainly composed of calcium carbonate polymorph aragonite and a minor quantity of biomolecules such as proteoglycans and glycoproteins (Campana 1999; Sollner et al. 2003). The process of otolith biomineralization is influenced by several genes and the fish may also use collagens to stimulate biogenesis (Murayama et al. 2002; Sollner et al. 2003). Moreover, it is reported that otolin-1 (meshwork-forming collagen) may contribute to forming biominerals composed of calcium carbonate (Murayama et al. 2002). The mineralized calcium carbonate is precipitated from the endolymph fluid which contains calcium and bicarbonate ions and it is reported that there is a daily pause in deposition caused by changes in endolymph pH cycles (Wright et al. 1992; Campana and Thorrold 2001). Thus, the chemical composition of endolymph surrounding otolith is an important factor for otolith growth.

Otolith chemistry reflects the permanent record of physical and chemical qualities of the ambient environment (Campana 1999). The whole dissolved otolith composition reveals the average of the lifetime exposure to both the ambient environment and its physiological processes thus delineating based on differences among the groups of fishes exposed to different environments (Campana et al. 2000). The variations in the elemental composition of whole otoliths cannot be used to infer the time period when the groups of fishes remained separated because the arbitrary inhabitancy in different environmental conditions can lead to an evident change in the otolith elemental composition (Campana 2005). The application of beam-based instruments such as laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) has revolutionized the understanding of temporal patterns in otolith composition (Di Franco et al. 2014).

Several studies have reported that the otolith chemistry is influenced by environmental factors (e.g., water chemistry, temperature, salinity, and their interactive effects) and physiological processes (e.g., growth, metabolism, and reproductive stage) (Elsdon and Gillanders 2003; Gaetani and Cohen 2006; Walther et al. 2010; Sturrock et al. 2014; Stanley et al. 2015; Mazloumi et al. 2017; Walsh and Gillanders 2018). Therefore, the knowledge of how exogenous and endogenous factors affect otolith chemistry is essential to reconstruct the life history events and the migration pattern of fish species (Elsdon et al. 2008; Reis-Santos et al. 2013). The predominant source of the elements incorporated into the otoliths is the ambient water but for some elements such as Zn the main source is diet thereby the relative contribution may vary for different elements (Walther and Thorrold 2006; Doubleday et al. 2013). The ambient water chemistry is regulated by several factors such as underlying geology, anthropogenic influences, precipitation, agricultural runoff, mixing of different water bodies, etc. (Elsdon et al. 2008). Therefore, the chemical composition may vary between water bodies or even within a water body at temporal and spatial scales (Kerr et al. 2007; Elsdon et al. 2008). Moreover, otoliths permanently record spatial and temporal variability in water chemistry (Campana et al. 2000; Dorval et al. 2005; Miller 2007; Mateo et al. 2010).

The shape of otoliths is species-specific and shows less variation in growth comparative to somatic growth (Campana and Casselman 1993; Lombarte and Lleonart 1993). Otolith shape variation of fish from different geographic areas is supposed to provide evidence that the fish species occupied distinct regions during life history, thus demonstrating a phenotypic method of stock identification (Ihssen et al. 1981). Otolith shape is a more reliable tool because it is comparatively more stable than external morphometric characters to the short-term variations caused by changes in fish condition (feeding or spawning conditions) or environmental variations (Vieira et al. 2014; Mahe et al. 2018). Several studies have reported that the variations in otolith shape may be correlated with differences in growth rate (Campana and Casselman 1993), as noted in Atlantic mackerel, *Scomber scombrus* Linnaeus, 1758 (see Castonguay et al. 1991); king mackerel, *Scomberomorus cavalla* (Cuvier, 1829) (see DeVries et al. 2002); and blue whiting, *Micromesistius poutassou* (Risso, 1827) (see Keating et al. 2014). Vignon and Morat (2010) reported that the left and right otoliths are influenced symmetrically by the environment and genetics. This may have key implications because any unsystematic deviations from perfect symmetry of otoliths may be used to study the developmental stress and hence could be a potential sign of fitness (Lemberget and McCormick 2009; Palmer et al. 2010).

Several studies have used otolith chemistry (Edmonds et al. 1991; Campana et al. 1994; Campana 2005; Volpedo and Cirelli 2006; Pangle et al. 2010; Khan et al. 2012; Khemiri et al. 2014; Miyan et al. 2014; Miyan et al. 2016; Avigliano et al. 2017; Moreira et al. 2018; Wright et al. 2018; Nazir and Khan 2019) or shape of otoliths (Campana and Casselman 1993; Begg and Brown 2000; DeVries et al. 2002; Pothin et al. 2006; Stransky et al. 2008; Aguera and Brophy 2011; Keating et al. 2014; Sadighzadeh et al. 2014; Vieira et al. 2014; Bacha et al. 2016; Ider et al. 2017; Khemiri et al. 2018) as a tool for stock discrimination. However, the understanding of the ecological and evolutionary processes that sustain the fish population structure requires a multidisciplinary approach (Abaunza et al. 2008; Taillebois et al. 2017). Therefore, the environmental markers (e.g., otolith microchemistry) and genetic markers may provide important information to assess stock structure especially if they show high agreement when used in union (Welch et al. 2015; Tanner et al. 2016). Several researchers have concurrently used otolith chemistry and shape to indirectly validate the percentage of correct classification of individuals to their original location(s) (Turan 2006; Longmore et al. 2010; Ferguson et al. 2011; Soeth et al. 2019). Further, it is preferable to conduct similar investigations to generate conclusive information on population structure and to assess the generality of the results (Longmore et al. 2010).

Several review papers based on otolith chemistry have been published both at the global and regional level (Campana 1999; Campana and Thorrold 2001; Elsdon and Gillanders 2003; Elsdon et al. 2008; Sturrock et al. 2012; Pracheil et al. 2014; Avigliano and Volpedo 2016; Tanner et al. 2016; Walther et al. 2017; etc.). However, more information is warranted on all exogenous and endogenous factors influencing otolith chemistry and shape. Therefore, the objectives of this review paper were to provide comprehensive information on various factors influencing the otolith chemistry and shape, and the utility of otolith chemistry and shape for fish stock discrimination with an emphasis towards the research areas needing additional studies.

Factors affecting otolith chemistry

Otolith chemistry reflects the habitat conditions at different life-history stages of a fish species (Elsdon and Gillanders 2003). The geographic variations in the ambient environmental conditions may result in regional patterns in otolith chemistry (Chang and Geffen 2013) and significant differences in otolith chemistry at spatial scales were reported (Kennedy et al. 2005; Miyan et al. 2016; Wright et al. 2018; Nazir and Khan 2019). The taxonomic relations or ecological resemblances between fish species may result in phylogenetic patterns in otolith chemistry

(Chang and Geffen 2013), thereby limiting the implementation of a single common model (Martin and Wuenschel 2006). The physiological regulation has a greater impact on otolith chemistry particularly when the source of elements is other than water (Walther et al. 2017). Therefore, a complete understanding of exogenous and endogenous factors and their relative effects on the accretion of elements in otolith is needed (Izzo et al. 2018).

Effect of temperature

Temperature variations at both spatial and temporal scales influence otolith chemistry because it affects precipitation reactions of trace elements. Morse et al. (2007) reported that the solubility of all pure carbonates decreases with increasing temperature. Temperature affects the crystal precipitation process because it influences the pH of the blood plasma and endolymph fluid (Romanek and Gauldie 1996; Elsdon and Gillanders 2003). The complete information of the life history events and the migration pattern of the fish species may be possible by understanding the relation between temperature and otolith chemistry (Mazloumi et al. 2017). Previously, several studies through controlled experiments have reported the effects of temperature on otolith chemistry (Miller 2009; DiMaria et al. 2010; Reis-Santos et al. 2013). Moreover, a number of studies have reported the effects of temperature on otolith chemical composition, which has produced both positive and negative effects of temperature on otolith chemistry (DiMaria et al. 2010; Barnes and Gillanders 2013; Reis-Santos et al. 2013; Stanley et al. 2015; Walsh and Gillanders 2018). The variations in the results may be due to the temperature ranges used in the experiments which were generally narrower than the fish typically experience in nature (Elsdon and Gillanders 2003; Reis-Santos et al. 2013). Recently, Izzo et al. (2018) reported that the experiments undertaken at low temperature (<5°C) or short duration experiments (<20 days) produced varying results, thus emphasizing that the experimental conditions may have an influential effect on the final conclusion of the study. Therefore, it is suggested to undertake future experiments at different life stages and by keeping in view the temperature conditions experienced by the fish species under natural conditions (Sturrock et al. 2012; Sturrock et al. 2015; Izzo et al. 2018).

Several studies have reported the effects of temperature on otolith chemistry, but a full understanding of the relation is lacking because it is complicated by other factors that affect otolith chemistry (such as salinity, diet, species-specific physiology, etc.) and many empirical studies do not replicate the range of conditions that the species of interest experience naturally (Sturrock et al. 2012; Barnes and Gillanders 2013; Walsh and Gillanders 2018). Therefore, it is required that more studies should be undertaken to validate and evaluate the temporal environmental changes which influence otolith chemistry (Miller 2011) and to examine the mechanism through which temperature affects otolith chemistry across a full reproductive cycle (Sturrock et al. 2012; Reis-Santos et al. 2013).

