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Neogobius melanostomus (Pallas, 1814) lacking the black spot on the first dorsal fin – a morphometric and meristic comparison

Neogobius melanostomus (Pallas, 1814) ohne schwarzen Fleck auf der ersten Rückenflosse – ein morphologischer und meristischer Vergleich

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Summary: In 2014 a total of 488 specimens of round gobies was captured in the lower River Weser. These specimens exhibited two distinct pigmentation patterns of the first dorsal fin. Whereas in approximately 80 % of the individuals the characteristic large oblong black spot on the posterior part of the first dorsal fin was present, it was lacking in about 20 % of the specimens. This black spot is regarded as an important feature in species identification. 200 voucher specimens, 100 individuals with spot and 100 lacking the typical spot, were analyzed morphologically and meristically and compared statistically. Despite some scattered differences (i.e. ratio of preventral distance/standard length, the number of mid-lateral scales and number of mid-lateral scales of the caudal fin) the two groups broadly corresponded. The results are consistent with findings from invasive *N. melanostomus* in Lake Erie/North America, where a similar variation in pigmentation pattern was observed. Thus we assign the specimens of gobies exhibiting morphological characteristics of round gobies but lacking the black spot on the dorsal fin to the species *N. melanostomus*.

Keywords: *Neogobius melanostomus*, round goby, pigmentation, lacking black spot, first dorsal fin, lower River Weser

Zusammenfassung: Im Jahr 2014 wurden in der Unterweser in Bremen 488 Schwarzmundgrundeln gefangen. Die Individuen wiesen zwei verschiedene Pigmentierungsmuster der ersten Dorsale auf. Etwa 80 % der Individuen hatten den typischen, schwarzen Fleck am posterioren Ende der ersten Dorsale, bei etwa 20 % der Individuen fehlte dieses auffällige Merkmal. Bisher gilt der schwarze Fleck an der ersten Dorsale als eines der wesentlichen Merkmale für die Artbestimmung. 200 Belegexemplare, 100 mit Fleck und 100 ohne Fleck, wurden auf morphometrische und meristische Merkmale untersucht und mittels statistischer Analysen verglichen. Beide Gruppen wiesen eine große Übereinstimmung auf, geringe Unterschiede gab es z. B. bei Präventrallänge/Standardlänge oder der Anzahl der Schuppen entlang der mittleren Lateralebene. Die Ergebnisse stimmen mit den Resultaten einer Studie über *N. melanostomus* aus dem Eriesee in Nordamerika überein, in dem auch Schwarzmundgrundeln ohne Fleck auf der ersten Dorsale gefunden wurden. Aufgrund der morphologischen Merkmale wurden die Grundeln ohne den schwarzen Fleck an der ersten Dorsale der Art *Neogobius melanostomus* zugeordnet.

Schlüsselworte: *Neogobius melanostomus*, Schwarzmundgrundel, Pigmentierung, Färbung, fehlender schwarzer Fleck, erste Dorsalflosse, Unterweser

1. Introduction

The round goby, *Neogobius melanostomus* (Teleostei; Gobiidae), is native to the Ponto-Caspian region (Kottelat & Freyhof 2007, Kornis et al. 2012). Since the end of the 20th century it has successfully colonized a wide range of brackish and freshwater ecosystems on both sides of the Northern Atlantic (Corkum et al. 2004; Leslie & Timmins 2004; Kottelat & Freyhof 2007; Kornis et al. 2012; Hempel & Thiel 2013). Although the natural migration rate appears to be limited and is reported to vary between < 1 km/yr (Bergstrom et al. 2008) and

30 km/yr (Azour et al. 2015), the actual dispersal rate outruns suggested natural spreading rates. Fast expansion of invasive *N. melanostomus* to non-native ecosystems is attributed to three main factors: (1) interconnection of previously separated drainage systems; (2) ships acting as vectors by dispersal of eggs and larvae via uptake and discharge of ballast water (Brownscombe et al. 2012; Kornis et al. 2012; Brandner et al. 2013a) and (3) artificial river regulations such as shoreline stabilization consisting of riprap (Brandner et al. 2015). In Europe *N. melanostomus* initially invaded water systems in South-Eastern Europe and Eastern-Central Europe (e.g. the Baltic Sea, the upper River Danube and the River Vistula) (Corkum et al. 2004; Sapota 2004; Paintner & Seifert 2006). Within a few years colonization extended westwards towards the North Sea basin (Stemmer 2008, Brunken et al. 2012). Important milestones in colonization of the round goby in German water systems refer to: (1) the first record of *N. melanostomus* in the Baltic Sea close to the island of Rügen in 1998 (Nehring et al. 2010); (2) first record in the German part of the River Danube (Paintner & Seifert 2006); (3) records in the North Sea – Baltic Sea canal in 2007 (Neukamm o.J.); (4) catches in the tidal River Elbe in 2008 (Hempel & Thiel 2013); (5) records in the River Rhine in 2008 (Stemmer 2008); (6) the first record of *N. melanostomus* in the lower River Weser in 2012 (Brunken et al. 2012).

Records of *N. melanostomus* in the lower River Weser in 2012 were exclusively captured at sampling sites characterized by shoreline stabilization elements consisting of riprap (Brunken et al. 2012), which is known as the preferred habitat of invasive round gobies (Brandner et al. 2013b). All specimens exhibited the typical morphological features for *N. melanostomus*, including the presence of a large black spot on the posterior part of the first dorsal fin (Brunken et al. 2012).

A large black spot on the first dorsal fin is regarded as characteristic feature of *N. melanostomus*, when comparing invasive goby species (Pinchuk et al. 2003a; Kottelat & Freyhof 2007; Kornis et al. 2012). However, individuals of *N. melanostomus* lacking such a black spot on the first dorsal fin were reported in: (1) St. Clairs River/North America (total length < 9 mm, Leslie & Timmins 2004); (2) Lake Erie/North America (total length unknown) (Cavender et al. 1996; Marsden et al. 1996) and (3) River Elbe/Europe (a single adult specimen, total length = 140 mm, Hempel & Thiel 2013). To our knowledge *N. melanostomus* lacking the black spot on the first dorsal fin have not yet been recorded in its native region.

In 2014 a total of 488 individuals of *N. melanostomus* were captured by seine net fishing in the lower River Weser at sampling sites characterized by sandy substrate. About a quarter of these specimens lacked a black spot on the posterior part of first dorsal fin, whereas habitus, morphological and meristic features supported an identification as *N. melanostomus*.

