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Here to stay: evaluating establishment of eastern tubenose goby *Proterorhinus semipellucidus* (Kessler, 1877) in the Baltic Sea with otolith microchemistry and structure

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Abstract In 2020, the presence of a novel non-indigenous species, eastern tubenose goby *Proterorhinus semipellucidus* (Kessler, 1877), was documented in the Gulf of Finland, Baltic Sea. As tubenose goby invasion may have a comprehensive ecological impact on the local ecosystem, it has to be confirmed whether the species has established an independently reproducing population or persists through continuous invasions in this area. We examined the otolith microstructure to determine the age of specimens sampled from the southern and northern coasts of the Gulf of Finland. Additionally, otolith microchemistry analysis was carried out on the southern coast specimens. Otolith microstructure revealed the population's age structure, showing the presence of different age classes, including younger individuals. The microchemistry analyses suggested that the specimens had hatched in brackish water salinities similar to those found in the sampling areas. This indicates local reproduction instead of introduction from the virtually freshwater Neva Bay, which is most likely the donor area of the studied populations. These data confirm the establishment of a self-sustaining reproducing population of eastern tubenose goby in the Gulf of Finland, eastern part of the brackish Baltic Sea.

Keywords Gulf of Finland · Brackish environment · Non-indigenous species · Otolith studies · Total length back calculations · Tubenose goby

Introduction

To date, there are more than 200 non-indigenous species recorded from the Baltic Sea, and the number of invasions continues to increase (AquaNIS 2023). One of the newest known invaders is eastern tubenose goby *Proterorhinus semipellucidus* (Kessler 1877), which was first detected in the Gulf of Finland in 2020 and identified based on both morphological and molecular data (Truuverk et al. 2021). However, not every newly discovered invader establishes in the area, i.e., does achieve an independently reproducing population (Soto et al. 2024). Determination of the establishment of a non-indigenous species in a region is crucial for the assessment of the possible impact and further spread of invasion. It can be assumed that a species that has rapidly established an

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independently reproducing population has the potential to spread further. In the genus *Proterorhinus* Smitt, 1900, novel populations have been reported to establish within two (Slynko 2010) to 5 (Uspenskiy 2020) years, yet it has to be noted that the length of confirmed establishment may depend on the time gap between the actual introduction, first records, and further research. Therefore, confirming the establishment of eastern tubenose goby in the Gulf of Finland is crucial for studying its ecological impact.

Many of the Baltic Sea invaders originate from the Ponto-Caspian region (Black Sea, Sea of Azov, Caspian Sea), including eastern tubenose goby, which most likely reached the Baltic Sea through the northern invasion corridor (see Bij De Vaate et al. 2002). This new invader holds many characteristics typical of gobiids and other species from the original area. These characteristics often contribute to the success of invasion and establishment, making Ponto-Caspian species well-known non-indigenous species in the Northern Hemisphere (e.g., Verliin et al. 2017; Copilaş-Ciocianu et al. 2023). One such trait is a fast generation cycle: species of the genus *Proterorhinus* reach maturity within the first year, consequently, 1 + fish are ready to breed in the spring after they have hatched (Valová et al. 2015). Furthermore, tubenose gobies are typical batch spawners, ensuring the success of the breeding period (Grabowska et al. 2019). The species of the genus *Proterorhinus* are quite flexible in their salinity tolerance (Cuthbert and Briski 2021) and food requirements (Ondračková et al. 2019). Also, passive larval drift helps them pass through unsuitable environmental conditions and widen their distribution (Kocovsky et al. 2011). These abilities contribute significantly to the dispersal and development of independently reproducing populations and, therefore, to the success of establishment.

Up to now, the eastern tubenose gobies have been described to inhabit the southern shore of the Gulf of Finland in the Baltic Sea, at least since 2020 (Truuverk et al. 2021). There have been no studies conducted on the invasion status in other parts of the brackish Gulf of Finland. While tubenose gobies invaded the easternmost part of the Gulf of Finland (essentially freshwater Neva Bay) in 2006 or earlier, the species was identified as *P. marmoratus* (Pallas, 1814) (Antsulevich 2007). However, in 2021, the tubenose gobies in Neva Bay were identified as *Proterorhinus semilunaris* (Heckel, 1837) (Demchuk et al. 2021). Yet, the species were determined solely based on morphological characters (Antsulevich 2007; Demchuk et al. 2021), which can be misleading in this genus (e.g., Neilson and Stepien 2009). Truuverk et al. (2021) identified the species in the southern shore of the Gulf of Finland as *Proterorhinus nasalis* (De Filippi, 1863) based on molecular data. However, other recent sources consider the species spreading through the northern invasion corridor to be *P. semipellucidus* (Zarei et al. 2021, 2022). Moreover, Zarei et al. (2021) attribute the name *P. nasalis* exclusively to the material recorded from the Southern Caspian Sea basin (cf. Zarei et al. 2021: fig. 3), despite the type specimen originating from nearby Baku (Azerbaijan) (Canestrini et al. 1862). Nevertheless, the Eschmeyer's Catalog of Fishes (Fricke et al. 2023) suggests *P. nasalis* for the species recorded by Truuverk et al. (2021), whereas *P. semipellucidus* is simultaneously considered an invasive species in the Volga River basin and the northeastern Baltic Sea. This discrepancy in naming has led to confusion regarding the appropriate species designation. In accordance with Echmeyer's Catalog of Fishes, we refer to the species spreading in the Gulf of Finland as *Proterorhinus semipellucidus* (Kessler, 1877)—eastern tubenose goby. Certainly, the current status appears ambiguous and requires clarification through future research.