During the assessment of the literature, we found some specific study designs that were particularly good to understand the factors influencing the otolith chemistry, for example, Sturrock et al. (2015) carried out a 1-year controlled experiment across immature and mature reproductive stages of European plaice, *Pleuronectes platessa* (Linnaeus, 1758), at nearly natural conditions to assess the influence of environmental and physiological variables to establish a basis for modeling the uptake and transport pathways of elements to the otoliths. In another controlled laboratory experiment on the fingerlings of mulloway, *Argyrosomus japonicus* (Temminck et Schlegel, 1843), the temperature and salinity ranges were set similar to the natural conditions to study the influence of environment and genetics on the chemical composition of otoliths with a caution that a range of environmental conditions to which the fish may be exposed should be considered (Barnes and Gillanders 2013). The elements such as Sr, Ba, and Mn are the most frequently used elemental markers in otolith chemistry studies based on a positive correlation between incorporation rates, ambient concentrations, and/or temperature (Reis-Santos et al. 2013; Sturrock et al. 2015).

Effect of salinity

Salinity changes occur due to precipitation and evaporation processes within water bodies, leading to a stratification of the water column which often delimits boundaries between different water bodies (Tomczak and Godfrey 1994). Mucci (1983) investigated the effect of salinity on the stoichiometric solubility products and reported that the solubility products of aragonite tend to increase with increasing salinity. It is also described that the aragonite precipitation rates tended to decrease (about five times) with increasing salinity, although this decrease was only observed for salinity ≥ 35‰ (Zhong and Mucci 1989). The uptake of elements from blood-endolymph-otolith is affected by salinity (McCormick 2001). In the case of highly migratory fish species (diadromous species), which moves across different salinity environments, the gill membrane osmoregulates the movement of ions (Miller 2011; Sturrock et al. 2012). Thus, the understanding of the effects of salinity on otolith chemistry is very essential to study the migration pattern of fish species. The influence of salinity on elements such as Sr and Ba has been frequently studied in deciphering the migration between habitats exhibiting strong gradients of salinity (Milton and Chenery 2005; Reis-Santos et al. 2013).

Several studies have reported strong positive and negative effects of salinity on otolith chemistry (Kraus and Secor 2004; Dorval et al. 2007; Sturrock et al. 2012; Reis-Santos et al. 2013; Panfili et al. 2015; Mazloumi et al. 2017; Walsh and Gillanders 2018). A number of studies also reported no significant effect of salinity on Sr, Ba, Mg, and Mn concentrations in the otoliths (Elsdon and Gillanders 2002; Elsdon and Gillanders 2005; Martin and Wuenschel 2006; Gillanders and Munro 2012). This disagreement of results suggests that other factors may interact with salinity to affect the elemental incorporation and also, the salinity does not influence all elements equally and concurrently; consequently, the species may accrete elements within their otoliths in different ways (Elsdon and Gillanders 2003). Several reasons may explain this disagreement or complexity of the results. The elements such as Ba, Sr, Mn, Mg, and Li are mainly found as hydrated free ions both in seawater and blood, and these ions are relatively constant in seawater and mostly vary with salinity changes (Sturrock et al. 2012). Moreover, the physiological processes, the kinetic growth effect, and the synthesis of protein throughout somatic growth are the pivotal factors that affect the ion uptake into otoliths (Sinclair 2005; Trudel et al. 2010; Sturrock et al. 2012). The Dynamic Energy Budget Models (e.g., biokinetic and bioenergetic models) can be used to further investigate a suite of environmental and/or biological factors to evaluate the rate of uptake of individual elements (Fablet et al. 2011; Izzo et al. 2018). Thus, additional validation experiments are needed to understand the influence of salinity and other abiotic and biotic factors influencing the incorporation rate of individual elements into otoliths (Reis-Santos et al. 2013).

Interactive effects of temperature and salinity

The water chemistry, temperature, and salinity are the three key environmental factors influencing the otolith chemistry and these variables are usually not independent of each other (Elsdon and Gillanders 2003). The temperature and salinity have a significant influence on the otolith chemistry especially in estuaries where more than one factor can vary (Elsdon et al. 2008). Several studies have reported that the Ba:Ca ratio usually shows positive and negative relation with temperature and salinity, respectively (Elsdon and Gillanders 2005; Dorval et al. 2007; Marohn et al. 2011; Reis-Santos et al. 2013; Stanley et al. 2015; Mazloumi et al. 2017; Nelson and Powers 2019). The Sr:Ca ratio generally shows positive relation with both temperature and salinity (Bath et al. 2000; Zimmerman 2005; Martin and Wuenschel 2006; Brown and Severin 2009; Miller 2009; Reis-Santos et al. 2013; Mazloumi et al. 2017; Nelson and Powers 2019). Further, the Mn:Ca and Mg:Ca usually show no relation with temperature and salinity (Elsdon and Gillanders 2002; Martin and Thorrold 2005; Tanner et al. 2011; Gillanders and Munro 2012; Mazloumi et al. 2017). Therefore, the interactive influence of temperature and salinity should be considered for accurate reconstruction of the environmental history of fish species (Elsdon et al. 2008; Miller 2011).

Laboratory-based validation experiments have been performed to analyse the accretion of elements on the otoliths for a number of species, but a limited number of studies have examined the interactive effect of temperature and salinity (Walsh and Gillanders 2018). Therefore, the interpretation of environmental histories of fishes based on single environmental factor may provide imprecise information as the elements are possibly confounded by the effect of other variables thereby highlighting the importance of studying interactive effects of environmental factors (Martin and Wuenschel 2006). Moreover, the interactive effects of environmental variables may also represent species-specific responses to these variables (Tzeng 1996; Chesney et al. 1998). Evaluating the interactive effect of temperature and salinity on otolith chemistry by using a multi-element approach can enhance our interpretation of migration patterns and stock assessment (Elsdon and Gillanders 2003).

Effect of crystal structure

Several studies have reported that the otoliths vary considerably in the crystal structure and the differences in elemental composition were found among $CaCO_3$ polymorphs (Melancon et al. 2005; Tzeng et al. 2007; Ma et al. 2008; Veinott et al. 2009). All the elements which are incorporated from the water into the aragonite and vaterite do not follow the same pattern (Pracheil et al. 2017). Typically, the vaterite contains lower elemental concentrations than aragonite (Pracheil et al. 2019). The elements such as Sr and Ba have lower elemental concentrations in vaterite portions than aragonite portions, for example, the otoliths of European eel, *Anguilla anguilla* (Linnaeus, 1758) (see Tzeng et al. 2007). Further, the elements such as Mg and Mn have increased concentrations in vaterite compared to aragonite sections, for example, the otoliths of lake trout, *Salvelinus namaycush* (Walbaum, 1792) (see Melancon et al. 2005), brown trout, *Salmo trutta* (Linnaeus, 1758), and brook charr, *Salvelinus fontinalis* (Mitchill, 1814) (see Morat et al. 2008). Moreover, it is reported that aragonite otolith chemistry reflects water chemistry whereas vaterite otolith chemistry does not reflect ambient water chemistry thereby vaterite otoliths may be of limited use in stock identification and reconstructing the environmental history of fishes (Bath et al. 2000; Gillanders and Kingsford 2000; Pracheil et al. 2017). Usually, otoliths have aragonite or vaterite crystal structure, however, Campana (1983) first documented the coprecipitation of aragonite and vaterite in the same otolith but at different growth zones and later it was reported that the environmental stress and/or changes within soluble proteins of the endolymph are mainly responsible for this to occur especially in hatchery-reared fishes (Sweeting et al. 2004; Tomas et al. 2004; Ma et al. 2008; Morat et al. 2008). Gauldie (1986) reported that the water temperature was a prime cause in producing a shift between aragonite and vaterite in otoliths of chinook salmon, *Oncorhynchus tshawytscha* (Walbaum, 1792). Aragonite and vaterite otoliths differ in their densities and lattice structure; vaterite is less dense than aragonite (Tomas and Geffen 2003; Chakoumakos et al. 2016; Neves et al. 2017), resulting in otolith mass asymmetry (Vignon and Aymes 2020). The vaterite precipitation has a negative impact on auditory sensitivity in fishes (Reimer et al. 2016). Moreover, the functional, behavioural, and ecological implications of vaterite deposition at the organismal level are usually untested experimentally (Vignon and Aymes 2020). The calcium and other trace element concentrations may vary from one polymorph to another thereby the data should be normalised accordingly otherwise it will lead to inaccurate results (Pracheil et al. 2017). To increase the accuracy of the otolith chemistry technique, there is a need to understand the link between calcium polymorphs and incorporation rate of elements, and the occurrence of several calcium polymorphs across the hatchery-reared and wild fish species (Pracheil et al. 2019).