Beside *N. melanostomus* four other gobiid species have been recorded in the drainage basin of the lower River Weser so far, i.e. the naked goby – *Gobiosoma bosc* (Thiel et al. 2012), the racer goby – *Babka gymnotrachelus* (Meyer, LAVES, Dez. Binnenfischerei, pers. comm. 2013), the common goby – *Pomatoschistus microps* and the sand goby – *Pomatoschistus minutus* (Scholle et al. 2006). The monkey goby – *Neogobius fluviatilis*, the bighead goby – *Ponticola kessleri* (Günther 1861) and the tube-nosed goby – *Proterorhinus semilunaris* are gobies that might co-occur with the round goby (Corkum et al. 2004) but have by now not been verified in the lower River Weser.

In this study the question is addressed whether specimens of *N. melanostomus* lacking the characteristic black spot on the first dorsal fin exhibit the same morphological and meristical features as individuals with the typical color pattern.

2. Material and methods

A total of 200 gobiid specimens, obtained from the ichthyologic collection of the City University of Applied Sciences Bremen, were analyzed. The specimens were captured by seine net fishing in the lower River Weser in 2014 on habitats characterized by sandy

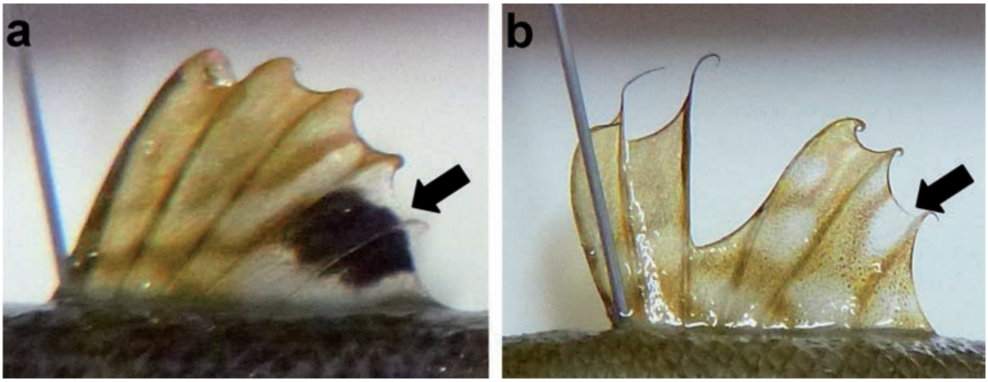

Fig. 1: Pigmentation pattern in the dorsal fin of the gobiid voucher specimens obtained from the ichthyologic collection of the City University of Applied Sciences Bremen. **a** Specimens exhibiting a conspicuous black spot on the posterior part of dorsal fin. **b** Individual lacking a black spot on the dorsal fin; arrows indicate spot/lacking spot.

Abb. 1: Pigmentierungsvarianten der ersten Dorsale der Grundeln der Ichthyologischen Sammlung der Hochschule Bremen, die ihm Rahmen der vorliegenden Studie untersucht wurden. **a** Exemplar mit schwarzem Fleck am posterioren Ende der ersten Dorsalen. **b** Individuum ohne schwarzen Dorsalfleck. Die Pfeile verweisen auf den schwarzen/fehlenden Dorsalfleck.

substrate. After capture the individuals were euthanized, cleaned and fixed in 40 % ethanol which was subsequently changed to a final concentration of 70 % ethanol within three days. All individuals were pre-identified as *Neogobius melanostomus* directly after capture. 100 individuals exhibited the typical pigmentation of the dorsal fin of the round goby, i.e. a large oblong black spot on the posterior part of the first dorsal fin (hereinafter referred to as NMS), whereas in 100 specimens the conspicuous black spot on the dorsal fin was lacking (hereinafter referred to as NML) (fig. 1).

The species pre-identification was later verified by the examination of eleven external characteristics, i.e. fused pelvic fins, first branched ray of second dorsal about as long as the penultimate ray (Kottelat & Freyhof 2007), anterior nostrils not elongated beyond the upper lip (Pinchuk et al. 2004), shape of body elongated and round in cross section (Kornis et al. 2012), protruding dorsolateral eyes (Leslie & Timmins 2004), oblique snout convex in profile positioned slightly subterminal, angle of the jaw below anterior quarter of the eye, cycloid scales on the nape and part of the abdomen, weakly ctenoid scales on the rest of the body and pelvic disc with weakly defined lateral lobes on the anterior membrane (Pinchuk et al. 2003a), congruency of pigmentation pattern (Pinchuk et al. 2003a). The pigmentation of the first dorsal fin, i.e. the lack of the conspicuous black spot, was not taken into account.

Morphometrical, morphological and meristical data of the NMS and NML from the lower River Weser were compared with those of (1) *N. melanostomus* from native and non-native populations, (2) *Gobiosoma bosc*, (3) *Babka gymnotrachelus*, (4) *Pomatoschistus microps*, (5) *Pomatoschistus minutus*, (6) *Ponticola syrman*, (7) *Neogobius fluviatilis*, (8) *Ponticola kessleri* and (9) *Proterorhinus semilunaris* (tab. 1). The data for morphometrical and meristical comparison were conducted from literature (Miller 2003; Pinchuk et al. 2003a, b, c, d; Vasil'eva & Vasil'ev 2003; Pinchuk et al. 2004; Miller 2004; Stranai & Andreji 2004; L'avrinčiková et al. 2005; Kovác & Siryová 2005; Kottelat & Freyhof 2007; Marsden et al. 2007; Capova et al. 2008; Neilson & Stepien 2011; Kornis et al. 2012; Thiel et al. 2012; Hempel & Thiel 2013) (tab. 1). Nomenclature was based on Eschmeyer et al. (2016).