The invasion process, including establishment, should be documented and studied thoroughly, to prevent drawing wrong conclusions (García-Berthou 2007). Although establishment is usually affirmed by the abundance of YOY (young-of-the-year) fish (Uspenskiy 2020), the possibility of larval drift may lead to wrong conclusions especially when the donor population is located nearby. Considering the challenges in the species determination in the genus *Proterorhinus*, it is possible that the tubenose gobies inhabiting Neva Bay and named as *P. marmoratus* sensu lato (Uspenskiy 2020) may also be the same taxon as described by Truuverk et al. (2021). Therefore, as the conspecificity of the populations cannot be ruled out, possible larval drift to Estonian and Finnish waters from the Neva Bay population cannot be ruled out either. The aim of the present study is to clarify the status of invasion and reveal the possible establishment of eastern tubenose goby in the Gulf of Finland. In addition, the study intends to provide preliminary knowledge for future research. Hereby, we combine age structure and microchemistry analyses to assess the state of eastern tubenose goby invasion in the Gulf of Finland. Age structure analysis is the basis for the typical assessment of establishment, where the presence of younger specimens and different age classes indicate a self-sustaining population (e.g., Slynko 2010). Microchemical analysis aimed to determine whether there is a trace of freshwater, which would provide evidence of larval drift from Neva Bay. This is a novel approach to estimate the establishment of non-indigenous species in the Baltic Sea.

Materials and methods

During fieldwork at the Kalvi sampling site on the northern coast of Estonia (Fig. 1), 35 eastern tubenose gobies were collected. Of these, 11 were designated for back-calculation studies, 7 for otolith studies, and 17 for other research purposes. Additionally, 22 tubenose gobies were collected from Finnish sampling sites (Fig. 1), with 8 used in this study and 14 in other research. The total length of the specimens ranged from 16 to 75 mm. Seven specimens from the northern coast of Estonia and eight specimens from the southern coast of Finland underwent age determination based on the examination of otolith microstructure. Microchemistry analysis was performed exclusively on the Estonian specimens.

The specimens were caught on 9 and 10 June 2022 from Syvänhiekanpohja (60.48278N; 27.41106E) and Karjalanniemi (60.48946N; 27.65976E) in Finland and on the 23rd of September 2022 from Kalvi near the mouth of Padajõgi river (59.49682N; 26.77614E) at salinity 5.65 PSU in Estonia (Fig. 1, Table 1). Fish were caught with a beach seine in Finland and a push net in Estonia. The seine's net dimensions were 1.3 m in height, 2.2 m in length, and 1.2 m in width. Fifteen-meter-long and 1.3-m-wide wings surrounded the seine mouth on each side. Both wings had a mesh size of 10 mm for the first 8 m, decreasing to 5 mm

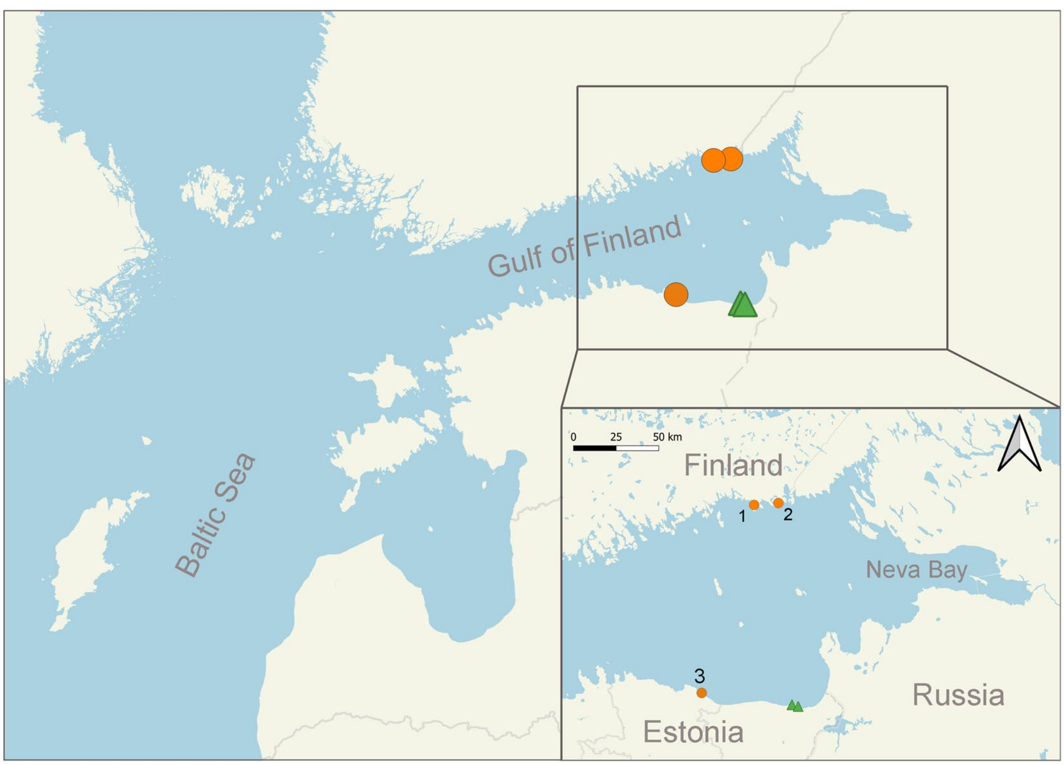

Fig. 1 Sampling sites in Syvänhiekanpohja (1) and Karjalanniemi (2) on the northern coast and Kalvi (3) on the southern coast of the Gulf of Finland (orange dots) together with first findings of eastern tubenose gobies in the Gulf of Finland (green triangles). Basemap: Estonian Land Board