Effects of growth, diet, and ontogeny

The growth rate of a fish species is known to influence the elemental incorporation in otolith (Martin and Thorrold 2005; Sturrock et al. 2012). Further, it is reported that the individuals which live in the same water body may have different otolith compositions if they exhibit different growth rates (DiMaria et al. 2010). However, several studies have reported that the chemical composition of otoliths usually shows a negative or no relation with growth (Bath et al. 2000; Martin et al. 2004; Martin and Thorrold 2005; Lin et al. 2007; Miller 2009). The variations in growth rate among the groups of fish should be determined if whole otoliths are analysed (Elsdon et al. 2008). Thus, it is necessary that the fish of known or same sex, size, and age should be collected among areas for analysis (Wells et al. 2003; Sturrock et al. 2012). In general, two hypotheses have been put forward, such as the kinetic and the physiological hypothesis to explain the influence of growth rate differences on the accretion of elements in otoliths (Walther et al. 2010). The kinetic hypothesis describes the calcification rate such as precipitation and substitution in the otolith (Sinclair 2005). Also, the kinetic hypothesis suggests that the pH influences the level of calcium concentration in the endolymph and it also changes the relative abundance of bivalent ions in the calcifying fluid especially when the incorporation rate is fast (Sinclair 2005; Sinclair and Risk 2006). Kinetic hypothesis needs to be completely resolved experimentally, because of the incongruities among the kinetic hypothesis models of calcification (Walther et al. 2010). A fish species physiologically change the relative concentration of ions during transport across interfaces from the ambient environment to crystal lattice (Campana 1999; Walther et al. 2010). However, the majority of the studies focus on the applicability of otolith chemistry instead of mechanisms of incorporation of elements in otoliths (Hussy et al. 2020). The physiological processes (e.g., growth) are known to affect the otolith chemistry and it is known that the faster growth rate could alter the concentration of ions in endolymph because of protein synthesis especially calcium-binding proteins during somatic growth (Trudel et al. 2010; Walther et al. 2010). The growth rate is the most important factor among physiological factors that show significant relation with otolith chemistry (Stanley et al. 2015). Furthermore, the growth rate effects confound efforts to spatially discriminate fishes based on the variations in otolith chemistry; therefore, to understand the influence of growth rate on the composition of otoliths further studies should be undertaken to elucidate its effects. The elemental incorporation in otoliths is highly element-specific and varies among life history stages, species, and ecosystems (Hussy et al. 2020) therefore, it is in general difficult to separate and identify the respective roles of kinetic and physiological hypotheses.

The elemental signatures in otoliths are accreted usually from water but the effect of diet is also significant for few elements such as Sr and Ba (Woodcock et al. 2012; Doubleday et al. 2013; Woodcock and Walther 2014). Several studies reported that the diet influences Sr and Ba concentration in otoliths of freshwater, marine, and estuarine fish species by manipulating diet; though, the reported results show contradiction (Hoff and Fuiman 1995; Limburg 1995; Milton and Chenery 2001; Buckel et al. 2004; Marohn et al. 2009; Engstedt et al. 2012). Tanner et al. (2016) reported that the diet shifts with life history stages in the same habitat, or when individuals from geographically distinct populations feed on different preys, show different otolith compositions. The bioaccumulation of elements during the dietary shift towards higher trophic level makes it very difficult to evaluate the influence of diet on otolith chemistry (Gray 2002). Other than water chemistry and diet, several factors may affect otolith chemistry at varying degrees such as fish species, temperature, salinity, growth rate, ontogeny, etc. The combined effect of these variables is seldom tested and may obscure the relative contributions of water chemistry and diet (Doubleday et al. 2013).

Otolith chemistry can vary with life history stages (growth from larval to juvenile and juvenile to adult stages) and metamorphosis (Toole et al. 1993; Elsdon and Gillanders 2003). If the variations in otolith chemistry of a fish species bridge with life-history stages then the elemental profile of otoliths may be a reflection of ontogenetic effects rather than changes in the environmental conditions (Elsdon et al. 2008). Fish species such as eels show metamorphosis thereby are very susceptible to ontogenetic changes in the chemical composition of otoliths which could be misinterpreted as migration patterns (Arai et al. 2002; Correia et al. 2003). To understand how ontogeny affects otolith chemistry, validation experiments by rearing fish at constant or known environmental conditions during ontogenetic and/or physiological changes (Fowler et al. 1995; Elsdon and Gillanders 2005; Zimmerman 2005) can elucidate such effects.

Other factors affecting otolith chemistry

Stress can influence otolith chemistry but the trend and mechanism driving such results are still not clearly known (Kalish 1992; Walther et al. 2010). Mohan et al. (2014) hypothesized that hypoxic stress can affect otolith chemistry because of physiological changes in blood chemistry resulting from changes in blood proteins. Further, the authors observed without a clear mechanism that constant hypoxia exposure over few weeks does not affect otolith chemistry whereas the periodic hypoxia influences otolith chemistry in Atlantic croaker, *Micropogonias undulatus* (Linnaeus, 1766). Manganese can be used as an environmental indicator to study hypoxic stress because Mn:Ca ratio is not affected by endogenous hypoxic stress (Mohan et al. 2014; Limburg et al. 2015). Laboratory validation experiments are required to address the mechanism underlying the effect of stress on otolith chemistry.

The reproductive conditions can influence the ion transport, flux of elements into the blood plasma, and consequently the availability of elements for uptake into otolith (Sturrock et al. 2014). Laboratory experiments separating their influences from other prominent factors such as temperature, salinity, and growth could provide critical evidence for the accurate interpretation of uptake into otolith (Sturrock et al. 2014). Further work is warranted to experimentally address the effect of a full reproductive cycle on the otolith chemistry.

Factors affecting otolith shape

The exogenous and endogenous factors determine the shape of otoliths and these variables may change between populations thereby resulting in stock-specific characteristics of otoliths (Campana and Neilson 1985). However, a complete understanding of the environmental and genetic factors which determine otolith shape is lacking (Vignon and Morat 2010). Several studies have reported that both genetic and environmental influences may be responsible for otolith shape variations in a particular fish species (Cardinale et al. 2004; Burke et al. 2008; Vignon and Morat 2010). However, few empirical studies have investigated the influence of both environmental and genetic factors on otolith shape. For example, Cardinale et al. (2004) released hatchery cod, *Gadus morhua* Linnaeus, 1758, into the wild and after some years recaptured the species to validate the genetic and environmental influences on the otolith shape. Hussy et al. (2016a) reported the combined effect of environment, ontogenetic and genetic influence on otolith shape of Baltic Sea cod, *Gadus morhua*. Several studies have suggested that local environmental conditions are responsible for otolith shape variations in absence of genetic differences (Simoneau et al. 2000; Katayama and Isshiki 2007; Legua et al. 2013). The introduction of non-native fish species having distinct evolutionary history and genetics could provide a remarkable method to analyse both genetic and environmental influence on otolith shape particularly when the species were introduced in the same habitat (Vignon and Morat 2010). The environmental conditions, genetics, and their interaction may act symmetrically on both left and right otolith thereby carrying the same kind of information (Vignon and Morat 2010). Additional studies should be undertaken to elucidate the effects of other confounding factors such as sex, size, age, stock, food availability, temperature, etc. on the shape of otoliths.

Several studies have reported abnormal otoliths which have different size, shape, and density as compared to normal otoliths in a number of freshwater and marine fishes (Sweeting et al. 2004; Oxman et al. 2007; Ma et al. 2008; Reimer et al. 2016). In the case of abnormal otoliths, the aragonite is replaced by vaterite but in some species, calcite may replace aragonite (Gauldie 1993; Campana 1999; Ma et al. 2008; Reimer et al. 2017). Various factors are responsible for aberrant otoliths such as stress, genetic and neuroendocrine factors but a limited number of studies have tested the effect of these factors (Tomas et al. 2004; Ma et al. 2008; Reimer et al. 2017). The replacement of aragonite by vaterite is usually higher in fish species that are hatchery-reared but this may also occur in wild fishes (Tomas and Geffen 2003). Currently, there are high incidences of culture fishes entering into the natural water bodies; therefore, it becomes necessary to evaluate otolith abnormality and to verify its effects before analyzing otolith shape for fish stock discrimination.

Diet is also known to influence otolith shape because the composition of the diet may affect saccular endolymph proteins which play an essential role in otolith biomineralization (Mille et al. 2016). However, there is a paucity of information regarding the relation between diet and otolith shape. Oceanic acidification is known to change the carbonate structure morphologically in invertebrates and fish otoliths (Checkley et al. 2009; Bignami et al. 2013; Reveillac et al. 2015). Mirasole et al. (2017) reported the effects of ocean acidification on otolith shape and suggested that the fish species which show high site fidelity/territorial behaviour are more influenced as compared to pelagic and more mobile species. The effect of ocean acidification on otolith shape depends on exposure time, levels of CO_2, and species behaviour (Munday et al. 2011). The complete understanding of the variations in otolith shape on fish physiology and behavior needs further investigation in acidified oceans (Munday et al. 2014; Mirasole et al. 2017).

