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RESEARCH ARTICLE

Spatial and intra-host distribution of myxozoan parasite *Tetracapsuloides bryosalmonae* among Baltic sea trout (*Salmo trutta*)

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Abstract

Proliferative kidney disease caused by the myxozoan parasite *Tetracapsuloides bryosalmonae* has been actively studied in juvenile salmonids for decades. However, very little is known about parasite prevalence and its geographical and intra-host distribution at older life stages. We screened *T. bryosalmonae* among adult sea trout (*Salmo trutta*) ($n = 295$) collected along the Estonian Baltic Sea coastline together with juvenile trout from 33 coastal rivers ($n = 1752$) to assess spatial infection patterns of the adult and juvenile fish. The parasite was detected among 38.6% of adult sea trout with the prevalence increasing from west to east, and south to north, along the coastline. A similar pattern was observed in juvenile trout. Infected sea trout were also older than uninfected fish and the parasite was detected in sea trout up to the age of 6 years. Analysis of intra-host distribution of the parasite and strontium to calcium ratios from the otoliths revealed that (re)infection through freshwater migration may occur among adult sea trout. The results of this study indicate that *T. bryosalmonae* can persist in a brackish water environment for several years and that returning sea trout spawners most likely contribute to the parasite life cycle by transmitting infective spores.

KEYWORDS
anadromous salmonid, aquatic pathogens, brown trout, endoparasite, sea trout

1 | INTRODUCTION

Tetracapsuloides bryosalmonae is a malacosporean endoparasite and causative agent of proliferative kidney disease (PKD) among salmonid fish (Canning et al., 1998). In Europe, native brown trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*), European grayling (*Thymallus thymallus*), Arctic charr (*Salvelinus alpinus*) and European whitefish (*Coregonus lavaretus*), as well as non-native rainbow trout (*Oncorhynchus mykiss*), chinook salmon (*Oncorhynchus tshawytscha*), cutthroat trout (*Oncorhynchus clarkii*), pink salmon (*Oncorhynchus gorbuscha*) and brook trout (*Salvelinus fontinalis*) are susceptible to *T. bryosalmonae* (for review see Ros et al., 2022). The parasite has a two-host life cycle, cycling between freshwater bryozoans and salmonids (Anderson et al., 1999). Fish become infected when *T. bryosalmonae*

spores enter through the skin or gills (Longshaw et al., 2002). After entering the vascular system, extrasporogonic parasite stages multiply and reach kidney interstitium, provoking an inflammatory response and damage to kidney tissue (Okamura et al., 2011). Clinical symptoms, such as pale and anaemic gills, abdominal swelling, renal proliferation and anaemia develop typically within 4–8 weeks after infection if the water temperature is above 15°C (Clifton-Hadley et al., 1986; Ros et al., 2021; Wahli et al., 2002). After proliferation in the kidney interstitium, some extrasporogonic stages of the parasite migrate into the kidney tubules of the fish and remain there, even after recovery of clinical PKD (Morris et al., 2000). When sporogenesis is complete, fish excrete mature spores via urine (Hedrick et al., 2004). Subsequently, these parasite spores infect bryozoans, the primary host of *T. bryosalmonae*, which then completes the life cycle (Morris & Adams, 2007).

PKD primarily affects juvenile salmonids during their first growth season in freshwater habitat (Dash & Vasemägi, 2014; Mo et al., 2011; Schager et al., 2007). Infections can also occur among older fish who have not been previously exposed to the parasite (Feist & Longshaw, 2006; Lauringson et al., 2022). However, compared with young-of-the-year fish, older specimens are typically less susceptible to the disease (Bailey et al., 2021). While surviving juveniles acquire resistance against subsequent infections, extrasporogonic stages of the parasite stay in kidney tubules for a prolonged period, resulting in surviving fish becoming long-term carriers of the parasite (Abd-Elfattah et al., 2014; Morris et al., 2000; Okamura et al., 2011; Soliman et al., 2018). It has been suggested that this subclinical phase of PKD, with the production of infectious spores, can continue indefinitely among brown trout and grayling (Okamura et al., 2011). Recent experimental investigations have revealed that brown trout can be the carrier of *T. bryosalmonae* for up to 5 years (Soliman et al., 2018). Specifically, the 5-year-old brown trout kept in controlled conditions was capable of infecting an introduced bryozoan colony, and low number of sporogonic and pre-sporogonic *T. bryosalmonae* stages were found in the kidney, spleen and liver tissues (Soliman et al., 2018). This suggests that older life stages of freshwater or anadromous salmonids, infected by *T. bryosalmonae*, may represent an important means for parasite transmission within and between waterbodies (Morris & Adams, 2007; Okamura et al., 2011). However, for successful establishment of a new parasite population in previously uninfected watercourse, *T. bryosalmonae* needs a bryozoan host to complete its life cycle (Schmidt-Posthaus et al., 2021).