Tab. 1: Literature review of morphometric and meristic data on *Neogobius melanostomus*, *Neogobius fluviatilis, Ponticola syrman, Babka gymnotrachelus*, *Gobiosoma bosc*, *Ponticola kessleri, Pomatoschistus microps*, *Pomatoschistus minutus* and *Proterorhinus semilunaris.*

Tab. 1: Übersicht von Literaturangaben zu morphometrischen und meristischen Daten von *Neogobius melanostomus*, *Neogobius fluviatilis*, *Ponticola syrman, Babka gymnotrachelus*, *Gobiosoma bosc*, *Ponticola kessleri, Pomatoschistus microps*, *Pomatoschistus minutus* und *Proterorhinus semilunaris.*

Species	Source	type of scales on		height of D2	anterior nostril	black spot on D1	range							
		nape	body				D1	D2	A	scales mls	PDD/SL [%]	PVD/SL [%]	PAD/SL [%]	OE/SL [%]
Neogobius melanostomus native	KORNIS et al. 2012	cycloid	ctenoid	.	.	yes	VII - VIII	I 12 - 17	I 9 - 14					
Neogobius melanostomus	PINCHUK et al. 2003a	cycloid	ctenoid	uniform	.	yes	V - VII	I 12 - 17	I 10 - 14	45 - 57	34.2 - 37.8			
	KOTTELAT & FREYHOF 2007	scaled	scaled	uniform	not projecting forward beyond lip	yes				45 - 54				
	HEMPEL & THIEL 2013	scaled	.	.	.	yes; no	VI - VII	I 15 - 16	I 12	54 - 55	32.4 - 37.2	29.2 - 31.5	56.4 - 58.3	5.3 - 8.0
	STRÁNAI & ANDREJI 2004	cycloid	ctenoid	.	.	yes	VI	I 15 -16	I 12- 13	54 - 56	34.2 - 36.5	29.9 - 32.6	56.4 - 65.4	
	KORNIS et al. 2012	cycloid	ctenoid	.	.	yes	V - VI	I 15 - 17	I 11 - 15	42 - 59				
	MARSDEN et al. 2007	cycloid	.	.	.	yes; no	V - VII	I 13 - 16	I 11 - 14	45 - 57				
	L'AVRINČÍKOVÁ et al. 2005	.	.	.	.	.					32.7 - 39.0	28.2 - 36.3	54.7 - 63.4	5.3 - 10.4
Neogobius fluviatilis	PINCHUK et al. 2003b	ctenoid	ctenoid	descending in profile	.		V- VII	I 14 - 17	I 12 - 16	55 - 61	31.8 - 36.5			
	CAPOVA et al. 2008	.	.	.	.	.					31.0 - 36.6	26.5 - 34.6	46.9 - 57.6	4.4 - 6.4
	NEILSON & STEPIEN 2011	.	.	.	.	.	V - VII	I 14 - 16	I 13 -15	54 - 70	22.4 - 39.4		36.5 - 58.8	
Ponticola kessleri	MILLER 2003	ctenoid	ctenoid	uniform		no	V - VII	I 16 - 19	I 13 - 16	64 - 79	33.8 - 39.4			
	KOVÁČ & SIRYOVÁ 2005	.	.	.							35.5 - 40.4	27.8 - 41.3	58.9 - 70.9	
Ponticola syrman	PINCHUK et al. 2003c	ctenoid	ctenoid	usullay uniform		no	VI - VII	I 16 - 18	I 11 - 15	57 - 70	33.5 - 38.4			
Babka gymnotrachelus	PINCHUK et al. 2003d	none	cycloid or ctenoid	uniform		no	V - VII	I 15 - 18	I 12 - 15	51 - 77	29.5 - 33.7			
Gobiosoma bosc	THIEL et al. 2012	none	none	.	.	.	VII	I 12 - 13	10 - 11	none				
Pomatoschistus microps	MILLER 2004	none	scaled	.	short		IV - VII	I 7 - 11	I 7 - 10	39 - 52	32,5 - 36,8	25,0 - 30,2	53,8 - 59,4	
	KOTTELAT & FREYHOF 2007	none	scaled						8 - 10,5	39 - 52				
Pomatoschistus minutus	MILLER 2004	none		.	short					55 - 75				
	KOTTELAT & FREYHOF 2007									55 - 75				
Proterorhinus	MILLER 2004	cycloid	cycloid	uniform		no	VI - VII	I 15 - 20	I 11 - 17	42 - 50	31.8 - 36.2			
					projecting									

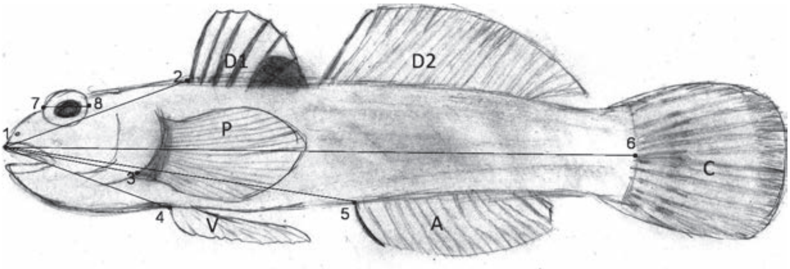

Fig. 2: Scheme of *Neogobius melanostomus* illustrating morphology and landmarks for morphometrics. PDD (predorsal distance 1-2), PPD (prepectoral distance 1-3), PVD (preventral distance 1-4), PAD (preanal distance 1-5), SL (standard length 1-6), ØE (eye diameter 7-8) and fins D1 (first dorsal fin), D2 (second dorsal fin), P (pectoral fin), V (ventral fin), A (anal fin) and C (caudal fin).
Abb. 2: Schematische Zeichnung von *Neogobius melanostomus* mit Angabe der untersuchten morphometrischen Eigenschaften. PDD (Predorsallänge 1-2), PPD (Prepectorallänge 1-3), PVD (Preventrallänge 1-4), PAD (Preanallänge 1-5), SL (Standardlänge 1-6), ØE (Durchmesser Auge 7-8) und den Flossen D1 (erste Dorsale), D2 (zweite Dorsale), P (Pectorale), V (Ventrale), A (Anale) und C (Caudale).

Sex was determined by shape and length of the urogenital papilla (Marsden et al. 1996; Kornis et al. 2012). The specimens were assigned to: (1) females, when the urogenital papilla was broad and blunt, (2) males, when the urogenital papilla was long and pointed, and (3) juveniles, when shape and length differed from the previously described features and individuals were smaller than 32 mm SL (Azizova 1962).

Standard length (SL), prepectoral distance (PPD), preventral distance (PVD), predorsal distance (PDD), preanal distance (PAD) and the eye diameter (ØE) were measured to the nearest 1 mm using a digital caliper (fig. 2). Weight was measured to the nearest 0.1 g (digital balance Sartorius cP 6201). Additionally spines and soft rays of the first dorsal fin (D1), second dorsal fin (D2) and anal fin (A), scales on the midlateral series on the body (scales mls) and midlateral series on the caudal fin (scales mls caudal) were enumerated.