Otoliths as a tool in stock discrimination

Otolith chemistry

Traditionally, the artificial tagging of fish and telemetry are applied to study the life history events (Landsman et al. 2011). The main challenges with tagging are the high investment required (both in costs and human time) and the comparatively small resulting amount of data collected. Even if tagging is cheaper there is still the issue of diminishing returns of tagged individuals (Carlson et al. 2017). Currently, the information contained within otolith chemistry has been used to infer the stock structure and environmental history of the fish species (Clarke et al. 2007; Allen et al. 2009; Reis-Santos et al. 2018). The otolith chemistry has been effectively used to identify the natal origin or dispersal pattern of a number of marine, freshwater, and anadromous fishes (Schaffler and Winkelman 2008; Zeigler and Whitledge 2010; Turner and Limburg 2014; Bailey et al. 2015; Garcez et al. 2015). A major disadvantage of using otolith chemistry is the temporal variability in water chemistry within the natal origin of fish populations (Pangle et al. 2010). Moreover, large temporal variations may restrict the use of otolith chemistry for stock discrimination of fish populations of several age classes because it may influence the classification accuracy at spatial scales (Hamer et al. 2003; Dorval et al. 2005; Pangle et al. 2010). Therefore, it is suggested to construct an annual baseline of water chemistry and otolith chemistry signatures of cohorts of known origin to assess variability over time.

The utility of otolith chemistry for stock discrimination has risen effectively in the past two decades. The otolith chemistry and its utility in stock discrimination have appeared in nearly 1500 peer-reviewed papers from 2000 to 2019 (Web of Science™, search on 17 September 2020; search term: otolith chemistry OR otolith microchemistry OR otolith elemental composition (black bar), and otolith chemistry OR otolith microchemistry OR otolith elemental composition AND stock discrimination OR stock delineation OR stock deciphering (grey bar)). Approximately 39% of these papers were published in the past five years and the number of papers based on this parameter is increasing with time (Fig. 1). Jonsdottir et al. (2006b) used otolith elemental composition to study the stock structure of Icelandic cod, *Gadus morhua*. Moreover, the otolith chemistry (especially Ba, Li, and Sr at all locations) showed differences in the spawning area of cod in the north and south of Iceland. Similarly, the spawning cod showed different otolith chemistry in the south of Iceland at the main spawning ground below and above 125 m depth. The Icelandic cod was managed as a single stock; however, several studies have indicated more than two stock residing north and south of Iceland by using a holistic approach such as otolith shape and insights from microsatellites, Syp I locus, Pan I locus, and tag-

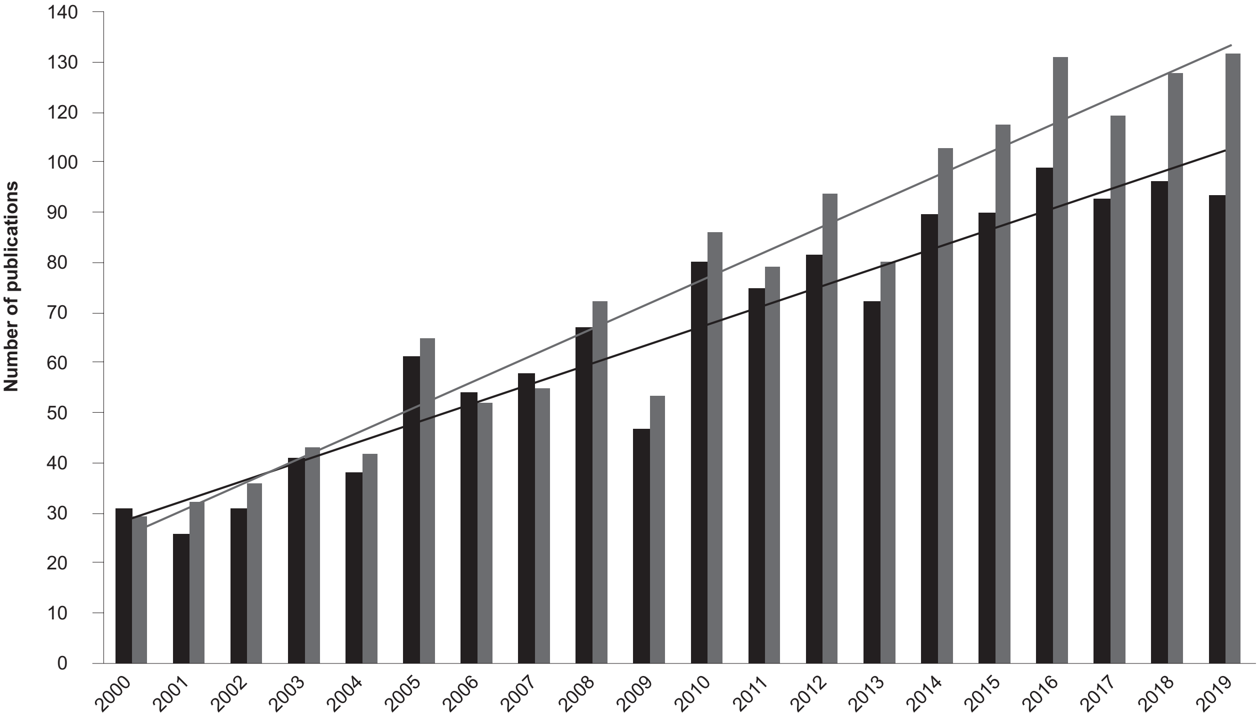

Figure 1. Number of publications per year featuring otolith chemistry (Web of Science, search on 17 September 2020, search term: otolith chemistry OR otolith microchemistry OR otolith elemental composition (black bar), and otolith chemistry OR otolith microchemistry OR otolith elemental composition AND stock discrimination OR stock delineation OR stock deciphering (grey bar)). Lines indicate the trends and results were not cross-checked.

ging experiments (Jonsdottir et al. 2002; Jonsdottir et al. 2006a, 2006b; Pampoulie et al. 2006; Petursdottir et al. 2006). The management strategies developed for a single cod stock around Iceland is not effective because the connectivity of the cod populations (northeast and southwest) and the migration to and from feeding grounds were not taken into consideration (Pampoulie et al. 2012). Dou et al. (2012) reported that the otolith chemistry of tapertail anchovy, *Coilia nasus* Temminck et Schlegel, 1846 varied considerably among five Chinese estuaries (Liaohe River estuary, the Haihe River estuary, the Yellow River estuary, the Daguhe River estuary, and the Yangtze River estuary). They observed that the Sr and Ba composition in the otolith nuclei exhibited inter-site differences and can be used as a successful natural marker for discriminating stocks with an overall classification accuracy rate of 72.7%. Although, the authors recommended that further studies on the interactions of environmental factors in the spawning sites as well as the physiological effects on the elemental uptake into the otoliths are necessary. Miyan et al. (2016) reported that the variations in Sr, Ba, Li, Cu, Fe, Pb, Zn, Mn, Ni, and Mg were important to isolate the stocks of the giant river-catfish, *Sperata seenghala* (Sykes, 1839), in the Gangetic River system (rivers Ganga, Yamuna, and Gomti) with a mean classification accuracy of 83.2%. Moreover, site-specific elemental variances in *S. seenghala* otoliths showed a high level of fidelity to its feeding/growing area. Furthermore, the barrages at Narora and Kanpur locations along the Ganga River could have restricted the movement which consequently could have led to the stock separation in the Ganga River. The microsatellite markers showed different populations of the giant river catfish among the rivers Ganga, Brahmaputra, Godavari, Mahanadi, and Narmada, and therefore separate management plans should be formulated for these populations (Acharya et al. 2019). Wright et al. (2018) reported that in the North Sea, the lesser sandeel, *Ammodytes marinus* (Raitt, 1934), shows significant spatial differences in otolith chemistry although overall classification accuracy was low (48.8%). Further, the elements such as Mn and Rb were important in stock discrimination of *A. marinus* and likely reflect the physico-chemical environment. The authors also reported that the *A. marinus* is currently managed as seven stocks discriminated based on biophysical model predictions of the restricted larval mixing among the stocks. It is reported that the lesser sandeel shows weak genetic differentiation although strong functional genomic signal in the North Sea, however, the use of several genetic markers can increase the power to characterize the genetic population structure (Jimenez-Mena et al. 2020). Nazir and Khan (2019) reported site-specific four stocks of long-whiskered catfish, *Sperata aor* (Hamilton, 1822), from the Ganga River; furthermore, the otolith chemistry showed comparatively low temporal variations as compared to spatial variations thereby the classification success (overall correct classification was 83.5%) remained constant over the three selected sampling years (2013, 2014, and 2015). The elements such as Ba, Sr, K, and Mg were used to correctly classify the individuals to their original location of *S. aor* from the Ganga River. The otolith chemistry, truss morphometry of the fish body, and microsatellite markers showed non-significant variation in percentage classification accuracy of *S. aor* stocks from the Ganga River (Nazir 2018). However, these methods showed three to four stocks among the selected locations across the Ganga River (cf. Nazir and Khan 2017; Khan and Nazir 2019; Nazir and Khan 2019).