Because of the severity and ecological consequences of the PKD, *T. bryosalmonae* has been frequently examined in juvenile salmonids during the first growth season (Dash & Vasemägi, 2014; Mo & Jørgensen, 2016; Skovgaard & Buchmann, 2012; Sterud et al., 2007; Vasemägi et al., 2017). However, considerably fewer studies have analysed the prevalence and distribution of *T. bryosalmonae* at older life stages of salmonid fish. For example, recent work in Northern Norway demonstrated that brown trout and Atlantic salmon become infected by *T. bryosalmonae* after the first growing season (Lauringson et al., 2022). Similarly, Dash and Vasemägi (2014) found higher *T. bryosalmonae* prevalence among 1+ and older anadromous brown trout compared with 0+ fish, indicating that the (re)infections may also occur after the first growing season. Additionally, the presence of *T. bryosalmonae* has been reported among brown trout and Atlantic salmon sea-migrating smolts with (sub)clinical signs of PKD (Mo et al., 2011). However, very little information exists on *T. bryosalmonae* occurrence among anadromous salmonids when feeding in the sea or returning to freshwater. To our knowledge, only a single study has reported *T. bryosalmonae* among returning sea trout spawners in rivers (Dash & Vasemägi, 2014). Unlike juvenile fish, which typically show consistent *T. bryosalmonae* infection across the whole kidney, parasite DNA was observed only in the middle and posterior sections of the kidney in all three examined adult spawners (Dash & Vasemägi, 2014). Thus, only the sporogonic stages of the parasite at the excretory section of the kidney may be able to survive for extended time, while trout may be able to eliminate extrasporogonic stages in the lymphatic anterior section. As a result, the spatial distribution of parasite along the kidney can potentially provide useful temporal information about the timing of (re)infections of adult fish, since (re)infection can occur if adult sea trout migrate to freshwater, which exposes them to the parasite.

The distribution of *T. bryosalmonae* between rivers of the same region and even within the same river network can be highly heterogeneous (Carraro et al., 2018; Dash & Vasemägi, 2014; Schmidt-Posthaus et al., 2021). In Northern Estonia, parasite prevalence varies from zero to 100% between and, notably, within rivers and streams, reflecting high variation in parasite presence on a microgeographical scale (Dash & Vasemägi, 2014). Within a river network, heterogeneous *T. bryosalmonae* spread may be influenced by fish migration and hydrological spore transport mechanisms (Carraro et al., 2018). However, the links between *T. bryosalmonae* prevalence among juvenile brown trout in rivers and adult fish during their sea-migratory life-stage are currently not well understood. Furthermore, while a few studies have shown that *T. bryosalmonae* is capable of persisting in brown trout for prolonged periods in experimental freshwater conditions (Abd-Elfattah et al., 2014; Soliman et al., 2018), it is unclear how common the malacosporean parasite is among anadromous brown trout (sea trout) feeding at sea, and if the prevalence of the parasite varies spatially, or relative to fish age.

This study aimed to: (i) assess *T. bryosalmonae* prevalence in adult sea trout feeding in the coastal areas of the Baltic Sea; (ii) determine individual intra-host distribution of *T. bryosalmonae* and compare it to migratory behaviour inferred from otolith microchemistry; (iii) evaluate the effect of host age on *T. bryosalmonae* prevalence in sea trout; (iv) characterize spatial variation in *T. bryosalmonae* prevalence among sea trout and compare it with the occurrence of parasite among juvenile fish in nearby rivers and streams. To achieve the objectives, we utilized end-point multiplex PCR to characterize *T. bryosalmonae* infection frequency among adult sea trout ($n = 295$) and combined it with published (15 rivers and 1039 individuals) (Bruneaux et al., 2017; Dash & Vasemägi, 2014; Lauringson et al., 2021) and newly obtained parasite prevalence data (30 rivers

and 713 individuals) on trout juveniles collected from coastal rivers and streams in Estonia.

2 | MATERIALS AND METHODS

2.1 | Sampling of adult sea trout in the Baltic Sea

Twelve voluntary recreational anglers were involved in sampling using rod-and-reel-type fishing with artificial lures to catch sea trout ($n = 135$) and collect kidney tissue samples during the period from 2014 to 2022. Nine commercial fishers collected whole fish ($n = 140$) using gillnets from 2018 to 2020. In addition, the authors of the study were issued a special gillnet fishing permit in 2017 (Estonian Ministry of Environment Permit no. 2/2017), which allowed to collect trout under the legal size limit ($n = 20$). In total, kidney tissue samples were collected from anterior and posterior kidneys of 295 adult sea trout caught from coastal areas of Estonia (Table 1, Figure 1a). The complete sampling protocol is described in Appendix A.

2.2 | Collection of juvenile brown trout from streams and rivers

To enable a comparison of the occurrence of *T. bryosalmonae* among sea trout feeding in coastal areas with the prevalence of the parasite in coastal streams and rivers, we incorporated data on parasite prevalence from 33 coastal spawning rivers in Estonia, which consisted of data from 1752 salmonids. Data for 15 rivers have been previously published by Dash and Vasemägi (2014), Bruneaux et al. (2017) and Lauringson et al. (2021). Additional samples from 30 (partially overlapping) rivers were gathered in 2014, 2020 and 2021 and screened as in Dash and Vasemägi (2014). Juveniles were caught in late summer, early autumn (Aug-Sept) using standard electrofishing equipment (permits issued by Estonian Ministry of Environment) and euthanized according to the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Further information about sampled rivers, fish and estimated parasite prevalence is presented in Appendix B.