Table 1 Studied specimens. *TL* total length, R_o otolith radius. Fish numbers correspond to the original numbering of the specimens

Fish nr	Age (y)	TL (mm)	R_o (µm)	Sampling site	Sampling date
1	4+	66	919.1	Kalvi	23 Sep 2022
2	1+	21	492.1	Kalvi	23 Sep 2022
3	2+	42	666.6	Kalvi	23 Sep 2022
4	3+	67	980.3	Kalvi	23 Sep 2022
5	2+	49	593.6	Kalvi	23 Sep 2022
6	3+	44	397.3	Kalvi	23 Sep 2022
8	2+	39	482.4	Kalvi	23 Sep 2022
9*	2+	37	464.4	Syvänhiekanpohja	9 Jun 2022
10*	3+	43	557.2	Syvänhiekanpohja	9 Jun 2022
12*	4+	75	1088.8	Karjalanniemi	10 Jun 2022
15*	2+	36	417.9	Karjalanniemi	10 Jun 2022
27*	2+	38	505.2	Karjalanniemi	10 Jun 2022
28*	3+	37	498.6	Karjalanniemi	10 Jun 2022
29*	1+	27	311.2	Karjalanniemi	10 Jun 2022

*Asterisks denote fish whose TL were corrected for the fixation in ethyl alcohol

near the cod-end, which was cone-shaped and had a mesh size of 2 mm. Using 15 m ropes, the seine was hauled towards or along the shore with a mean hauled area of 392 m^2. The push net was 0.62 m in height and 0.82 m in length, with mesh size of 3 mm and a mean hauled area of 23 m^2.

Specimens from Kalvi were retained frozen for further analysis, while fish from Finland were preserved in ethyl alcohol (96.5%) since microchemistry analysis was not originally planned. Afterwards, frozen specimens were thawed, total lengths (TL) of all fish were measured, and otoliths (sagitta) were removed, dried with paper, and stored in microtubes until further preparation in October and November 2022. The choice of left or right otolith was made randomly. Individual otoliths were manually grinded with abrasive paper (grit size P2500) until the core area was exposed and finally polished (grit size P4000). Otolith thin sections were mounted on a microscope slide with a drop of Loctite superglue.

Microchemical analysis was conducted using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) at the University of Tartu, Department of Geology. Firstly, otoliths were ultrasonically cleaned in NanoPure water for 15 min and air-dried. Thereupon, LA-ICP-MS was used to measure ^{43}Ca, ^{55}Mn, ^{88}Sr, ^{137}Ba signals which were later reduced to element:Ca in mmol/mol using NIST612 as reference material following the method described in Rohtla et al. (2014). Continuous line scans were traced from otoliths' core to edge with scanning speed of 5 µm/s and spot size of 40 µm. A nine-point running mean was used to reduce the noise and smooth the data.

Finally, otoliths were repolished, etched, and stained for age estimation. Neutral red solution (0.8%) was used based on the standard procedures (Richter and McDermott 1990). Otoliths were photographed and otolith radii measured using a stereomicroscope (model SZX10, Olympus Corporation, external light source Olympus KL 1500 LED) with×94.5 magnification. Otolith growth rings were blindly counted twice by three independent readers to reach consensus. If differences persisted, the otolith was discarded, constituting 1 otolith out of 15. Growth rings were compared to the otolith elemental profiles to expose potential patterns and movements of fish, as well as environmental conditions the fish had lived in. Linear regression analysis was used to explore relationships between fish total length (TL), age, and otolith radius (Ro).

It is known that fixation in ethyl alcohol reduces individual TL of fish (Melo et al. 2010). Eleven eastern tubenose gobies from the Kalvi sampling point were preserved in ethyl alcohol to assess the precise effect of fixation on the species' TL. These individuals were exclusively employed to examine TL variations and were not involved in otolith studies. Length changes were measured over a 4-week period in October 2022 using a measuring board with a 1-mm accuracy. After a month of being preserved in ethyl alcohol, the length decrease of the studied fish ceased (Fig. 2).

Friedman ANOVA was used to assess the changes in fish total length over time, revealing a significant

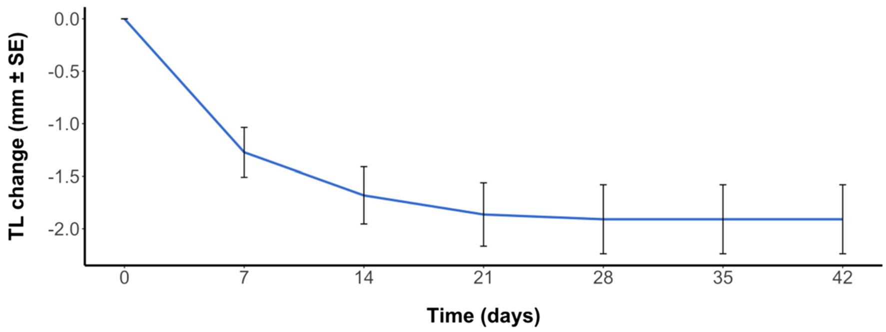

Fig. 2 Effect of ethanol fixation on the total length (TL) of eastern tubenose gobies

difference among the treatment conditions (χ^2=35.19; df=4; $p<0.001$). Regression analysis was conducted to establish a relationship between fish original TL and the TL after being fixed for 4 weeks. This allowed for future back calculations in subsequent studies. The equation obtained from the regression analysis ($y=0.76+0.93x$; R^2=0.998; n=11; where x represents the original TL and y represents the fixed TL) was utilized for corrected total length in this study (Fig. 3). This statistical measure of precision allowed for the estimation of their original total length based on observed data. Wilcoxon signed-rank test showed a significant difference between original measurements after fixation and corrected length (n=11; W=0; $z=-2.93$; p=0.003), prompting the use of corrected total length instead of original measurements for further analysis of relationships (Table 1, fish nr 9–29).