The majority of the stock discrimination studies using otolith chemistry have not considered the effects of multiple environmental and biological factors that govern the incorporation of elements in otoliths. Therefore, future studies at spatial and temporal scales to disentangle the relative influence of these factors should be undertaken to strengthen our understanding of otolith chemistry and its field applications. Moreover, studies based on otolith chemistry (about 83%) have not described the applications of otolith chemistry for fisheries management (Carlson et al. 2017) because of several limitations mostly in adult fishes such as brief residence time (spawning migration) and slow growth in older fish (Pracheil et al. 2014). Carlson et al. (2017) suggested that the information gap can be filled through descriptive case studies (e.g., identifying natal origins and stock assignment, larval dispersal and population connectivity, stock enhancement, etc.) that elucidate management applications of otolith chemistry both in freshwater and marine ecosystems. Pracheil et al. (2014) suggested that the information produced by otolith chemistry can be used in fisheries management when integrated with other methods (genetics, telemetry, and/or tagging). The otolith chemistry provides information on fish stock structure, life-history, and habitat use which are very useful for fisheries management and conservation thereby fisheries managers can use this method to develop science-based management plans (Pracheil et al. 2014; Tanner et al. 2016; Carlson et al. 2017).

Otolith shape

Otolith shape variation has become widely used for stock discrimination (Burke et al. 2008; Aguera and Brophy 2011; Ider et al. 2017; Moreira et al. 2019) and has advanced from simple distance measurements to geometric morphometry with the improvements in image analyzing tools (Cadrin and Friedland 1999; Stransky 2014). The otolith shape and its utility in stock discrimination have appeared in almost 801 peer-reviewed papers from 2000 to 2019 (Web of Science^{TM}, search on 17 September 2020; search term: otolith shape OR otolith morphometry (black bar), and otolith shape OR otolith morphometry AND stock discrimination OR stock delineation OR stock deciphering (grey bar)) (Fig. 2). Otolith morphometry is preferred over the traditional method of morphometric and meristic characters of the fish body because otoliths are usually not affected by the short-term changes in fish

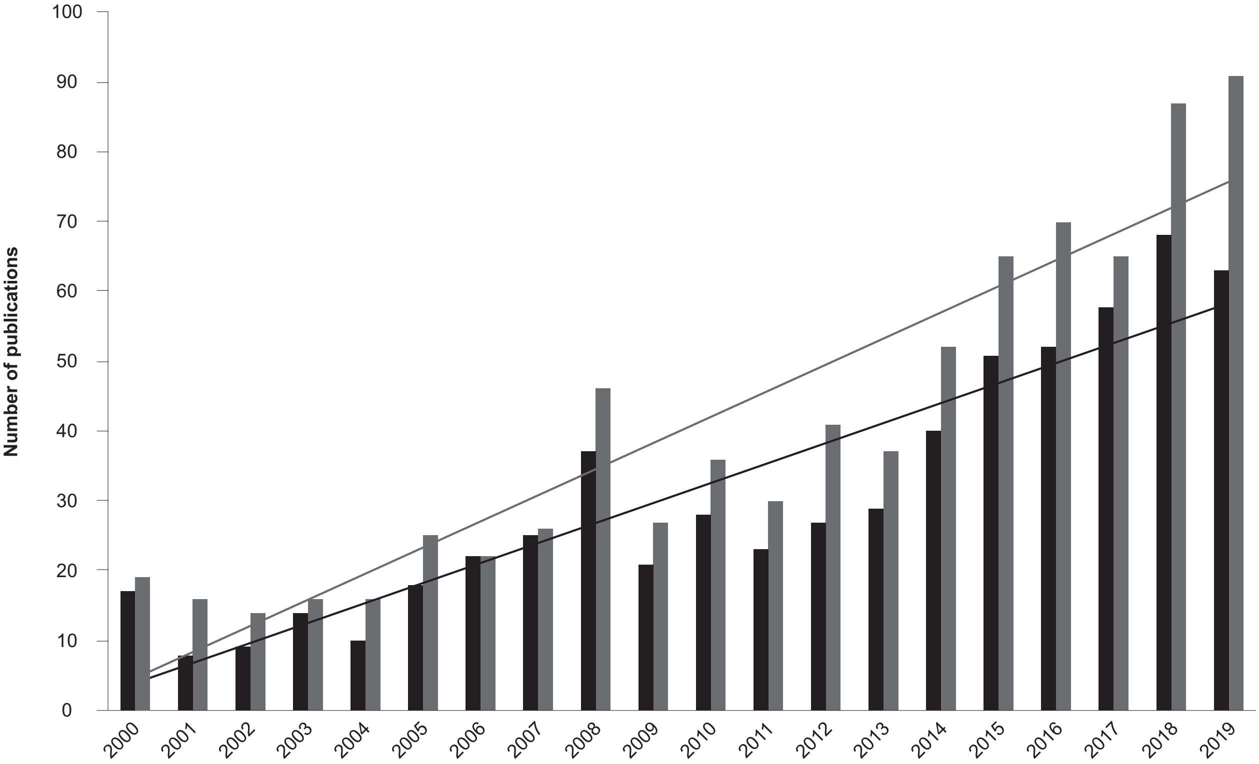

Figure 2. Number of publications per year featuring otolith shape (Web of Science, search on 17 September 2020, search term: otolith shape OR otolith morphometry (black bar), and otolith shape OR otolith morphometry AND stock discrimination OR stock delineation OR stock deciphering (grey bar)). Lines indicate the trends and results were not cross-checked.

condition or preservation of samples (Campana and Casselman 1993). Geometric outline and landmark methods are commonly used to study otolith shape variations between or among fish groups by removing size-dependent variation using an allometric approach (Stransky 2014). Otolith shape variation analysis by geometric outline method involves capturing otolith outline and deriving Cartesian coordinates (x, y) using image analysis software such as ImageJ (http://rsbweb.nih.gov/ij/) and tpsDIG (http://life.bio.sunysb.edu/morph/) (Stransky 2014). Several methods are being used for fitting outlines; however, the Fourier analysis (FA) is most commonly employed (Stransky 2014) but has limited applicability for otoliths which show significant and complex curvatures. The issues with Fourier analysis can be alleviated by using Elliptical Fourier analysis (EFA) which decomposes the complex curves of otoliths by generating the sum of harmonically related ellipses (Crampton 1995; Tracey et al. 2006; Stransky 2014). The Wavelet transform can be used as a substitute to the usually applied Fourier transform. Moreover, the Wavelet transform can resolve the problem of poor estimation of sharp edges of otoliths associated with Fourier transform (Libungan and Palsson 2015). The software products for FA of 2D outlines are HANGLE, HMATCH, and HCURVE (Crampton and Haines 1996) while for EFA, EFAwin (Isaev and Denisova 1995), SHAPE package (Iwata and Ukai 2002), and ShapeR and Momocs packages in the R environment (Libungan and Palsson 2015; Brophy et al. 2016; Denechaud et al. 2020) are commonly used. The ShapeR package is more common as it has been specifically designed for otolith shape study while Momocs on the other hand is used for general image and shape analysis.

Otolith shape analysis has been used for stock discrimination in a number of fish species. For example, Aguera and Brophy (2011) reported that the Atlantic saury, *Scomberesox saurus saurus* (Walbaum, 1792), larvae exposed to different ambient conditions in the Mediterranean Sea and the North-eastern Atlantic and irrespective of where they have spawned show distinct stocks with a cross-validated correct classification of 86%. Moreover, the Mediterranean and Atlantic show considerable difference in environmental factors, such as temperature, salinity, and food availability (Patarnello et al. 2007) which could have affected the growth rates of the fish that exist there and may be responsible for significant changes in fish condition between saury from these two regions. The stock structure of Atlantic saury is still unclear because of limited studies and therefore, this species should be managed like other short-lived forage species (Aguera and Brophy 2012). Paul et al. (2013) described that the two stocks of cod, *Gadus morhua*, in the Baltic Sea show significantly different otolith shape and more than 90% of the individuals were correctly classified to one of the two stocks. These findings highlight that the otolith shape analysis can be effectively used to discriminate adults of Baltic cod stocks. Similarly, two genetic stocks of Baltic cod with a classification accuracy of

92% were reported but these two stocks show considerable mixing, thereby future studies should evaluate the mixing dynamics of populations to fully understand the ecology of the species (Weist et al. 2019). Keating et al. (2014) stated that the blue whiting, *Micromesistius poutassou* (Risso, 1827), population in the North-eastern Atlantic was classified into two morphotypes (99% correct classification success) with a strong latitudinal influence despite the complex stock structuring at the spawning grounds. Furthermore, consistent with earlier studies of stock differentiation in blue whiting, the results further suggested the blue whiting to be considered as separate stocks because of separate feeding and breeding grounds with varying degree of mixing in the common spawning grounds. Previous studies have reported some degree of genetic differentiation among blue whiting spawning groups, however, these differences reflect only a snapshot in time and there are enough chances of intermixing of populations at the breeding grounds (Mork and Giaever 1995; Was et al. 2006). Vasconcelos et al. (2018) studied the population structure of blue jack mackerel, *Trachurus picturatus* (Bowdich, 1825), in the North-eastern Atlantic (Peniche, Madeira, and Canary islands) using otolith shape and they found three populations with an overall 73.3% correct classification. Further, the authors reported that the remaining percentage represents misclassification which may be due to the migration driven by feeding and spawning requirements. The use of parasites as a tag revealed the presence of three stocks (Portuguese mainland, Madeira archipelago, and Canary archipelago) of blue jack mackerel in the North-eastern Atlantic (Vasconcelos et al. 2017). In another study on the blue jack mackerel, four stocks namely Portugal mainland, Azores, Madeira, and the Canaries were identified from North-eastern Atlantic with an overall classification success of 81% using otolith microchemistry (Moreira et al. 2018).