TABLE 1 Descriptive statistics of analysed sea trout samples. The mean values are presented with standard deviations in brackets.

	Tetracapsuloides Bryosalmonae DNA	
	Detected	**Undetected**
Number of individuals	114	181
Number of females; males	63; 45	118; 50
Mean total length (mm)	545.3 (70.9)	540.5 (80.4)
Mean total mass (g)	1968 (758.6)	1878.6 (971.9)
Mean condition factor	1.18 (0.21)	1.12 (0.18)
Mean age (years)	3.97 (0.97)	3.46 (0.80)
Min; Max age (years)	2; 6	2; 6

2.3 | Age estimation and otolith chemistry

Otoliths were removed from 127 sea trout and a routine age estimation was performed. Otoliths were placed in clear epoxy resin, ground using P1000, P2500 and P4000 grit grinding papers in increasing order until the core was exposed and polished using Metkon™ Forcipol 1V polishing machine. Individual otoliths were glued to glass plates, cleaned with 1% hydrochloric acid and stained using acidified Neutral Red. Age was estimated from stained otolith annuli formations with a Nikon Eclipse 50i Microscope using 2× magnification. To determine individual migration patterns between fresh- and marine water environments, the otolith Sr:Ca ratio was determined in another subset of samples ($n = 48$). For each fish, one otolith was randomly chosen and prepared for microchemical analyses as described in (Rohtla et al., 2017). Prior to the microchemical analyses the otolith thin sections were ultrasonically cleaned for 15 min in ultrapure water and then dried in a laminar flow hood. Otoliths were analysed for ^{86}Sr and ^{43}Ca using laser ablation inductively coupled plasma mass spectrometry at the University of Tartu, Department of Geology. The laser was set 10 hz with a 40 µm ablation spot size and scan speed of 5 µm/s. A continuous line scan was traced from the otolith core to the edge. Data reduction to Sr:Ca mmol/mol was carried out by following the methods of Miller (2011) as described in Svirgsden et al. (2018).

2.4 | DNA extraction and molecular detection of *T. bryosalmonae*

Molecular genetic detection of *T. bryosalmonae* was conducted in the Fish Genetics Laboratory of Chair of Aquaculture, Estonian University of Life Sciences. Total genomic DNA was extracted from the posterior and anterior kidney tissues of each specimen using two protocols. For 230 specimens, DNA was extracted using the QIAamp 96 DNA QIAcube HT kit and QIAcube® HT Instrument for automated nucleic acid purification (QIAGEN). For 65 specimens, DNA was extracted using a one-step Chelex double-stranded DNA extraction protocol (Casquet et al., 2011; Lauringson et al., 2022). The quality and quantity of DNA was measured using a NanoDrop™ 2000 spectrophotometer (Thermo Scientific). Following DNA extraction, multiplex PCR was conducted as in Lauringson et al. (2022), using *T. bryosalmonae*-specific primers described in Dash and Vasemägi (2014). This multiplex reaction amplifies 2–3 parasite (166, 298 and 756 bp) and a single salmonid-specific fragment (ca 500 bp). *T. bryosalmonae* positive and negative controls were included on each PCR plate to test the assay and detect potential contamination. The PCR products alongside size standards were visualized on 2% agarose gel stained with ethidium bromide using UV transilluminator UVItec FireReader (Figure 1).