Results

TL of all the studied specimens differed between 21 and 74.4 mm (mean 44.3 mm ± SD 15.2). The radius of otoliths ranged from 311 to 1088 µm, with 598.2 µm as the mean (Table 1, Fig. 4). Relationships between fish age and total length (R^2=0.73; $p<0.0001$) (Fig. 5), otolith radius and total length (R^2=0.83; $p\leq 0.0001$), and otolith radius and age (R^2=0.52; p=0.004) were all significant (Fig. 6). The age of the studied specimens varied from 1+ to 4+, the median age being 2+. No YOY eastern tubenose gobies were detected.

There was no trace of freshwater in the otolith strontium to calcium (hereinafter abbreviated as Sr:Ca) profiles of eastern tubenose gobies collected from Kalvi. Sr:Ca concentration smoothed values (blue line Fig. 7) stayed between 2.2 and 5.0 mmol/mol, which are typical brackish water values (e.g., Rohtla et al. 2017; Matetski et al. 2022). In most of the otoliths Sr:Ca concentrations increased slightly in the outer parts and fluctuated clearly (Fig. 7). Fluctuations were also present in barium to calcium (Ba:Ca) profiles, smoothed values varying from 0.002 to 0.028 mmol/mol (Fig. 8).

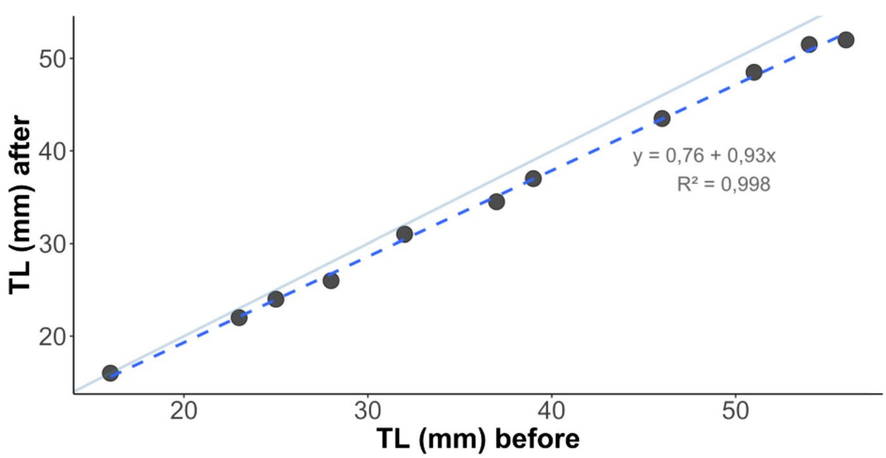

Fig. 3 Regression of fish total length (TL) before versus after fixation in ethyl alcohol (dashed blue line). The solid light blue line represents the 1:1 relationship between TL values

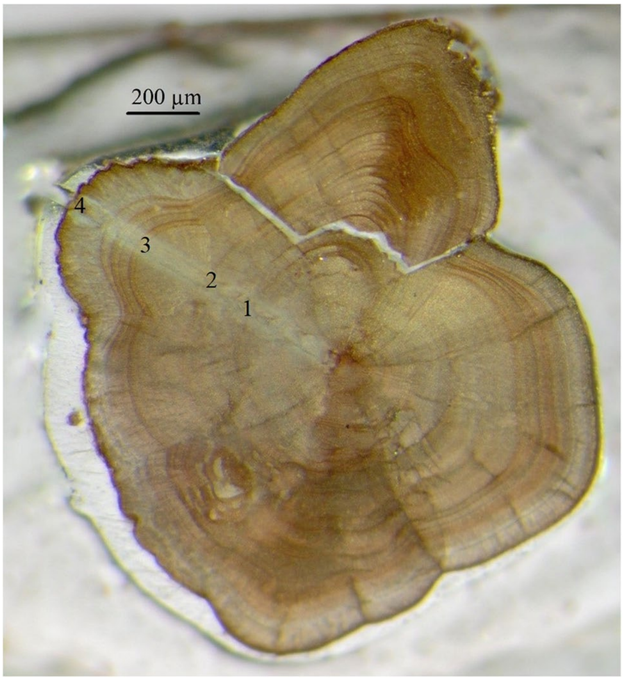

Fig. 4 Otolith nr 1 from Kalvi: age = 4 +; R_o = 919.14 µm. Numbers denote annuli

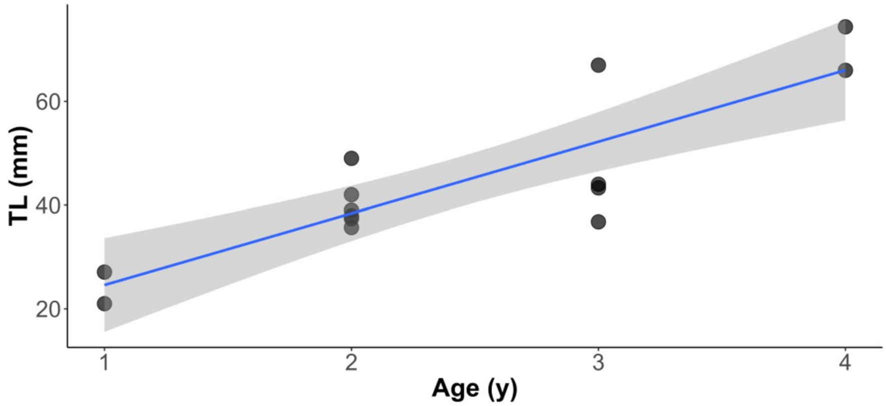

Fig. 5 Sampled fish age and total length (TL) relationship, where $R^2 = 0.73$ and $p = 0.0001$. The gray area marks 95% confidence intervals