During the review of literature, the interest in population discrimination was much lower in freshwater ecosystems where exploitation is less in scale and populations are often well spatially-separated compared to marine ecosystems. In general, the otolith shape method itself is not comparably less used but there are simply fewer analyses of population discrimination in freshwater ecosystems. The utility of otolith shape for stock discrimination has shown an increasing trend because it can be used as a complementary technique that certainly can improve our understanding of the stock structure (Begg and Waldman 1999; Cadrin 2000; Begg et al. 2005; Campana 2005). Further, it is a less expensive tool as compared to genetic markers or otolith chemistry.

Otolith chemistry and otolith shape: a complementary approach

In the past two decades, the number of publications per year based on otolith chemistry and otolith shape is showing an increasing trend (Web of Science™, search on 17 September 2020, search term: otolith chemistry OR otolith microchemistry OR otolith elemental composition (black bar), otolith shape OR otolith morphometry (grey bar), and otolith chemistry OR otolith microchemistry OR otolith elemental composition AND otolith shape OR otolith morphometry (light grey bar)) (Fig. 3). Several studies

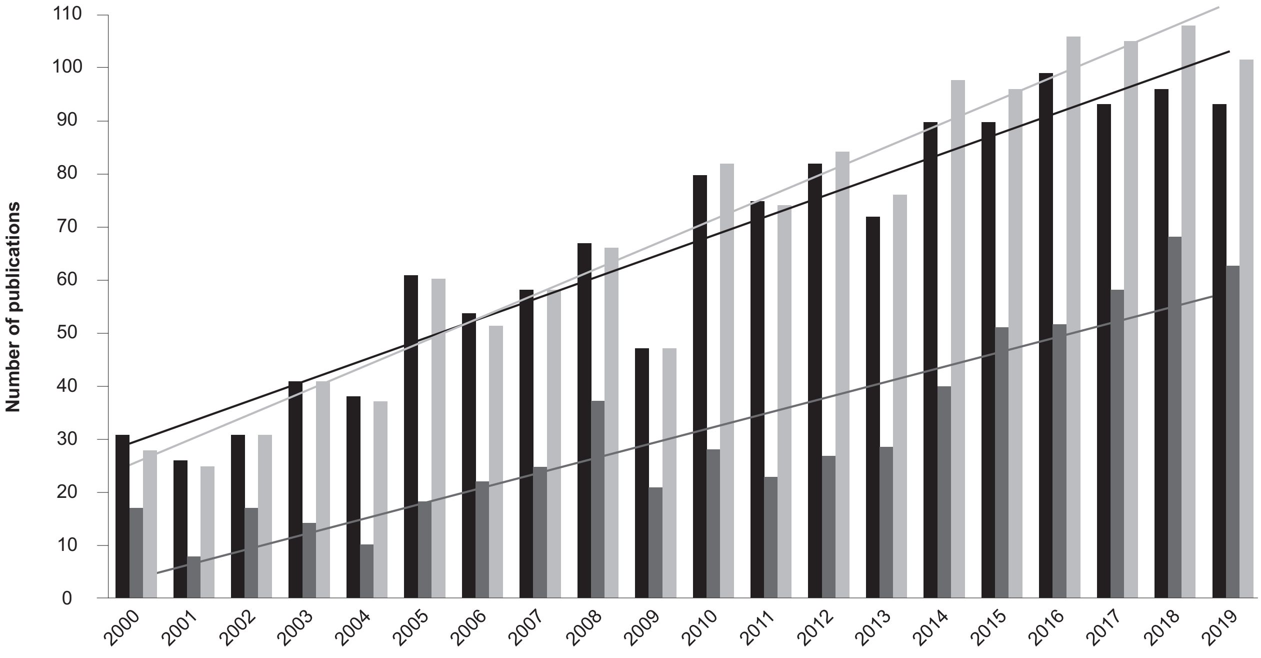

Figure 3. Number of publications per year featuring otolith chemistry and otolith shape (Web of Science, search on 17 September 2020, search term: otolith chemistry OR otolith microchemistry OR otolith elemental composition (black bar), otolith shape OR otolith morphometry (grey bar), and otolith chemistry OR otolith microchemistry OR otolith elemental composition AND otolith shape OR otolith morphometry (light grey bar)). Lines indicate the trends and results were not cross-checked.

have reported that the otolith chemistry and shape provide concordance in results but some workers have argued that otolith chemistry is more efficient in discriminating fish stocks (Turan 2006; Longmore et al. 2010; Ferguson et al. 2011). Turan (2006) demonstrated that the otolith shape and chemistry can be used as complementary techniques and both these methods showed two stocks (the central Black Sea and Aegean Sea stocks) of the Mediterranean horse mackerel, *Trachurus mediterraneus* (Steindachner, 1868). However, no stock separation was found in a previous study based on morphometric and meristic data (Turan 2004). Turan (2006) also found no significant correlation between geographical distances with the Euclidean distances for both the selected methods thereby showing that the geographic distance does not restrict the movement of *T. mediterraneus* populations among selected areas. Moreover, the otolith shape revealed 79% while otolith chemistry showed 83% correct classification thereby the individuals of this species may have spent a significant part of their lives in different environmental conditions. Longmore et al. (2010) examined spatial variations in a deep-sea teleost, *Coryphaenoides rupestris* Gunnerus, 1765, collected from different areas in the North Atlantic using otolith chemistry and shape. Overall, otolith chemistry (92% classification success) was comparatively more successful in identifying individual fish from different areas as compared to otolith shape (43% classification success). The high percentage of correct classification obtained using otolith chemistry showed that the individual fish inhabiting different deep sea areas can be accurately delineated to their original stock. The otolith shape showed low classification accuracy (<50%) and similar results were found in another deep sea fish, the beaked redfish, *Sebastes mentella* Travin, 1951 (see Stransky 2005). The strong variations in classification accuracy between otolith chemistry and shape in deep sea fishes may be because of slow growth and deep sea do not show strong variations in environmental conditions (Longmore et al. 2010). The authors also recommended further studies to evaluate the extent of otolith chemistry reflect actual stock structure by investigating chemical signatures across different life history events and to compare the results with genetic data. In another study, a similar approach was used to discriminate stocks of mulloway, *Argyrosomus japonicus*, collected from western, central, and eastern coasts of South Australia (Ferguson et al. 2011). The stock discrimination methods provided complementary results although allocation success was lower for otolith shape and morphometric indices (83%) compared to the elemental composition of the otolith edge (94%). Although, the otolith shape and morphometric indices showed that the regional differences were temporally stable but the authors recommended the comparison of otolith shape between sexes, size-classes, and multiple spatial and temporal scales to understand the potential utility of this method in stock discrimination. Barnes et al. (2016) investigated the genetic stock structure of mulloway, *A. japonicus*, within Australian waters and between Australia and South Africa, and reported strong genetic variations between Australia and South Africa using microsatellite markers. Izzo et al. (2017) studied age-related and temporal patterns of the stock structure of sardine, *Sardinops sagax* (Jenyns, 1842), collected from South Australia and the east coast through combined analysis of otolith chemistry and shape using archived otoliths. The integrated analysis of otolith chemistry and shape revealed the presence of separate stocks of young sardine (1–3 years of age) in northern and southern New South Wales. However, the occurrence of age-related and temporal patterns of stock sub-structuring of sardine in Australian waters complicates the separation of discrete stocks (Izzo et al. 2017). Soeth et al. (2019) studied the stock structure of Atlantic spadefish, *Chaetodipterus faber* (Broussonet, 1782), from the South-western Atlantic Ocean and found spatially structured semi-discrete groups between 23°S and 27°S with the possibility of intermixing. The authors further suggested that the local populations of *C. faber* should be considered as different stocks even with the possibility of an intermingling of populations and without knowing the recruitment sources in the Brazilian South-western Atlantic coast. Further, the overall classification success was 59% and 76% as shown by otolith shape and chemistry, respectively. However, when the authors combined the data of both the techniques the overall classification success increased to 83%. Therefore, the concurrent use of otolith chemistry and shape is desirable to maximize the likelihood of correct classification of individuals to their original populations. Machado et al. (2017) reported tropical and subtropical clades of *C. faber* based on mitochondrial DNA and haplotype distribution showed peripheral isolation throughout Southwestern Atlantic between 2°S and 27°S.