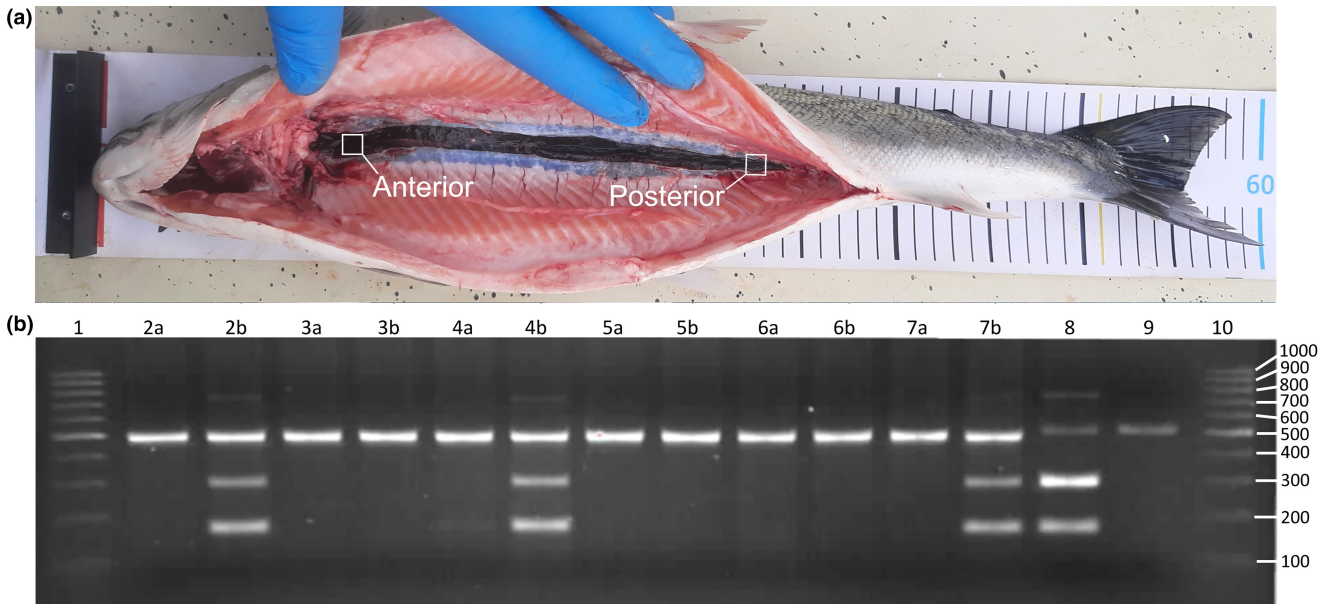

FIGURE 1 Sampled sea trout with anterior and posterior sampling sections (a) and image of 2% agarose gel with *Tetracapsuloides bryosalmonae* (166, 298 and 756 bp) and brown trout (500 bp) amplicons (b). Lanes 1 and 10 consist of 100–1000 bp DNA Ladder (Thermo Fisher Scientific Inc). Lanes 2a to 7b contain multiplex PCR products from six sea trout kidneys (one fish per two consecutive lanes, first for anterior (a) and second for posterior kidney (b). Lanes 8 and 9 include brown trout samples with and without *T. bryosalmonae*, respectively. A sample was regarded as *T. bryosalmonae* positive if, at least, the 166 and 298 bp parasite fragments were clearly visible (i.e. lanes 2b, 4b and 7b).

2.5 | Statistical analysis

Statistical analyses were performed, and figures were constructed with statistical software R 4.2.2 (R Foundation for Statistical Computing) and PAST 4.12 software (Hammer et al., 2001). Fulton's condition factor (*K*) was calculated using the formula $K = 100 \times (W/L^3)$ (Nash et al., 2006). The *t*-test using Monte Carlo permutation (Permutation $n = 1{,}000{,}000$) was used to compare the average length, mass, condition factor and age of *T. bryosalmonae* infected and uninfected fish, and the Chi-squared test was used to study the relationship between the prevalence of *T. bryosalmonae* infection and sex. The prevalence of *T. bryosalmonae* along the Estonian coast together with estimated parasite prevalence in coastal rivers was visualized using packages *mapplots* and *rworldmap*, and the changes in the probability of *T. bryosalmonae* infection depending on the age, and longitude and latitude were modelled with logistic regression. All results were considered statistically significant at $p \leq .05$.

3 | RESULTS

3.1 | *T. bryosalmonae* prevalence

Out of the 295 studied adult sea trout, 114 specimens (38.6%) were infected with *T. bryosalmonae* (Table 1). For the majority of infected fish (83.3%, $n = 95$), only the posterior kidney was infected. Both the posterior and anterior kidneys were infected in 16 specimens and two fish showed a positive parasite signal only in the anterior part of the kidney. There was a marginally insignificant difference in infection status between males and females (χ^2-test, $p = .057$), whereby 34.4% of females and 47.4% of males were infected with *T. bryosalmonae*. The total length and mass of infected and non-infected fish were similar (*t*-test with Monte Carlo permutation, $p = .604$ and .404 respectively). Fulton's condition factor was significantly higher for infected fish (*t*-test with Monte Carlo permutation, $p = .009$). Based on published and new data, 17 out of the 33 coastal spawning rivers were found to host the parasite (Appendix B). Within these 17 rivers, *T. bryosalmonae* was found, on average, in 67.2% of the fish studied.

3.2 | Age estimation and otolith chemistry

The mean age of infected sea trout was significantly higher (3.97 years, $n = 34$) compared to uninfected fish (3.46 years, $n = 93$, *t*-test with Monte Carlo permutation, $p = .004$). The increase in probability of *T. bryosalmonae* infection with age was also supported by logistic regression (Figure 2; $y = \exp(0.36468x - 2.6759)$, $p = .005$). Infected female fish were significantly older than uninfected sea trout of the same sex, and infected male fish were marginally older than uninfected sea trout of the same sex (*t*-test with Monte Carlo permutation, female $p = .020$, male $p = .050$). The oldest infected sea trout were 6 years old ($n = 3$).

Sr:Ca ratio in otoliths were determined for 48 specimens, of which 17 individuals were confirmed to be infected with *T.*

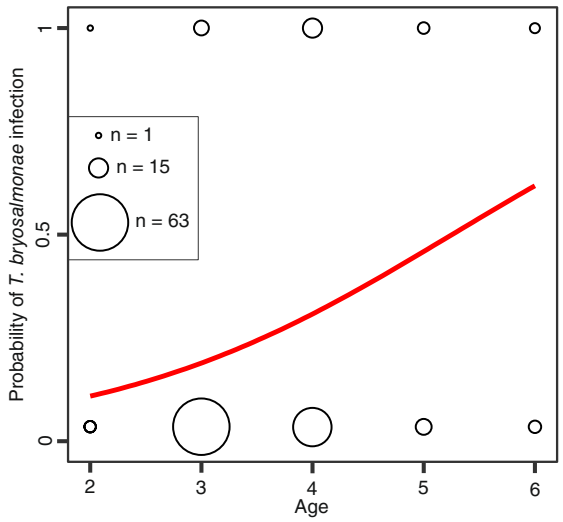

FIGURE 2 Increase in probability of *Tetracapsuloides bryosalmonae* infection in relation to fish age based on logistic regression analysis. Circles indicate the number of studied samples in each age group.