All measurements were plotted against SL and indicated in percent, i.e. PPD/SL, PVD/SL, PDD/SL, PAD/SL and ØE/SL, except TW/SL which was calculated in g/mm. Data were analyzed using the statistical software WinSTAT® for Microsoft®Excel (univariate statistical analysis) and MVSP (multivariate statistical analysis). Data were tested for normal distribution (Shapiro-Wilk test).

Statistical analyses were performed for (1) all individuals independent from sex (all), (2) males and (3) females. Pairwise comparisons were conducted using the Student's t-test when data were normally distributed (Shapiro-Wilk test: p value > 0.05). For all other data the Mann-Whitney-U test (hereinafter referred to as U test) was applied. Multivariate comparisons were conducted using Principal Components Analysis (PCA). Application of PCAs in grouping of morphometrical and meristical data between NML and NMS were carried out to verify possible differences and similarities in variation of NMS and NML. For the PCAs the data were standardized by $x' = ((x-\bar{x})/SD)+100$. Standardization was indicated to acquire comparability of meristical data (counts) and morphometrical data (ratios).

Specimens exhibiting damaged fins or loss of scales were excluded from the analyses when the missing feature was taken into account. For this reason the number of individuals (n) varied between the different analyses and was indicated in the results.

3. Results

All 200 voucher specimens were assigned to the species *Neogobius melanostomus* exhibiting the typical morphological characters (tab. 1). *Neogobius melanostomus* lacking a black spot on the dorsal fin (NML) appeared to have similar external characteristics as *N. melanostomus* exhibiting the

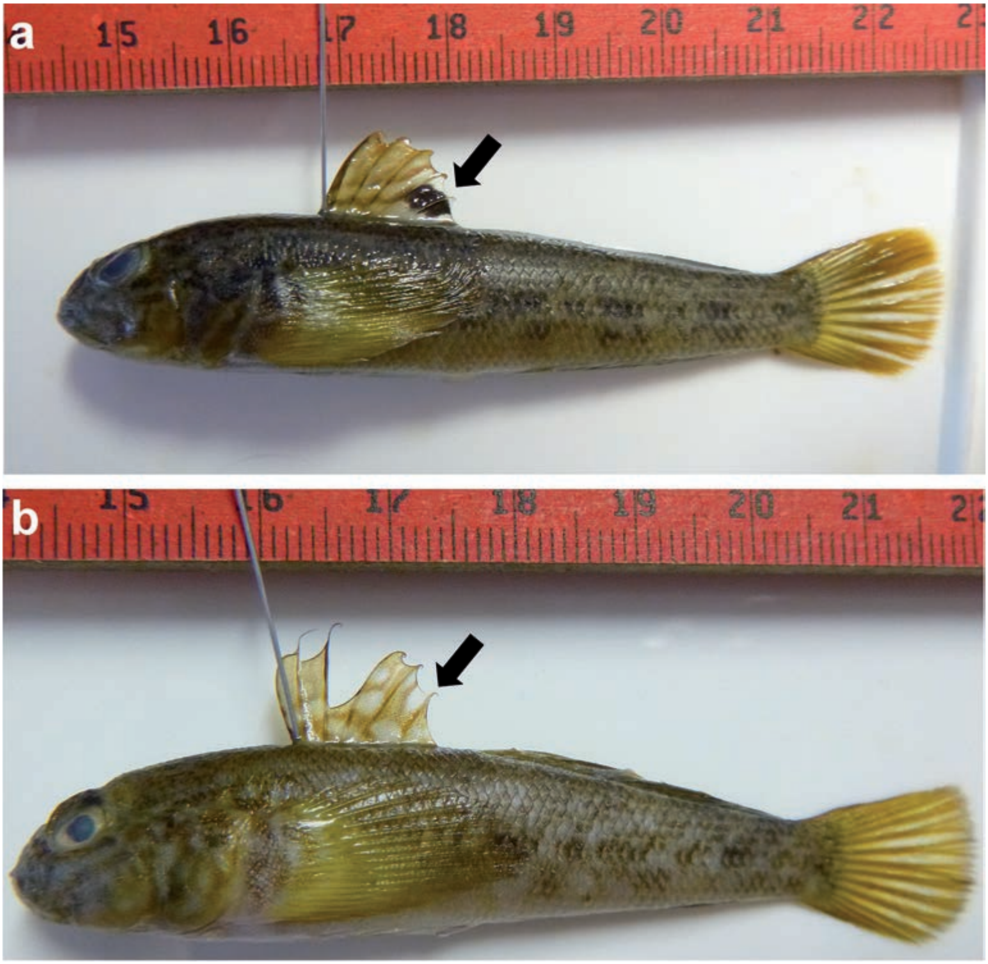

Fig. 3: *Neogobius melanostomus* from the lower River Weser. **a** Exhibiting a large oblong spot on the posterior end of first dorsal fin (NMS). **b** Lacking a black spot on the posterior end of first dorsal fin (NML); arrows indicate spot/lacking spot.
Abb. 3: *Neogobius melanostomus* aus der Unterweser. **a** Mit schwarzem Dorsalfleck (NMS). **b** Ohne Dorsalfleck (NML). Die Pfeile verweisen auf den schwarzen/fehlenden Dorsalfleck.

typical black spot on the first dorsal fin (NMS) (figs 3 a, b).

The standard length (SL) of the voucher specimens ranged between 15.1 and 82.4 mm. The majority of all specimens were smaller than 45 mm SL (184 specimens, 92 %), only 16 individuals (8 %) exceeded 45 mm (fig. 4). No significant difference between NMS and NLM was revealed for measurements of the SL ($p > 0.05$).

Sex determination revealed a total of 105 females (49 NMS and 56 NML) and 49 males (28 NMS and 21 NML), thus corresponding to a female/male ratio of roughly 2:1. In 46 juveniles (23 NMS and 23 NML) sex remained indeterminable; the SL of these specimens ranged between 15.1 and 27.5 mm.

There were no significant differences in morphometric ratios of NMS and NML, except for the ratio of PVD/SL (t-test, $p = 0{,}024$) when taking all 200 individuals into account (tab. 2).