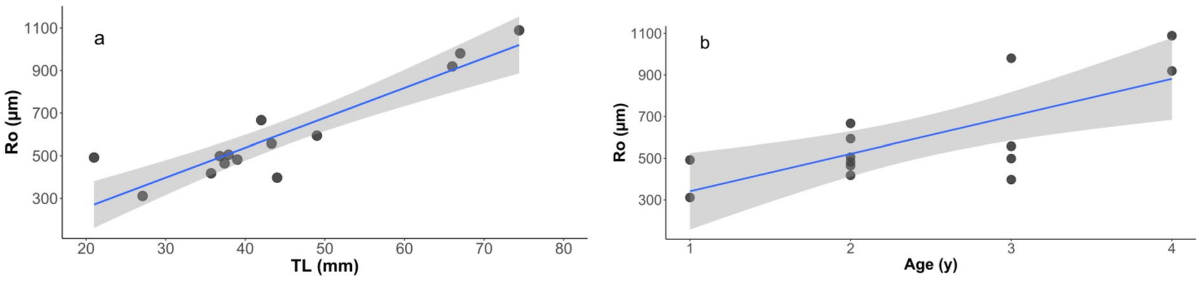

Fig. 6 **a** Otolith radius (*R*o) and fish total length (TL) relationship ($R2 = 0.83$; $p < 0.0001$). **b** *R*o and fish age relationship ($R2 = 0.52$; $p = 0.004$). Gray areas mark 95% confidence intervals

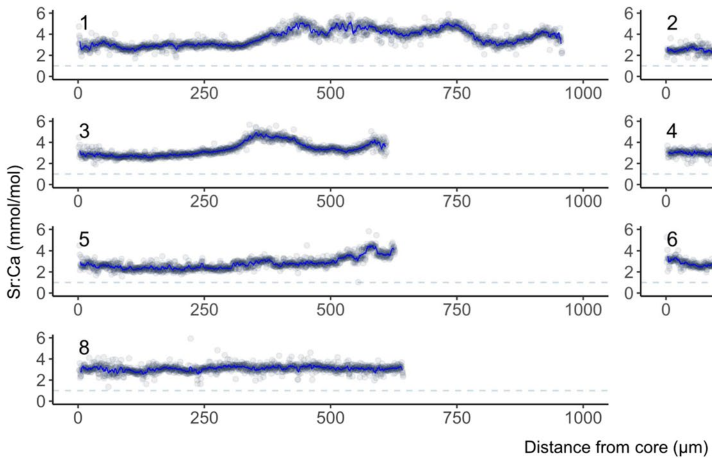

Fig. 7 Otolith Sr:Ca profiles of fish from different age groups. Age 1+ = fish nr 2; age 2+ = fish nr 3, 5, 8; age 3+ = fish nr 4, 6; age 4+ = fish nr 1. Light blue dashed lines represent the estimated maximum boundary for freshwater values in the Gulf of Finland

Discussion

The age structure and otolith elemental composition of eastern tubenose gobies, caught from Kalvi, indicate that the species has established along the southern coast of the eastern part of brackish Gulf of Finland. Several different year classes were found with the presence of 1- and 2-year-old individuals. Furthermore, otolith microchemistry indicated that all the analyzed fish had hatched in brackish water. The population age structure of eastern tubenose goby on the northern shore of the Gulf of Finland was similar to that of the Estonian population. Comparing the distances of populations from the potential introduction path, it is therefore highly likely that the population has become established in the eastern part of the Gulf of Finland as a whole.

No YOY eastern tubenose gobies were detected during the present study. This may have been caused by suboptimal sampling dates (Valová et al. 2015) and mesh size. In June, genus *Proterorhinus* specimens have not yet hatched, while in September, the YOY are still too small and may escape through the mesh of used gear. However, the presence of various size

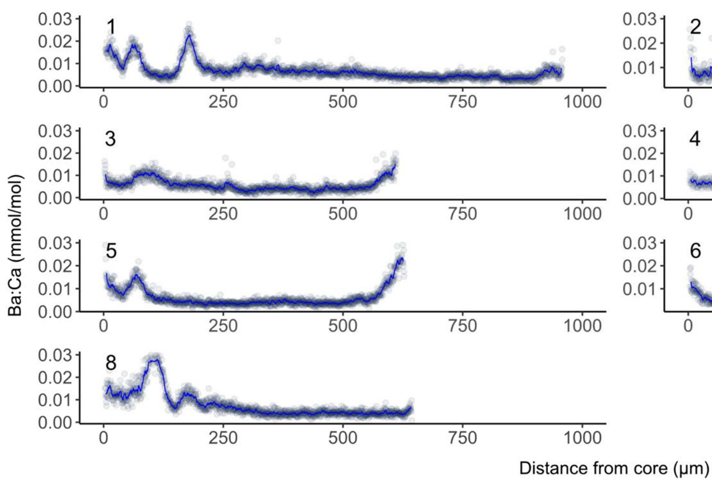

Fig. 8 Otolith Ba:Ca profiles of fish from different age groups. Age 1+ = fish nr 2; age 2+ = fish nr 3, 5, 8; age 3+ = fish nr 4, 6; age 4+ = fish nr 1

and year classes in both spring and autumn (Table 1) can be considered substantial evidence, as indicated by previous studies (e.g., Kocovsky et al. 2011), supporting the proposition that persistent reproduction of eastern tubenose gobies occurs in the Gulf of Finland. This finding aligns with the observed length ranges in other habitats invaded by the genus, such as the Dyje River in Czech Republic (Valová et al. 2015), The Great Lakes in the USA (Kocovsky et al. 2011), or Neva Bay in Russia (Uspenskiy 2020).