bryosalmonae. Among the infected fish, six individuals were determined to have been in freshwater habitat during last autumn based on the observed Sr:Ca nadir (Figure 3c,d,f). Additionally, a further six infected fish were visually determined as post-spawners. From these 12 individuals, *T. bryosalmonae* was found in both ends of the kidney in three specimens. Sr:Ca profiles of six individuals with different life history and infection statuses are presented in Figure 3 (infected fish: c,d). The elevated Sr:Ca ratio at the innermost core area is indicative of anadromous maternal origin and appears if a female parent of the studied specimen has been in the marine water environment during vitellogenesis, therefore incorporating the higher Sr concentration into the yolk (Figure 3a,c,e,f). The freshwater parr phase is characterized by a low Sr:Ca ratio which, when followed by marine life stage, is demonstrated by distinctly higher Sr:Ca ratio. However, time spent in freshwater varied considerably among the studied fish, and in some cases, juvenile fish made several short visits to brackish water and back (e.g. Taal et al., 2018) (Figure 3e). Interestingly, the Sr:Ca profiles also indicated that two individuals with positive amplification of *T. bryosalmonae* in both anterior and posterior kidney had been in a freshwater environment recently (one individual shown in Figure 3d).

3.3 | Spatial distribution of *T. bryosalmonae* infection

Spatial analysis demonstrated that *T. bryosalmonae* prevalence among feeding sea trout increased when moving from east to west and from south to north along the Estonian coast (Figure 4). Notably, the parasite prevalence pattern among adult sea trout correlated with the prevalence of *T. bryosalmonae* in Estonian coastal rivers and streams.

4 | DISCUSSION

Sea trout is one of the keystone species in the Baltic Sea, yet many populations in the region have deteriorated or have even been driven to extinction due to overfishing, habitat destruction and climate change (Donadi et al., 2023; HELCOM, 2018, 2022). Concurrently, PKD caused by *T. bryosalmonae*, has been identified as an additional emerging factor threatening wild salmonid populations in Europe (Burkhardt-Holm et al., 2005; Dash & Vasemägi, 2014; Gorgoglione et al., 2016; Ros et al., 2021). In the following, we discuss our main findings on *T. bryosalmonae* infection patterns among sea trout, linking them to the host–parasite interactions and life-history traits of the anadromous brown trout.

We found that more than one third of Estonia's sea trout were infected with *T. bryosalmonae* during their marine feeding phase. We also observed an increasing infection trend from south–north and west–east along the coast of Estonia, which mirrored *T. bryosalmonae* prevalence patterns in nearby rivers and streams. However, at the moment, it is not clear what are the specific factors responsible for the observed differences in parasite prevalence between regions and rivers. The similarity in prevalence patterns between juvenile and adult fish most likely reflects the limited dispersal of sea trout and is consistent with known migration patterns of Baltic sea trout. For example, sea trout feeding migrations in the Gulf of Finland are primarily restricted within the gulf and commonly do not exceed over 50 km from natal rivers (Kallio-Nyberg et al., 2017; Rannak et al., 1983). Similarly, sea trout in the Gulf of Bothnia tend to make relatively short feeding migrations (Degerman et al., 2012; Kallio-Nyberg et al., 2017; Lundqvist et al., 2006). Earlier studies have also shown that straying of sea trout is common and fish originating from smaller rivers frequently stray to non-natal rivers (Degerman et al., 2012). Therefore, straying sea trout can most likely act as parasite vectors and transmit *T. bryosalmonae* also to formerly uninfected waterbodies. However, the close proximity of infected and parasite-free rivers along the Estonian coast indicates that environmental factors, such as nutrient levels and water temperature influenced by differences in land use, riparian cover and agricultural activity likely have a more important effect than fish migration in the establishment of *T. bryosalmonae*. Thus, it is likely that the availability and abundance of bryozoans as primary hosts of *T. bryosalmonae* have a key role shaping the distribution of the parasite (Hartikainen et al., 2009; Okamura et al., 2011).

In experimental conditions, the excretion of *T. bryosalmonae* has been recorded for up to 5 years post-exposure in captive brown trout living in freshwater environment (Soliman et al., 2018). Based on age determination within a subset of samples ($n = 127$), we observed that *T. bryosalmonae* can be detected by multiplex PCR in up to 6-year-old sea trout. It is possible that these individuals were infected with the parasite as juveniles during the first or second growth seasons as otolith microchemistry analysis did not indicate that these fish have spent extended time in freshwater after smoltification. However, we cannot exclude the possibility that short-term