A comparison of the fin ray counts disclosed parity in the range of spines and rays of NMS

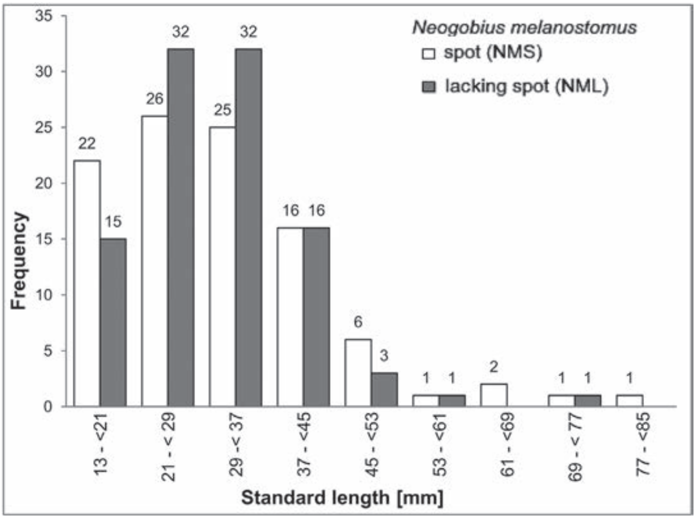

Fig. 4: Length frequency distribution of *Neogobius melanostomus* with black spot on the first dorsal fin (NMS) and lacking a black spot on the first dorsal fin (NML) for a) females, b) males and c) juveniles.
Abb. 4: Längen-Häufigkeits-Verteilung von *Neogobius melanostomus* mit schwarzem Dorsalfleck (NMS) und ohne Dorsalfleck (NML) für a) Weibchen (females), b) Männchen (males) und c) Juvenile (juveniles).

and NML in the first dorsal fin (D1), second dorsal fin (D2) and anal fin (A), when sex was not taken into account (range D1 V-VII, D2 I/14-17, A I/11-14). Minor but not significant differences in the number of spines and rays were revealed when the fin ray and spine count was analyzed specifically to sex (tab. 3). In contrast, significant differences were observed regarding the number of scales in the mid-lateral series (scales mls). The number of scales mls was lower in NML than in NMS. It ranged between 45 - 54 in NML (mean 47.3) and between 45 -55 in NMS (mean 48.6) when sex was not taken into account (all; $p = 0.001$; U test). Considering data of males only no differences in the number of scales mls were found ($p = 0.199$; U test), whereas the pairwise comparison of females by an U test revealed a high significant difference ($p = 0.003$). The range of the mid-lateral scales on the caudal fin (scales mls caudal) was similar in both groups of round gobies, nevertheless the mean number of these scales was higher in NMS than in NML (tab. 3).

PCAs revealed a high degree of overlap among NMS and NML. Axis one explained 16.1 % (eigenvalue 1.7) and axis two 13.7 % (eigenvalue 1.5) of the variation in the dataset, when sex was not considered. NML ($n = 92$) and NMS ($n = 93$) showed a similar scattering. An obvious grouping failed (fig. 5). The characters D2 soft rays and scales mls displayed the highest loadings (0.493 soft rays D2; 0.389 scales mls) on axis one but did not point towards a distinct pattern in variances for NML and NMS. The ratios of PDD/SL, PVD/SL and PAD/SL had a high impact on axis two (0.562 PPD/SL; 0.464 PVD/SL; 0.437 PAD/SL).

Considering data of males only (NMS $n = 28$; NML $n = 21$) axis one had an eigenvalue of 2.0 reflecting 17.8 % of the variation, whereas axis two explained 16.2 % and had an eigenvalue of 1.8 (fig. 6 a). The highest loadings for axis one resulted from D1 spines (0.433) and Scales mls caudal (0.425). For axis two the highest loading was represented by TW/SL (0.692). In females (NMS $n = 47$; NML $n = 55$), axis one had an eigenvalue of 1.5 and accounted for 15.6 % of the variation whilst axis two reached an eigenvalue of 1.5 and explained 15.0 % (fig. 6 b). D1 spines revealed the highest loading for axis one (0.748) and TW/SL for axis two (0.681).

Tab. 2: Ratio of prepectoral distance (PDD), preventral distance (PVD), predorsal distance (PDD), preanal distance (PAD), eye diameter (ØE) and total weight (TW) to standard length (SL) in percent with count (n), mean (m), standard deviation (SD) and range for *Neogobius melanostomus* with a spot on the first dorsal fin (NMS) and *N. melanostomus* lacking a black spot on first dorsal fin (NML). Legend: all = all specimens independent from sex; m = males; f = females; n = number of specimens: p value = level of significance; n. s. = not significant; * = significant; ** = highly significant; *** = highest significant; test = applied statistical test; U = U-test (Mann-Whitney); t = t-test for independent samples.
Tab. 2: Verhältnis von Präpectorallänge (PDD), Präventrallänge (PVD), Prädorsallänge (PDD), Präanallänge (PAL), Augendurchmesser (ØE) und Totalgewicht (TW) zur Standardlänge (SL) in Prozent. Angabe von Anzahl der untersuchten Individuen (n), Mittelwert (m), Standardabweichung (SD) und Spannweite (range) von *Neogobius melanostomus* mit schwarzem Dorsalfleck (NMS) und ohne Dorsalfleck (NML). All = alle Individuen; m = males; f = females; p value = Signifikanzniveau; n. s. = nicht signifikant; * = signifikant, ** = hoch signifikant; *** = höchst signifikant; test = angewandter statistischer Test, U = U-Test (Mann-Whitney); t = t-Test für unabhängige Stichproben.