In 2020, presence of eastern tubenose gobies was confirmed for the first time in the brackish Gulf of Finland (Truuverk et al. 2021). However, the otolith growth rings of the presently studied specimens revealed that the oldest were 4 + years old at capture. It is therefore likely that the species was already present at least a few years before its discovery. Tubenose gobies typically have a lifespan of 2–4 years (Harka and Farkas 2006); thus, it is impossible to determine the actual date of their settlement in the Gulf of Finland based on specimens captured in the present study.

While Sr:Ca ratios in otolith microchemistry correlate positively with the salinity in the environment (Elsdon et al. 2008), the Ba:Ca ratio shows a negative correlation with environmental salinity (Macdonald and Crook 2010). The otolith chemical profiles did not provide evidence of the prior presence of the sampled fish from Kalvi in freshwater conditions, as such circumstances would have yielded a significantly lower Sr:Ca ratio and markedly higher Ba:Ca ratio (e.g. Rohtla et al. 2014; Matetski et al. 2022). Tubenose gobies are nest spawners, meaning that larvae hatch at the same location where the eggs were fertilized (Grabowska and Przybylski 2015). The investigated individuals hatched in a saline environment, and it is probable that at least some of them have migrated between distinct water masses characterized by varying Sr:Ca ratios and therefore salinities. Sr:Ca concentration conspicuous fluctuations can also be explained by the variability in sampling site conditions at Kalvi. Fish were caught near Padajõgi river mouth, where prevailing winds can direct river water westward or eastward, affecting salinity levels in the area. Geochemical variations in inflowing river water can also contribute to changes in Sr:Ca and Ba:Ca ratios in the coastal sea and, consequently, fish otoliths (Vu et al. 2021).These factors can therefore influence fluctuations in the local salinity of nearby shallow coastal waters. Tubenose gobies hatch in shallow seas and migrate to winter in deeper waters (Valová et al. 2015). Hence, Sr:Ca fluctuations may also denote the seasonal movement of fish from the lower Sr:Ca shallow water to the higher Sr:Ca deep water (Tzeng et al. 1999; Rohtla unpublished data). Additionally, during winter when ice cover is present, it is likely that the influence of river water becomes more localized, as wind and wave action have less impact on the water.

While otolith Sr:Ca and water salinity have a positive relationship, otolith Ba:Ca concentration decreases with higher salinities (Macdonald and Crook 2010). Based on the studied otoliths, it seems that Sr:Ca and Ba:Ca profiles are in some way contrary: while the Sr:Ca concentration ratio is stable, Ba:Ca fluctuates and vice-versa. Otolith Ba:Ca ratios may also be influenced by fish diet composition (Elsdon et al. 2008) or seasonal variations in vegetation (Fisher et al. 1991). Nevertheless, no fundamental conclusions can be drawn about the fluctuations.

Generalist species typically succeed in variable environments. Tubenose gobies' high plasticity gives them an advantage in the rather vulnerable, semi-enclosed, low-diversity Baltic Sea. They can switch between different feeding strategies depending on the season and environmental conditions (Ondračková et al. 2019). This makes them more resilient to changes in their habitat and increases their chances of survival. Furthermore, their ability to adapt quickly may give them a competitive edge over native species. Given that tubenose goby and round goby (*Neogobius melanostomus* (Pallas, 1814)) occupy slightly different niches, their combined impact could potentially be more detrimental to local ecosystems (e.g., Mikl et al. 2017; Vašek et al. 2014). Whereas the round goby has dwelled in the coastal seas of Estonia and Finland for over 20 years, the performance and repercussions of eastern tubenose goby have yet to be revealed.

The obtained results suggest the establishment of eastern tubenose goby in the brackish Gulf of Finland. Still, additional research and the inclusion of other sampling points are needed to clarify the patterns observed in otolith elemental profiles as well as distribution and spreading of the species in the region. At least, current results indicate that eastern tubenose goby population in the Gulf of Finland is likely to persist and spread onwards in the Baltic Sea.

Author contribution Katariina Kurina: research conceptualization; sample design and methodology; investigation and data collection; data analysis and interpretation; and writing original draft, reviewing, and editing.

Mehis Rohtla: sample design and methodology, investigation, and data collection; data analysis and interpretation; and writing original draft, reviewing, and editing.

Imre Taal: sample design and methodology; investigation and data collection; data analysis and interpretation; and writing original draft, reviewing, and editing.

Anu Albert: sample design and methodology; investigation and data collection; and writing original draft, reviewing, and editing.

Roland Svirgsden: sample design and methodology; data analysis and interpretation; and reviewing and editing.

Sanna Kuningas: investigation and data collection and reviewing and editing.

Antti Lappalainen: investigation and data collection and reviewing and editing.

Redik Eschbaum: funding provision and reviewing and editing.

Lauri Saks: research conceptualization; sample design and methodology; investigation and data collection; data analysis and interpretation; ethics approval; and writing original draft, reviewing, and editing.

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Data availability All the raw data is available on request from the corresponding author.

Declarations

Competing interests The authors declare no competing interests.