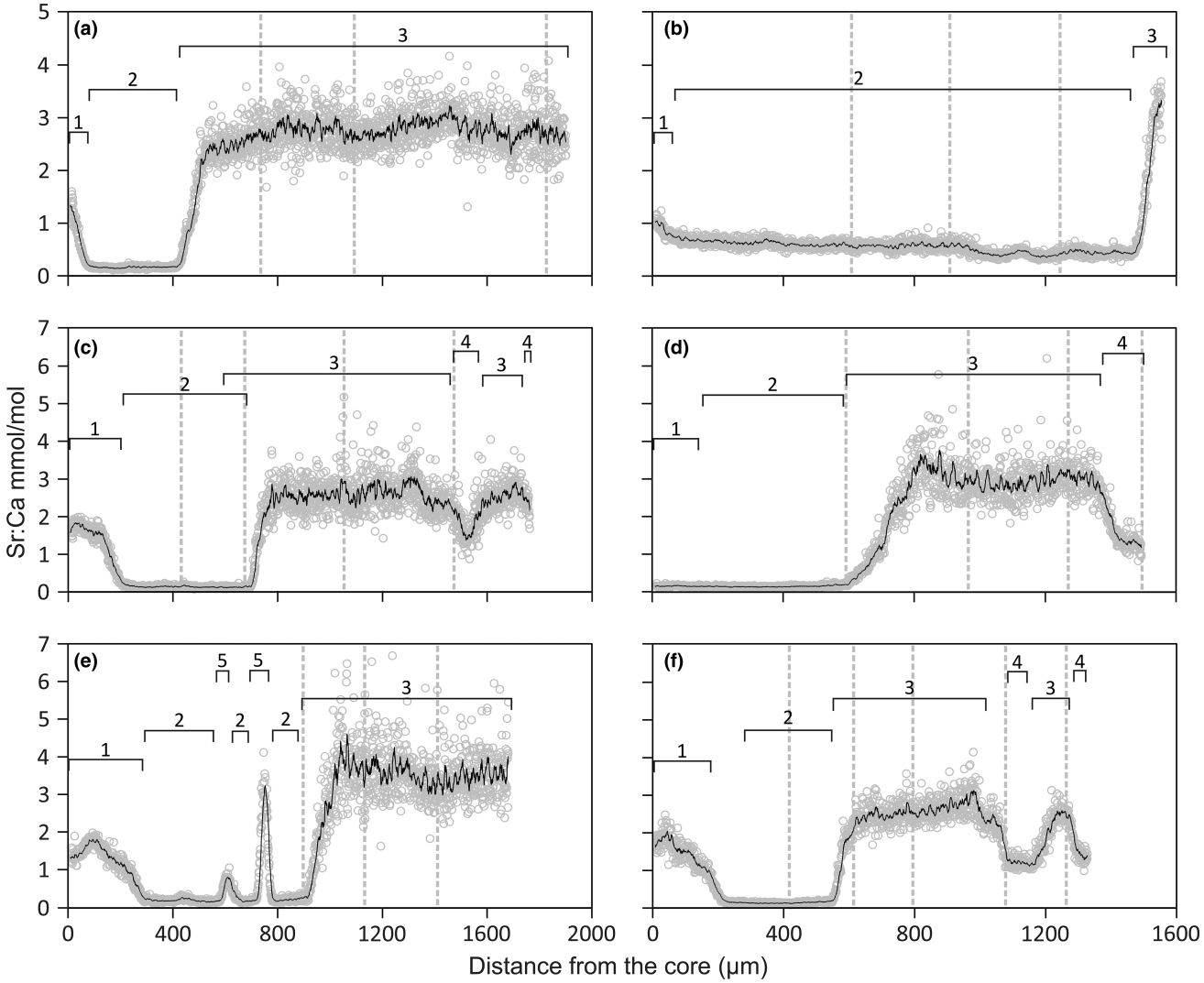

FIGURE 3 Sr:Ca profiles of six sea trout individuals (a–f) where grey circles represent single Sr:Ca point estimates and the moving average over 10 Sr:Ca ratio estimates is represented by the black line. The innermost core area (i.e. maternally influenced zone; elevated Sr:Ca values in this zone are indicative of anadromous maternal origin), is indicated as no. 1 (not present in b, d); Low Sr:Ca ratio indicative of riverine stage is marked as no. 2; high Sr:Ca ratio indicative of brackish water stage is marked as no. 3; no. 4 denotes likely spawning migrations from brackish water to fresh water; no. 5 denotes short migrations from freshwater to brackish water and back. Grey dotted lines represent the annuli. Total length of the fish from (a–f) (respectively): 635, 440, 540, 550, 560 and 570 mm.

movements from brackish to freshwater expose sea trout to *T. bryosalmonae* later in their life. For example, earlier studies on otolith elemental composition in juvenile fish have shown that it may be difficult to detect short-term changes in habitat salinity by using Sr:Ca ratio (Miller, 2011). For older fish, noticeable changes in otolith elemental composition most likely take even longer time since older fish have progressively slower otolith growth rates.