			Neogobius melanostomus spot (NMS)				*Neogobius melanostomus* lacking spot (NML)				p value	test
			n	m	SD	range	n	m	SD	range		
Percentage of	PPL/SL	all	100	23.3	2.4	16.7 - 30.0	100	22.9	2.2	17.2 - 27.2	n. s.	t
		male	28	22.8	2.6	16.7 - 30.0	21	22.5	2.0	17.2 - 25.6	n. s.	t
		female	49	23.8	2.0	19.0 - 27.8	56	23.0	2.1	18.1 - 26.4	n. s.	t
	PVL/SL	all	100	26.8	2.0	20.0 - 31.8	100	26.8	2.2	20.0 - 31.3	*	t
		male	28	26.8	1.8	23.1 - 30.3	21	26.2	2.4	20.1 - 30.8	n. s.	t
		female	49	26.7	1.8	22.6 - 31.8	56	26.0	1.9	20.7 - 29.6	n. s.	U
	PDL/SL	all	100	31.5	2.0	26.9 - 37.5	99	31.4	2.1	26.3 - 37.5	n. s.	t
		male	28	31.1	2.5	27.3 - 36.8	21	31.0	2.1	26.9 - 34.6	n. s.	t
		female	49	31.1	1.5	26.9 - 35.5	55	31.0	1.8	26.7 - 34.8	n. s.	t
	PAL/SL	all	100	52.2	2.6	43.8 - 58.8	100	52.7	3.3	47.1 - 73.7	n. s.	U
		male	28	51.4	2.1	47.4 - 57.1	21	52.5	2.2	48.3 - 56.0	n. s.	t
		female	49	52.4	2.3	47.6 - 57.8	56	52.4	2.1	48.4 - 57.1	n. s.	t
	ØE/SL	all	100	7.2	1.2	4.3 - 11.1	100	7.3	1.3	4.0 - 10.5	n. s.	t
		male	28	7.2	1.0	5.3 - 10.0	21	7.7	1.3	4.0 - 9.3	n. s.	t
		female	49	7.4	1.2	4.3 - 9.7	56	7.4	1.2	4.3 - 10.0	n. s.	t
TW/SL [g/mm]		all	100	2	2.2	0.3 - 14.5	100	1.7	1.7	0.3 - 10.6	n. s.	U
		male	28	2.4	2.9	0.5 - 14.5	21	1.9	1.1	0.4 - 4.0	n. s.	U
		female	49	2.4	2.1	0.4 - 10.9	56	2.1	2.0	0.5 - 10.6	n. s.	U

4. Discussion

We assign the gobies from the lower River Weser lacking the conspicuous black spot on D1 (NML) to the species *N. melanostomus*, even though the presence of a black spot on the posterior D1 is mandatory for the identification of *N. melanostomus* when using currently available identification keys (Miller 2003; Miller & Vasil'eva 2003; Kottelat & Freyhof 2007). The morphological characters of NML in the lower River Weser matched, apart from the pigmentation pattern of D1, the species description of the round goby (Pinchuk et al. 2003a; Kottelat & Freyhof 2007) and mismatched with those gobiid species which are abundant in the lower River Weser (Miller 2003; Miller & Vasil'eva 2003; Pinchuk et al. 2003c; Scholle et al. 2006; Kottelat & Freyhof 2007; Thiel et al. 2012; Meyer, LAVES Dez. Binnenfischerei, pers. comm. 2013) and with those which could potentially co-occur with *N. melanostomus* (Pinchuk et al. 2003b, d; Corkum et al. 2004; Miller 2004)

Tab. 3: Mean (m), standard deviation (SD) and range of meristic counts in *Neogobius melanostomus* with black spot on the first dorsal fin (NMS) and *N. melanostomus* lacking a black spot on the dorsal fin (NML). Legend: n = number of specimens; all = all specimens; m = males; f = females; D1 spines = number of spines in the first dorsal fin; D2 soft rays = number of soft rays in the second dorsal fin; A soft rays= number of soft rays in the anal fin; scales mls= number of mid-lateral scales; scales mls caudal= scales mid-lateral series in caudal fin; p value = level of significance; n. s. = not significant; * = significant; ** = highly significant; *** = highest significant; test = applied statistical test; U = U-test (Mann-Whitney).
Tab. 3: Anzahl (n), Mittelwert (m), Standardabweichung (SD) und Spannweite (range) der meristischen Untersuchungen an *Neogobius melanostomus* mit Fleck (NMS) und ohne Fleck (NML) auf der ersten Dorsale. All = alle Individuen; m = males; f = females; D1 spines = Hartstrahlen an der ersten Dorsale; D2 soft rays = Weichstrahlen an der zweiten Dorsale; A soft rays = Weichstrahlen an der Analen; scales mls = Anzahl Schuppen entlang der mittleren Lateralebene; scales mls caudal = Schuppenanzahl entlang der mittleren Lateralebene auf der Caudale; p value = Signifikanzniveau; n. s. = nicht signifikant; * = signifikant, ** = hoch signifikant; *** = höchst signifikant; test = angewandter statistischer Test, U = U-Test (Mann-Whitney).

			Neogobius melanostomus spot (NMS)				*Neogobius melanostomus* lacking spot (NML)				p value	test
			n	m	SD	range	n	m	SD	range		
Count of	D1 spines	all	100	6,0	0,2	5 - 7	97	6,0	0,2	5 - 7	n. s.	U
		m	28	6,0	0,2	6 - 7	55	6,1	0,2	6 - 7	n. s.	U
		f	49	6,0	0,2	5 - 7	21	6,1	0,2	6 - 7	n. s.	U
	D2 soft rays	all	100	15,6	0,7	14 - 17	98	15,6	0,7	14 - 17	n. s.	U
		m	28	15,8	0,6	15 - 17	56	15,6	0,7	14 - 17	n. s.	U
		f	49	15,7	0,8	14 - 17	21	15,8	0,6	14 - 17	n. s.	U
	A soft rays	all	100	14,0	12,0	11 - 14	98	12,8	0,7	11 - 14	n. s.	U
		m	28	13,0	0,7	12 - 14	56	12,7	0,7	11 - 14	n. s.	U
		f	49	15,4	17,2	12 - 14	21	12,9	0,7	11 - 14	n. s.	U
	Scales mls	all	94	48,6	2,8	45 - 55	94	47,3	2,3	45 - 54	***	U
		m	28	48,5	2,5	45 - 54	21	47,5	2,0	45 - 51	n. s.	U
		f	47	49,1	2,8	45 - 55	56	47,5	2,4	45 - 54	**	U
	Scales mls caudal	all	93	2,3	0,5	2 - 3	94	2,1	0,4	2 - 3	*	U
		m	28	2,4	0,5	2 - 3	21	2,1	0,3	2 - 3	*	U
		f	47	2,3	0,5	2 - 3	56	2,2	0,4	2 - 3	n. s.	U

(tab. 1). This conclusion broadly corresponded with the morphological data ascertained during this study. The number of (1) D1 spines; (2) D2 soft rays; (3) A soft rays; (4) scales mls are consistent with data reported in the literature (Pinchuk et al. 2003a; Stranai & Andreji 2004; Marsden et al. 2007; Kornis et al. 2012; Hempel & Thiel 2013). The range of the ratios of PDD/SL, PPD/SL, PVD/SL, PAD/SL and ØE/SL was wider in specimens from the lower River Weser than reported in the literature (Pinchuk et al. 2003a; Stranai & Andreji 2004; L'avrinčiková et al. 2005; Hempel & Thiel 2013) (tab. 1). However, numerous differences of external morphological characteristics have been reported for several *N. melanostomus* populations, differing between the various invasive populations as well as between native and invasive populations (Polačik et al. 2011; Kornis et al. 2012). Such differences are e.g. (1) size at age (Simonovic et al. 2001); (2) size at onset of maturation (L'avrinčiková et al. 2005); (3) number of pectoral fin rays; (4) upper jaw and lower jaw length; (5) eye diameter and (6) predorsal distance (Polačik et al. 2011).