The choice of stock discrimination methods is important because each method has its particular associated challenges such as ecological interpretation, spatio-temporal variations, discriminatory power, and related expenses (Tanner et al. 2016). Therefore, the comparison of methods is recommended because sometimes a single method may fail to identify stock separation. During the review of different studies, we found that a single method may not show stock discrimination because of several reasons such as the fish population is homogeneous especially in the marine environment, the marker may fail to detect changes at spatial and temporal scales, and the discrimination power of the method is very low. Therefore, two or more methods should be used complementarily to study the stock structure, and connectivity of fish populations at common breeding and feeding grounds (Randon et al. 2020). The concurrent use of several stock discrimination techniques operating over broader spatial and temporal scales may provide sufficient data to understand both evolutionary and ecological processes that sustain the fish stock structure (Abaunza et al. 2008; Taillebois et al. 2017). The holistic approaches combining otolith chemistry with different and potentially complementary tools may explicitly determine the stock structure and promote interconnection between management plans and biological processes (Welch et al. 2015; Tanner et al. 2016). Further, to investigate the utility of otolith chemistry and shape for stock discrimination, the genetic markers especially microsatellite markers or single nucleotide polymorphism (SNPs) should be used to complement the information of stock structure (Ferguson et al. 2011; Soeth et al. 2019). Currently, there is a need to develop new technology, statistics, and/or integrate methods to define the stocks and to include the information of population structure into fisheries management especially when there is a mismatch between population structure and management units (Kerr et al. 2017).

Otolith chemistry in association with growth increments can be used to document the environment information in which fish species live and habitat changes throughout ontogeny (Sturrock et al. 2012; Tanner et al. 2016). Further, the otolith chemistry provides complete information on fish migration which makes it unique when compared with other available tools (Secor 2010). Generally, the otolith chemistry studies have provided ample information on several processes such as natal origin, connectivity, migration pattern, life history events, spawning and nursery areas, etc. with cost-effective methodologies (Avigliano and Volpedo 2016). The seasonal pattern of trace elemental incorporation in otoliths is being used as a tool for age estimation and validation in the fish species which do not show significant contrast between growth bands (Hussy et al. 2016b; Heimbrand et al. 2020). However, more studies are warranted to validate this technique particularly if annual changes in fish growth rate do not occur. Currently, otolith chemistry has been used as an intrinsic proxy of fish metabolism to understand how a species interact with their environment to survive but it is very challenging to study in natural conditions (Chung et al. 2019; Martino et al. 2020).

Conclusion

In recent years, the information contained within otolith chemistry and shape has been commonly used for fish stock discrimination and to interlink management strategies and biological processes. However, the otolith chemistry and shape are influenced by several exogenous and endogenous factors. Several studies have reported that temperature and salinity are important environmental factors influencing otolith chemistry but these studies usually have not utilized a broad range of temperature and salinity typically the fish experience in nature. Comprehensive in situ validation experiments across a complete reproductive cycle should be undertaken to fully understand the relation between these factors and the otolith chemistry. The biokinetic and bioenergetics models can be used to evaluate a suite of environmental and biological factors influencing the uptake of elements in otoliths. The relation between calcium polymorphs and the rate of elemental uptake needs further assessment. To fully utilize otolith chemistry for stock discrimination at both spatial and temporal scales, it is recommended to build a multiyear database of otolith chemistry and water chemistry signatures. The complete understanding of local environmental conditions and genetic factors influencing otolith shape requires further investigations. The introduction of exotic fish species having distinct evolutionary histories can provide an effective method to analyse the influence of environment and genetics on otolith shape. A complementary approach is desirable because of the limitations and assumptions associated with any particular method and as far as proper fisheries management is concerned. Further, a collaboration between different stakeholders should be mediated by fisheries managers about the utility of otolith chemistry and shape to bridge the gap between research and ecosystem-based fisheries management.

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A new record of the brassy chub, *Kyphosus vaigiensis* (Actinopterygii: Perciformes: Kyphosidae), from the Mediterranean Sea

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Abstract

One individual of the brassy chub, *Kyphosus vaigiensis* (Quoy et Gaimard, 1825) (41.5 cm TL, 1.27 kg TW), was caught off Annaba, on the eastern coasts of Algeria in December 2013. This circumtropical fish is found for the first time on the south-western Mediterranean coasts. The chronology of its records in the Mediterranean supports the hypothesis of its Atlantic origin.

Keywords

alien species, first record, *Kyphosus vaigiensis*, Mediterranean, Algeria

Introduction

The taxonomy of sea chubs (Kyphosidae, *Kyphosus*) was confused for a long time (Orsi Relini 2017). The perciform family Kyphosidae currently accommodates 12 species in two genera: *Neoscorpis* Smith, 1931 and *Kyphosus* Lacepède, 1801 (see Knudsen and Clements 2013, 2016; Knudsen et al. 2019). Their morphology is uniform with only subtle variations among species (Humann 1994). The genus *Kyphosus* is represented by 11 species widely distributed in the Atlantic, Indian, and the Pacific oceans (Sakai and Nakabo 1995, 2014, 2016; Knudsen and Clements 2013). All of them are herbivorous fishes and occur in shallow waters (0–10 m depth) in tropical and temperate rocky reefs (Topp 1970).

In the Mediterranean, two species of Kyphosidae have been reported: the beaked chub, *Kyphosus sectatrix* (Linnaeus, 1758), and the brassy chub, *Kyphosus vaigiensis* (Quoy et Gaimard, 1825). The former is more common in subtropical to temperate seas, but can also be encountered in more tropical areas (Knudsen and Clements 2013). It was listed in the Mediterranean fish fauna (Tortonese 1975, 1986) based on old records (1846–1903) of a few individuals at Trieste, Palermo, and Genoa in Italy (Orsi Relini et al. 2011). Since then, it has been reported several times in the western and central Mediterranean, often under invalid names such as *Kyphosus saltatrix* (Linnaeus, 1758) (see Kiparissis et al. 2012; Lelong 2012; Elbaraasi et al. 2013) or *Kyphosus sectator* (Linnaeus, 1758) (see Hemida et al. 2004; Francour and Mouine 2008). Initially thought to be restricted to the Indo-Pacific, *K. vaigiensis* is a circumtropical species distributed along the Indian, Atlantic, and Pacific oceans, the Red Sea, and the Mediterranean (Knudsen and Clements 2013, 2016; Bañón et al. 2017; Evans et al. 2020). Not native to the Mediterranean, it was first reported three times under the species name *Kyphosus incisor* (Cuvier, 1831): twice in the vicinity of Almunécar (Granada, Spain) in the western Mediterranean Sea in June 1998 (Azzurro et al. 2013) and May 2013 (Peña-Rivas and Azzurro in Bilecenoglu et al. 2013) and along the Ligurian coast (Camogli, Italy) in July 2009 (Orsi Relini et al. 2011). Ligas et al. (2011)

had confused it with an individual of *Kyphosus saltatrix* (see Knudsen and Clements 2013; Mannino et al. 2015) found in August 2009 not far from the port of Livorno in Italy. Additional occurrences were reported thereafter in Sicily (Mannino et al. 2015), Malta (Vella et al. 2016), Israel (Goren et al. 2016), Cyprus (Michailidis and Rousou in Gerovasileiou et al. 2017), and Turkey (Kiyağa et al. 2019). With the exception of Orsi Relini et al. (2011) who found 2 individuals of this species (only one of which was measured), all the other authors report only one specimen.

Kyphosus vaigiensis is inshore species found over hard bottoms. It can also occur offshore under floating objects or following ships (Nelson 1994). It feeds mainly on algae, including sargassum (Carpenter 2002). Silvano and Güth (2006) highlight the omnivory of this species in a Brazilian subtropical reef. Some data on the biology of its reproduction on the Colombian coasts are given by Rueda et al. (2015), but its biology and ecology are in general poorly known (Silvano and Güth 2006).

The present note describes a new record of *K. vaigiensis* in the Mediterranean. This species is reported for the first time on the coasts of North Africa.

Methods

On 18 December 2013, one individual of *K. vaigiensis* was recorded from the Gulf of Annaba (Fig. 1). Caught using a trammel net, it was found at a fishmonger among a batch of salemas, *Sarpa salpa* (Linnaeus, 1758). It was photographed, weighed, and identified based on both meristic and morphometric characters. These were taken following standard procedures used in other descriptions of sea chubs (Carpenter 2002; Sakai and Nakabo 2004; Orsi-Relini et al. 2011; Azzurro et al. 2013). The specimen was fixed in formalin and deposited in the fish collection of the Marine Bioresources Laboratory at the Annaba University, Algeria. Since this report in 2013, a survey has been conducted with fishermen in the region to find out if other individuals of *K. vaigiensis* have been seen. This survey was carried out based on photographs.

Results

The meristic and morphometric data of the fished specimen (Fig. 2) are listed in Table 1. They agree with the different descriptions of *K. vaigiensis* given by other authors (Sgano 1981; Carpenter and Niem 2001; Carpenter 2002; Orsi Relini et al. 2011; Azzurro et al. 2013; Sakai and Nakabo 2014, 2016). Morphologically, its body is oval shaped and moderately deep. The head is short with a small and horizontal mouth whose maxilla is slipping under edge of preorbital bone. Head profile in front of eye is gently convex (instead of a bump in *K. sectatrix*). Each jaw is provided with a regular row of close-set incisor-like, round-tipped teeth. Their bases set horizontally, resembling a radially striated bony plate inside mouth. Scales are ctenoid, small and rough to touch, covering most of the head. Color is dorsally grey to silvery on the belly. Series of longitudinal yellow-golden stripes across the body. On head, there are two brassy horizontal bands separated by a silver stripe under the eye. Fins and opercular membrane are dark.