Our study also revealed that *T. bryosalmonae* prevalence is higher among older fish. Thus, it is possible that *T. bryosalmonae* re-infections occur when sea trout are returning from the marine environment to rivers and streams to spawn. Based on the analysis of *T. bryosalmonae* in both anterior and posterior sections of the kidney, we propose that such re-infections may be identified by evaluating the spatial distribution of the parasite along the kidney. In our dataset, the majority of detected infections were located only in the posterior kidney ($n = 95$). Altogether, 16 fish showed presence of parasite DNA at both anterior and posterior parts of the kidney, while only two individuals showed presence of *T. bryosalmonae* DNA at the anterior part of the kidney. These findings indicate that *T. bryosalmonae* persists at sporogonic stages in the excretory posterior kidney for extended period of time, while initial extra-sporogonic parasite stages are cleared in the lymphatic anterior section of the kidney. Therefore, future studies on *T. bryosalmonae* focusing on older fish should take into account potential differences in parasite presence across different sections of the kidney.

The observed intra-host infection pattern is also consistent with known life-history traits of sea trout. For example, only a small proportion of sea trout spawn multiple times (Källo et al., 2022; Kristensen et al., 2019); this is consistent with low level of potential (re)infections among adult fish (i.e. *T. bryosalmonae* present in

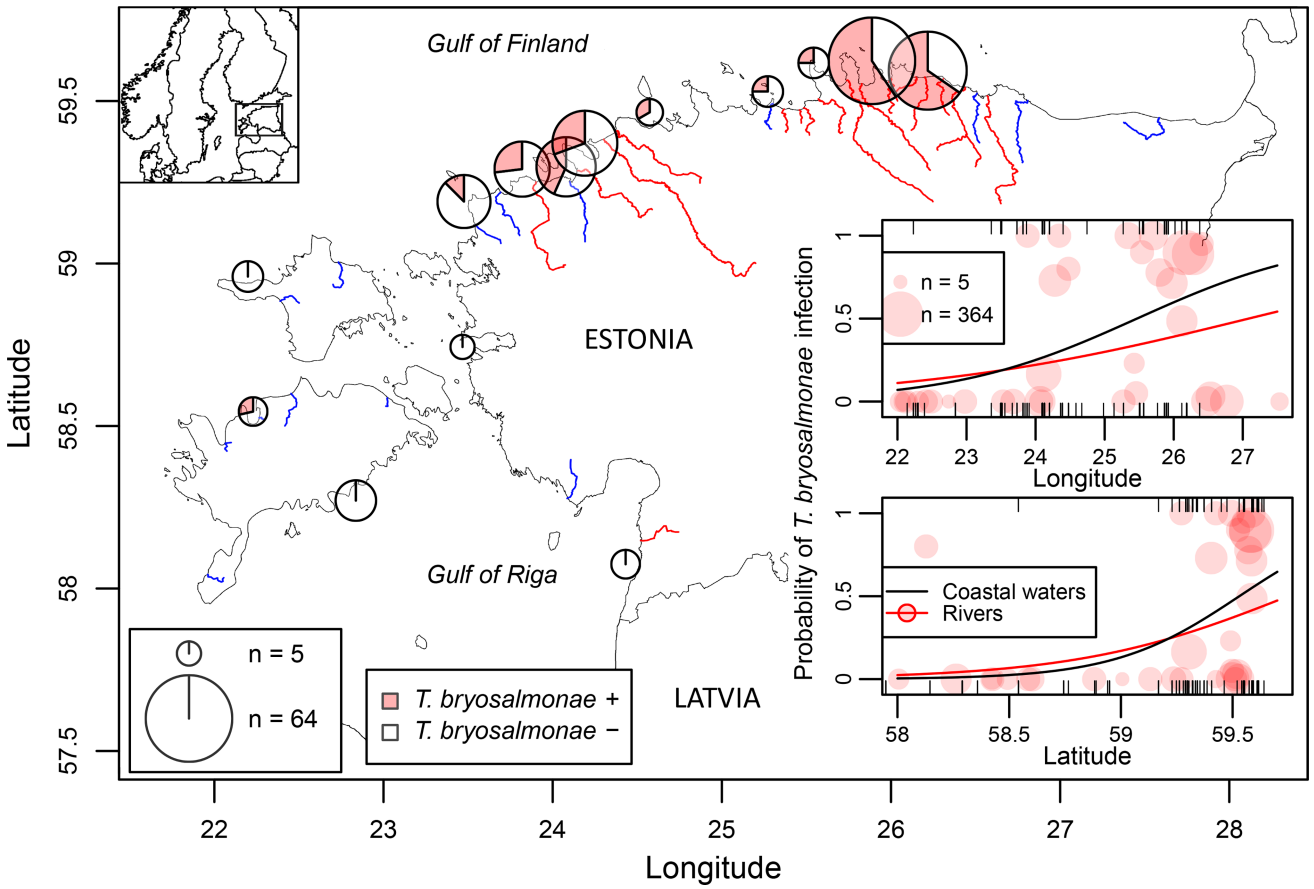

FIGURE 4 Proportion of fish with and without *Tetracapsuloides bryosalmonae* infection in Estonian coastal waters and rivers. Each location is represented by a pie chart showing the proportion of sea trout with (*T. bryosalmonae* +) and without (*T. bryosalmonae* −) infection and the size of the chart reflects the number of fish caught. Infected and non-infected rivers are marked with red and blue lines, respectively. The sub-plots show the expected change in the probability of *T. bryosalmonae* infection depending on the longitude and latitude of catching locations in coastal waters (black lines, based on sea trout data) and rivers' mouths (red lines, based on juvenile parasite prevalence data) estimated with logistic regression (for all models $p < .001$); small black dashes indicate individual sea trout with and without *T. bryosalmonae* infection caught in coastal waters, red circles mark the proportion of infected juvenile fish in rivers depending on the rivers' mouth coordinates, size of the circles reflects the number of juvenile fish.