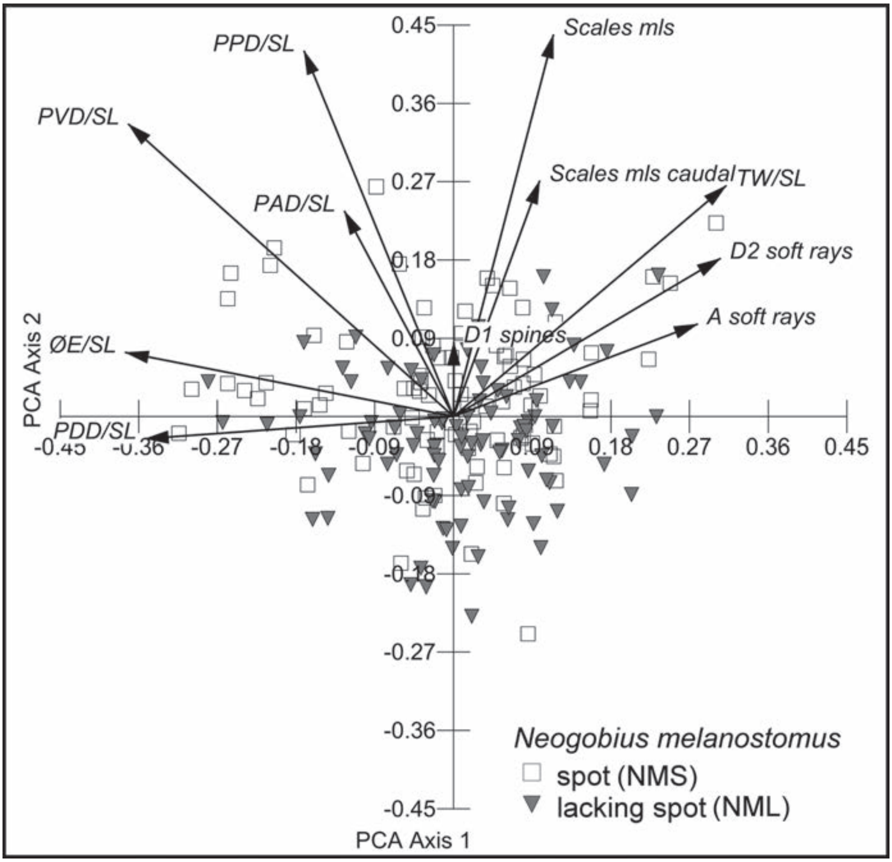

Fig. 5: Principal component analysis (PCA) of eleven morphometric and meristic variables of round gobies *Neogobius melanostomus* with spot (NMS; white square; n = 93) and lacking spot (NML; grey triangle; n = 92) from the lower River Weser, captured in 2014; Axis 1 16.1 %, eigenvalue 1.7; Axis 2 13.7 %, eigenvalue 1.5.
Abb. 5: Hauptkomponentenanalyse (PCA) von elf morphometrischen und meristischen Variablen der Schwarzmundgrundel *Neogobius melanostomus* mit Fleck (NMS, weißes Quadrat, n = 93) und ohne Fleck (NML, graues Dreieck, n = 92) aus der Unterweser, gefangen 2014; Achse 1 (Axis 1) 16,1 %, Eigenwert 1,7; Achse 2 (Axis 2) 13,7 %, Eigenwert 1,5.

Both types of voucher specimens, NMS and NML, displayed similarities regarding morphometrical and meristical characteristics. The univariate statistical analyses revealed minor differences only (i.e. PVD/SL, scales mls and scales mls caudal). This was also confirmed by the PCAs (figs 5, 6). As the range of the data was approximately the same in NMS and NML the few statistically significant differences between the two groups might have been either actual significant differences or triggered by (1) observational errors, (2) the relatively high number of immature specimens and specimens in the transition phase to maturity, (3) the random selection of specimens and (4) the sample size.

The assignment of NML to the species *N. melanostomus* is consistent of those of Cavender et al. (1996) and Leslie & Timmins (2004). Leslie & Timmins (2004) reported the presence of NML in the St. Clairs River/North America, remarking the absence of the black spot on D1 in "many" specimens < 9 mm SL (percentage not given). Cavender et al. (1996) revealed the presence of NML in the Lake Erie/North America and stated this variation in pigmentation to be uncorrelated to sex, size class and catchment

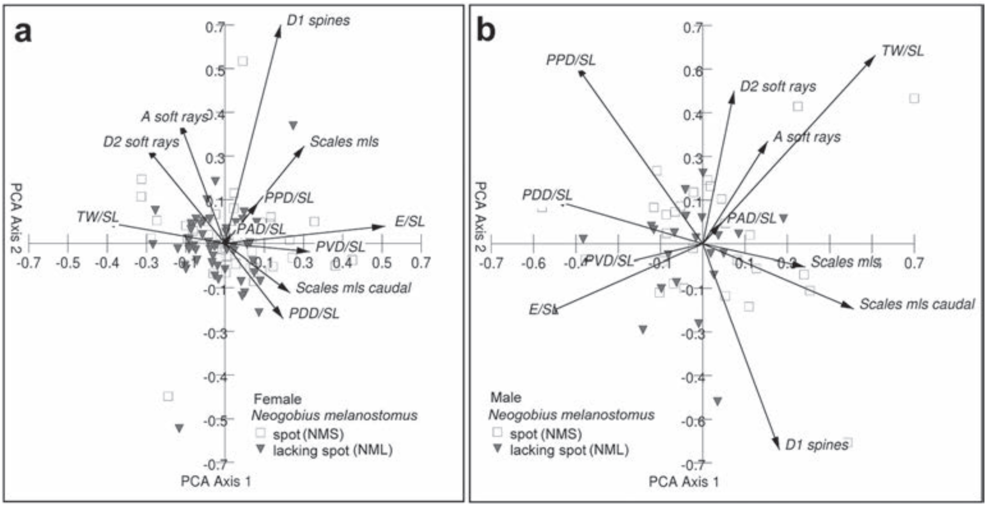

Fig. 6: Principal component analysis (PCA) of eleven morphometric and meristic variables of round gobies *Neogobius melanostomus* with spot (NMS; white square) and lacking spot (NML; grey triangle) from lower River Weser, captured in 2014. **a** Males (NMS n = 28, NML n = 21) Axis 1 17.8 %, eigenvalue 2.0; Axis 2 16.2 %, eigenvalue 1.8. **b** Females (NMS n = 47, NML n = 55) Axis 1 15.6 %, eigenvalue 1.5; Axis 2 15.0 %, eigenvalue 1.5.