References

AquaNIS (2023) Information system on aquatic non-indigenous and cryptogenic species. World Wide Web electronic publication. www.corpi.ku.lt/databases/aquanis. Accessed 27 Oct 2023

Antsulevich A (2007) First records of the tubenose goby *Proterorhinus marmoratus* (Pallas, 1814) in the Baltic Sea. Aquat Invasions 2:468–470. https://doi.org/10.3391/ai.2007.2.4.23

Bij De Vaate A, Jazdzewski K, Ketelaars HAM, Gollasch S, Van Der Velde G (2002) Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. Can J Fish Aquat Sci 59:1159–1174. https://doi.org/10.1139/f02-098

Canestrini G, Doria G, Ferrari PM, Lessona M (1862) Archivio per la zoologia, l'anatomia e la fisiologia /. s.l., [Genova . https://doi.org/10.5962/bhl.title.52252. (In Italian)

Copilaș-Ciocianu D, Sidorov D, Šidagytė-Copilas E (2023) Global distribution and diversity of alien Ponto-Caspian amphipods. Biol Invasions 25:179–195. https://doi.org/10.1007/s10530-022-02908-1

Cuthbert RN, Briski E (2021) Temperature, not salinity, drives impact of an emerging invasive species. Sci Total Environ 780:146640. https://doi.org/10.1016/j.scitotenv.2021.146640

Demchuk AS, Uspenskiy AA, Golubkov SM (2021) Abundance and feeding of fish in the coastal zone of the Neva Estuary, eastern Gulf of Finland. Boreal Env Res 26:1–16

Elsdon TS, Wells BK, Campana SE, Gillanders BM, Jones CM, Limburg CM, Secor DH, Thorrold SR, Walther BD (2008) Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences. Oceanogr Mar Biol 46:297–330

Fisher NS, Guillard RRL, Bankston DC (1991) The accumulation of barium by marine phytoplankton grown in culture. J Mar Res 49:339–354

Fricke, R, Eschmeyer, WN, Van der Laan, R (eds) (2023) Eschmeyer's catalog of fishes: genera, species, references. https://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp. Accessed 8 Mar 2024

García-Berthou E (2007) The characteristics of invasive fishes: what has been learned so far? J Fish Biol 71:33–55. https://doi.org/10.1111/j.1095-8649.2007.01668.x

Grabowska J, Przybylski M (2015) Life-history traits of non-native freshwater fish invaders differentiate them from natives in the Central European bioregion. Rev Fish Biol Fish 25:165–178. https://doi.org/10.1007/s11160-014-9375-5

Grabowska J, Błońska D, Marszał L, Przybylski M (2019) Reproductive traits of the established population of invasive western tubenose goby, Proterorhinus semilunaris (Actinopterygii: Perciformes: Gobiidae), in the Vistula River, Poland. AIeP 49:355–364. https://doi.org/10.3750/AIEP/02642

Harka A, Farkas J (2006) Growth and spawning period of the tubenose goby (*Proterorhinus marmoratus* (Pallas, 1811) in Lake Tisa (Eastern Hungary). Oesterr Fisch 59:194–201

Kocovsky PM, Tallman JA, Jude DJ, Murphy DM, Brown JE, Stepien CA (2011) Expansion of tubenose gobies *Proterorhinus semilunaris* into western Lake Erie and potential effects on native species. Biol Invasions 13:2775–2784. https://doi.org/10.1007/s10530-011-9962-5

Macdonald J, Crook D (2010) Variability in Sr: Ca and Ba: Ca ratios in water and fish otoliths across an estuarine salinity gradient. Mar Ecol Prog Ser 413:147–161. https://doi.org/10.3354/meps08703

Matetski L, Rohtla M, Svirgsden R, Kesler M, Saks L, Taal I, Hommik K, Paiste P, Kielman-Schmitt M, Kooijman E, Birzaks J, Saura A, Ziņģis M, Vaittinen M, Vetemaa M (2022) Variability in stream water chemistry and brown trout (*Salmo trutta* L.) parr otolith microchemistry on different spatial scales. Ecol Freshw Fish 31:438–453. https://doi.org/10.1111/eff.12642

Melo MT, Saturnino C, Santos JNS, Vasconcellos RM, Cruz-Filho AG, Araújo FG (2010) Correction of the weight and length for juveniles *Atherinella brasiliensis* (Actinopterygii: Atherinopsidae) after fixation in formalin and preservation in ethanol. Zool (Curitiba) 27:892–896. https://doi.org/10.1590/S1984-46702010000600009

Mikl L, Adámek Z, Všetičková L, Janáč M, Roche K, Šlapanský L, Jurajda P (2017) Response of benthic macroinvertebrate assemblages to round (*Neogobius melanostomus*, Pallas 1814) and tubenose (*Proterorhinus semilunaris*, Heckel 1837) goby predation pressure. Hydrobiologia 785:219–232. https://doi.org/10.1007/s10750-016-2927-z

Neilson ME, Stepien CA (2009) Evolution and phylogeography of the tubenose goby genus *Proterorhinus* (Gobiidae: Teleostei): evidence for new cryptic species: tubenose goby speciation and phylogeography. Biol J Linn Soc 96:664–684. https://doi.org/10.1111/j.1095-8312.2008.01135.x

Ondračková M, Všetičková L, Adámek Z, Kopeček L, Jurajda P (2019) Ecological plasticity of tubenose goby, a small invader in South Moravian waters. Hydrobiologia 829:217–235. https://doi.org/10.1007/s10750-018-3833-3

Richter H, McDermott JG (1990) The staining of fish otoliths for age determination. J Fish Biol 36:773–779. https://doi.org/10.1111/j.1095-8649.1990.tb04331.x