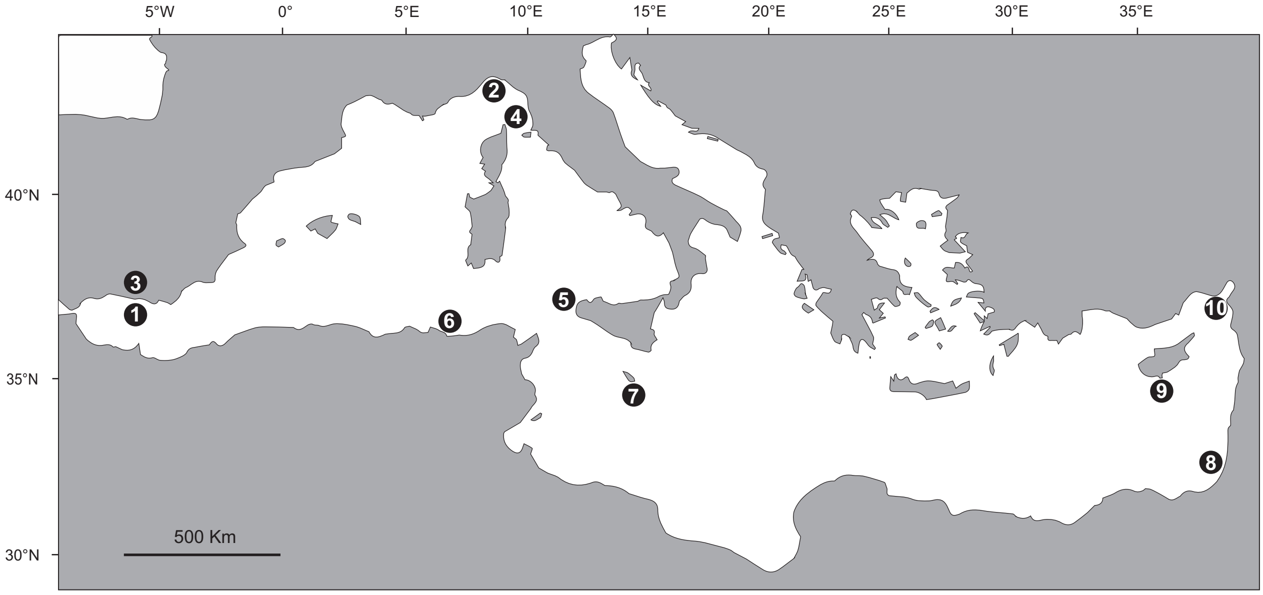

Figure 1. Map pointing out the capture locality of *Kyphosus vaigiensis* on Algerian coasts and chronology of the other records in the Mediterranean: 1 = Azzurro et al. (2013) (31 cm TL, 0.45 kg TW), 2 = Orsi Relini et al. (2011) (48 cm TL, 1.80 kg TW), 3 = Bilecenoglu et al. (2013) (46 cm TL, 1.64 kg TW), 4 = Ligas et al. (2011) (49.5 cm TL, 1.65 kg TW), 5 = Mannino et al. (2015) (47.5 cm TL), 6 = presently reported finding (45.1 cm TL, 1.27 kg TW), 7 = Vella et al. (2016) (15.7 cm TL, 0.49 g EW), 8 = Goren et al. (2016) (42.0 cm SL), 9 = Michailidis and Rousou in Gerovasileiou et al. (2017) (27.2 cm TL, 403 g TW), 10 = Kiyağa et al. (2019) (53.1 cm TL, 2.27 kg TW).

Figure 2. Specimen of *Kyphosus vaigiensis* (41.5 cm TL, 1.27 kg TW) caught in the Gulf of Annaba, Algeria in December 2013.

Table 1. Morphometric and meristic characters of *Kyphosus vaigiensis* captured off Annaba coast (eastern Algeria) in December 2013.

Morphometric characters [cm]	
Total length	41.5
Fork length	38.0
Standard length	31.0
Head length	9.2
Head depth at end of operculum	13.0
Head depth at end of orbit	9.5
Head width at operculum	6.2
Eye diameter	2.0
Pre-orbital length	3.0
Post-orbital length	4.3
Body width at dorsal origin	6.1
Body width at anal origin	5.0
Body height	15.5
Pre-dorsal length	11.2
Pre-anal length	21.0
Caudal peduncle depth	3.5
Caudal peduncle length	4.0
Dorsal fin base length	16
Pectoral fin length	6.5
Pectoral fin base length	2.0
Pelvic fin length	6.0
Pelvic fin base length	2.0
Anal fin base length	9.6
Caudal fin height (vertically extended)	16.0
Caudal fin length	9.0
Internasal space	2.4
Inter-orbital distance	4.2
Upper jaw length	3.0
Lower jaw length	1.8
6th dorsal fin spine (longest)	3.7
4th dorsal fin ray (longest)	2.9
Total weight [g]	1270
Meristic characters	
Dorsal fin spines and soft rays	XI + 13
Anal fin spines and soft rays	III + 12
Pectoral fin rays	17
Pelvic fin spines and soft rays	I + 5
Caudal fin rays	18
Pored scales in lateral line	60
Scales rows above lateral line	11
Scales rows below lateral line	17
Gill rackers on first arch (upper limb + lower limb)	10 + 19
Incisor-like teeth on upper jaws	31
Incisor-like teeth on lower jaws	32

Discussion

The morphology of the caught specimen, as well as its chromatic, meristic, and metric characteristics, confirm that it represents *K. vaigiensis*, as described in the literature (Tortonese 1975, 1986; Sgano 1981; Carpenter 2002; Knudsen and Clements 2013; Carpenter and De Angelis 2016; Sakai and Nakabo 2016) and by the authors of other records of this species in the Mediterranean (Ligas et al. 2011; Orsi Relini et al. 2011; Azzurro et al. 2013; Mannino et al. 2015; Goren et al. 2016; Vella et al. 2016; Michailidis and Rousou in Gerovasileiou et al. 2017; Kiyağa et al. 2019). In particular, Azzurro et al. (2013) insist on the morphological characters which differentiate *K. vaigiensis* from its congener *K. sectatrix*, two very similar species who cohabit in the Mediterranean: anal fin with 12 or 13 soft rays (11, rarely 10 or 12 in *K. sectatrix*), 19 to 22 gill rakers on the lower limb of first gill arch (16 to 19, rarely 19 in *K. sectatrix*) and the gently convex head profile in front of eye (a distinct bump in *K. sectatrix*).

Among the fifteen fishermen we surveyed, two confirmed that they had encountered this fish once. A single individual in the first case (date not specified) and two in the second (in 2019), both of size not exceeding 30 cm. However, given the morphologic similarity between the two species *K. vaigiensis* and *K. sectatrix*, it was not possible to confirm which of the two species it was.

This additional record of *K. vaigiensis* from the Mediterranean confirms its spread along the North-African coast. None of the ichthyological inventories carried out on the Algerian coasts had mentioned it (Dieuzeide et al. 1954; Djabali et al. 1993; Derbal and Kara 2001). This species is the tenth non-indigenous marine fish recorded in Algeria, but the first of circumtropical origin. All the other alien species that arrived are Lessepsian migrants of Indian or Indo-Pacific origin (Kara and Bourehail 2020).

The introduction of *K. vaigiensis* in the Mediterranean could be attributed to the natural expansion of its range

(Zenetos et al. 2012). Its adults can travel long distances (Azzurro et al. 2013) and its juveniles are pelagic and found associated with floating objects (Knudsen and Clements 2016). Thereby, it would have arrived actively or passively through the Suez Canal or the Strait of Gibraltar. However, the chronology of its occurrences which shows a clear spatial progression from west to east and a recent entry (after 2015) into the eastern Mediterranean supports the hypothesis of its Atlantic origin. Otherwise, Annaba being a port city, receiving hundreds of ships per year from all regions of the world (around 10 000 visit Algerian ports each year; Cheniti et al. 2018), its coasts are exposed to the introduction of exotic species. Thus, the recorded *K. vaigiensis* individual would have arrived by ships' ballast water from one of its natural geographic ranges. Indeed, sea chubs are often observed around ships in subtropical waters waiting for the dumping of garbage (Orsi Relini et al. 2011) and are commonly referred to as "rudderfish" (Evans et al. 2020). This behavior exposes them to being "embarked" on board the boats. Thus, their presence in the Mediterranean can also be considered as human-mediated as proposed by Lo Brutto (2017).

Until now, records of *K. vaigiensis* in Mediterranean are limited to one or two isolated individuals. As *K. sectatrix* (see Francour and Mouine 2008), it can be considered a neocolonizer species sensu Quignard and Tomasini (2000). However, in the current context of seawater warming, the ability of *K. vaigiensis* to travel over large distances and its thermophilic character could in the future facilitate its establishment in the Mediterranean. In this situation, its coexistence with the indigenous sparid *Sarpa salpa*, the only herbivorous fish species in the Mediterranean, would reproduce the interaction scenario between *Sarpa salpa*, *Siganus luridus* (Rüppell, 1829), and *Siganus rivulatus* Forsskål et Niebuhr, 1775 which happened in the eastern Mediterranean.

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