Abb. 6: Hauptkomponentenanalyse (PCA) von elf morphometrischen und meristischen Variablen der Schwarzmundgrundel *Neogobius melanostomus* mit Fleck (NMS, weißes Quadrat) und ohne Fleck (NML, graues Dreieck) aus der Unterweser, gefangen 2014. **a** Männchen (NMS n = 28, NML n = 21) Achse 1 (Axis 1) 17,8 %, Eigenwert 2,0; Achse 2 (Axis 2) 17,8 %, Eigenwert 2,0. **b** Weibchen (NMS n = 47, NML n = 55) Achse 1 15,6 % Eigenwert 1,5; Achse 2 15,0 %, Eigenwert 1,5.

area (Cavender et al. 1996; Marsden et al. 1996). Interestingly the ratio between NMS and NML was almost similar in the lower River Weser and Lake Erie/North America (lower River Weser: total catch 488 round gobies, 78 % NMS and 22 % NML; Lake Erie: total catch 245 round gobies, 80 % NMS and 20 % NML) (Cavender et al. 1996). Similarities in the results between the studies from Cavender et al. (1996) and this study are likely not coincidental. Several explanatory models can be taken in consideration, such as: (1) the introduction from specimens from Lake Erie to River Weser by ships acting as vectors, (2) founder effects where a mutation might have influenced the abundance of individuals with the described variation of pigmentation of the dorsal fin, (3) undervaluation of the prevalence of natural variation of *N. melanostomus*, (4) selective pressure which might favor a reduction of pigmentation when colonizing sandy habitats, (5) perturbation of melanoblast development, melanoblast migration or differentiation of melanophores during ontogeny and (6) hybridization.

The coherencies cannot be revealed for the moment. Transoceanic invasion corridors for alien species, such as e.g. *Petricola pholadiformis*, by ships acting as vectors have been point of discussion in the past decade (Gollasch et al. 2006). A premise for an introduction of NML from Lake Erie/North America would be an interconnecting corridor between Lake Erie/North America and the River Weser. In fact there are regular seaborne transfers between harbours in the St. Lawrence River/Canada, such as the ports of Quebec and Montreal, and the port of Bremerhaven/Germany in the lower River Weser. Despite an interconnection between the St. Lawrence River and Lake Erie (via the St. Lawrence Seaway) an introduction from NML of Lake Erie/North America to the lower River Weser is questionable, as transoceanic vessels do not pass Lake Erie but load and unload the cargo in the ports of the St. Lawrence River or the Gulf of St. Lawrence only, thus minimizing the possibility of introduction via a transoceanic corridor.

The fact that Leslie & Timmins (2004) reported the absence of a black spot on D1 for "many" specimens under 9 mm SL favors the hypothesis of a perturbation of melanoblast development, melanoblast migration or differentiation of melanophores during ontogeny. To our knowledge NML have not been reported from native habitats in the Ponto Caspian region so far. The different environmental conditions of native habitats and invaded regions might influence the developmental rate and thereby disturb the formation of the black spot on the dorsal fin. Heterochrony, a change in developmental rate and timing (Hall 1984) is assumed to trigger variation in morphology (Gould 1977), such as alterations of skeletal characters in teleosts by restructuring, loss and gain of elements (Kapitanova & Shkil 2014). So far the mechanisms underlying changes in pigmentation pattern are still poorly understood (Painter 2001), but hypothesizing that the developmental rate influences the pigmentation of the dorsal fin in *N. melanostomus,* we would have expected a pigmentation variation of the entire D1. Beside the lack of the black spot on the first dorsal fin in NML, examined in this study, the pigmentation of D1 was regular and could not be distinguished from the first dorsal fin of NMS (fig. 1).

Suggesting hybridization as a possible explanation for the lacking spot on D1 of NML presumes the presence of potential hybridization partners for *N. melanostomus* in the lower River Weser. Lindner et al. (2013) revealed two specimens of hybrids of *N. melanostomus* and *N. fluviatilis* catched in the River Rhine, one exhibiting a pale black spot on D1 while the other individual lacked the conspicuous spot. To our knowledge *N. fluviatilis* has not been recorded in the River Weser yet. So far *Gobiosoma bosc*, *Babka gymnotrachelus*, *Pomatoschistus microps* and *Pomatoschistus minutus* have been recorded in the River Weser basin. Males of *P. microps* and *P. minutus* both exhibit a black spot on D1 and thus it would be presumable that in male hybrids of *P. microps* or *P. minutus* and *N. melanostomus* a black spot on the dorsal fin would be present as well. *G. bosc* and *B. gymnotrachelus* could be considered as potential hybridization partners but the abundance of *G. bosc* in the River Weser basin appears to be low (Thiel et al. 2012) and only a single specimen of *B. gymnotrachelus* was recorded (Meyer, LAVES Dez. Binnenfischerei, pers. comm. 2013), thus reducing the probability of hybridization.

As the scenarios described above require a relatively high number of complex steps to ensure the preconditions, we favor the suggestion that the lack of a conspicuous black spot on D1 in about 20 % of *N. melanostomus* from the lower River Weser is promoted by an underevaluated prevalence of natural variation in pigmentation on the dorsal fin. The abundance of NML might additionally be triggered by the selective pressure, due to predation, when colonizing sandy habitats.

In a next step a comparative study between round gobies of Lake Erie and those of the lower River Weser, a DNA analysis and breeding experiments might elucidate the causal relation and inheritability of pigmentation variation in the first dorsal fin of the round gobies from the lower River Weser.

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