Rohtla M, Svirgsden R, Verliin A, Rumvolt K, Matetski L, Hommik K, Saks L, Vetemaa M (2017) Developing novel means for unravelling population structure, provenance and migration patterns of European whitefish *Coregonus lavaretus* s.l. in the Baltic Sea. Fish Res 187:47–57. https://doi.org/10.1016/j.fishres.2016.11.004

Rohtla M, Vetemaa M, Taal I, Svirgsden R, Urtson K, Saks L, Verliin A, Kesler M, Saat T (2014) Life history of anadromous burbot (*Lota lota*, Linneaus) in the brackish Baltic Sea inferred from otolith microchemistry. Ecol Freshw Fish 23:141–148. https://doi.org/10.1111/eff.12057

Slynko YuV (2010) Naturalization of tubenose goby *Proterorchinus marmoratus* (Pallas, 1814) (Pisces: Perciformes: Gobiidae) in the Rybinsk water reservoir. Russ J Biol Invasions 1:26–29. https://doi.org/10.1134/S2075111710010066

Soto I, Balzani P, Carneiro L, Cuthbert RN, Macêdo R, Serhan Tarkan A, Ahmed DA, Bang A, Bacela-Spychalska K, Bailey SA, Baudry T, Ballesteros-Mejia L, Bortolus A, Briski E, Britton JR, Buřič M, Camacho-Cervantes M, Cano-Barbacil C, Copilaș-Ciocianu D, Coughlan NE, Courtois P, Csabai Z, Dalu T, De Santis V, Dickey JWE, Dimarco RD, Falk-Andersson J, Fernandez RD, Florencio M, Franco ACS, García-Berthou E, Giannetto D, Glavendekic MM, Grabowski M, Heringer G, Herrera I, Huang W, Kamelamela KL, Kirichenko NI, Kouba A, Kourantidou M, Kurtul I, Laufer G, Lipták B, Liu C, López-López E, Lozano V, Mammola S, Marchini A, Meshkova V, Milardi M, Musolin DL, Nuñez MA, Oficialdegui FJ, Patoka J, Pattison Z, Pincheira-Donoso D, Piria M, Probert AF, Rasmussen JJ, Renault D, Ribeiro F, Rilov G, Robinson TB, Sanchez AE, Schwindt E, South J, Stoett P, Verreycken H, Vilizzi L, Wang Y, Watari Y, Wehi PM, Weiperth A, Wiberg-Larsen P, Yapıcı S, Yoğurtçuoğlu B, Zenni RD, Galil BS, Dick JTA, Russell JC, Ricciardi A, Simberloff D, Bradshaw CJA, Haubrock PJ (2024) Taming the terminological tempest in invasion science. Biol Rev : brv.13071. https://doi.org/10.1111/brv.13071

Truuverk A, Taal I, Eschbaum R, Albert A, Verliin A, Kurina K, Saks L (2021) Molecular analysis reveals the invasion of eastern tubenose goby *Proterorhinus nasalis* De Filippi, 1863 (Perciformes: Gobiidae) into the Baltic Sea. BioInvasions Rec 19:701–709. https://doi.org/10.3391/bir.2021.10.3.20

Tzeng WN, Severin KP, Wickström H, Wang CH (1999) Strontium bands in relation to age marks in otoliths of European eel *Anguilla anguilla*. Zool Stud 38:452–457

Uspenskiy AA (2020) Distribution and population characteristics of the invasive tubenose goby *Proterorhinus marmoratus* (Pallas, 1814) in the eastern Gulf of Finland. Proceedings ZIN 324:459–475. https://doi.org/10.31610/trudyzin/2020.324.4.459

Valová Z, Konečná M, Janáč M, Jurajda P (2015) Population and reproductive characteristics of a non-native western tubenose goby (*Proterorhinus semilunaris*) population unaffected by gobiid competitors. Aquat Invasions 10:57–68. https://doi.org/10.3391/ai.2015.10.1.06

Vašek M, Všetičková L, Roche K, Jurajda P (2014) Diet of two invading gobiid species (*Proterorhinus semilunaris* and *Neogobius melanostomus*) during the breeding and hatching season: no field evidence of extensive predation on fish eggs and fry. Limnology 46:31–36. https://doi.org/10.1016/j.limno.2013.11.003

Verliin A, Kesler M, Svirgsden R, Taal I, Saks L, Rohtla M, Hubel K, Eschbaum R, Vetemaa M, Saat T (2017) Invasion of round goby to the temperate salmonid streams in the Baltic Sea. Ichthyol Res 64:155–158. https://doi.org/10.1007/s10228-016-0537-4

Vu AV, Baumgartner LJ, Doran GS, Mallen-Cooper M, Thiem JD, Howitt JA, Limburg KE, Gillanders BM, Cowx IG (2021) Variability in water chemistry in the Lower Mekong Basin: considerations for fish life history reconstruction. Estuar Coast Shelf Sci 255:107355. https://doi.org/10.1016/j.ecss.2021.107355

Zarei F, Esmaeili HR, Schliewen UK, Abbasi K (2022) Taxonomic diversity and distribution of the genus *Proterorhinus* (Teleostei: Gobiidae) in the Caucasus biodiversity hotspot with conservation implications. Aquat Conserv 32:129–138. https://doi.org/10.1002/aqc.3728

Zarei F, Esmaeili HR, Schliewen UK, Abbasi K, Sayyadzadeh G (2021) Mitochondrial phylogeny, diversity, and ichthyogeography of gobies (Teleostei: Gobiidae) from the oldest and deepest Caspian sub-basin and tracing source and spread pattern of an introduced *Rhinogobius* species at the tricontinental crossroad. Hydrobiologia 848:1267–1293. https://doi.org/10.1007/s10750-021-04521-0