both the anterior and posterior part of the kidney). In addition, sea trout that enter fresh water before the spawning season are more likely to be (re)infected, while fish that ascend late are less likely to be (re)infected since lower water temperatures would reduce the production of infective spores by bryozoans (Tops et al., 2006). Therefore, slightly higher parasite prevalence among males, albeit non-significant ($p = .057$), may be linked to (re)infections and their earlier entry to spawning grounds since male sea trout tend to stay shorter at sea and migrate to freshwater earlier during the breeding season (Berg & Berg, 1989; Jensen, 1968). Furthermore, male spawners travel longer distances within rivers (Dębowski et al., 2011), which may also generate sex-specific differences in parasite prevalence. However, additional analyses are needed to confirm the occurrence of sex-related differences in *T. bryosalmonae* prevalence. In addition, to confirm the potential link between (re)infections and intra-individual heterogeneity of *T. bryosalmonae* infection in the kidney, additional experimental studies are needed. In particular, molecular and histological examination of different tissues and kidney sections combined with a detailed analysis of temporal dynamics of *T. bryosalmonae* spore release from fish and bryozoans (Carraro et al., 2017, 2018; Fontes et al., 2017) are needed to better understand *T. bryosalmonae* infection dynamics and the role of re-infections on host performance and parasite's life cycle.

Our analysis indicates that there appears to be no obvious negative effect of *T. bryosalmonae* infection on the condition factor of sea trout. On the contrary, infected fish showed higher condition factor in comparison with uninfected fish. Currently, we do not know the exact mechanism behind it, yet one potential cause for this unexpected outcome is survivorship bias (Szklo & Nieto, 2014). Thus, it is possible that most severely affected wild fish are underrepresented in our sample. An alternative mechanism, at least in theory, involves host manipulation, which is common among parasites for transmission enhancement in many host–pathogen systems (reviewed by Heil, 2016). Marine survival of sea trout is dependent on growth rate and mortality is higher in slow-growing individuals (Jensen, Finstad, & Fiske, 2017; Jensen, Finstad, Fiske, Forseth, et al., 2017). Thus, *T. bryosalmonae* may influence the probability of maturation and earlier return of the host to fresh water by manipulating energy intake and

food reserves to increase the condition factor of the host. Earlier return to fresh water, in turn, may increase the parasite's transmission likelihood.

In conclusion, this study revealed, for the first time, the frequent yet spatially heterogeneous distribution of *T. bryosalmonae* infection among and within adult sea trout feeding in marine environment. We show that parasite prevalence pattern of sea trout spatially correlates with *T. bryosalmonae* prevalence in rivers and streams. Our results also indicate that the parasite is capable of persisting in the excretory posterior kidney for extended periods of time, while initial extra-sporogonic parasite stages are cleared in the anterior section of the kidney. However, additional experimental work is needed to confirm the potential link between (re)infections, intra-individual heterogeneity of *T. bryosalmonae* infection in the kidney and the effectiveness of anadromous trout acting as parasite vectors.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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APPENDIX A

SAMPLING OF ADULT SEA TROUT BY RECREATIONAL ANGLERS

Twelve recreational anglers who volunteered to participate in sample collection (no. of sampled fish, $n = 135$) were provided with 15 mL Falcon tubes filled with 95% ethanol, forceps and plastic mini-grip bags. They were instructed to abide the following sampling and information collection protocol: (1) After catching sea trout, euthanize the fish with a sharp blow to the head; (2) record total mass of the fish to the nearest 10 g, total length to the nearest cm, catch location, date and any additional phenotypic information (i.e. if the fish showed darkened coloration indicative of post-spawning stage); (3) cut the fish with a clean scalpel or filleting knife from anal opening towards head and record the sex of the fish if gonads are visible; (4) remove the intestines; (5) clean the knife with hot water/ethanol and clean paper towel, make a 1 cm cut into anterior part of the kidney (Figure 1a); (6) remove the anterior part of the kidney from the area of incision with clean forceps and place it into a 15 mL Falcon tube filled with 95% ethanol; (7) clean the knife/scalpel and forceps with hot water/ethanol and paper towel, make a 1 cm cut into posterior part of the kidney near anal opening (Figure 1b); (8) remove the posterior part of the kidney from the area of incision with forceps and place it into the 15 mL Falcon tube filled with 95% ethanol; (9) place the tubes into plastic mini-grip bag together with written information on paper about mass, length, date and additional phenotypic information; (10) if possible, store head of the fish in separate bag for age determination in −20°C freezer until transportation on ice. All fish sampled were caught following regulations established by Fishing Act passed by Parliament of Estonia (https://www.riigiteataja.ee/en/eli/ee/513062016002/consolide/current).

Collection of fish by commercial anglers and scientists

Nine commercial anglers collected whole fish (no. of sampled fish, $n = 140$) using gillnets, recorded mass, total length and date. The fish were then packed and stored in −20°C until collection. The fish were subsequently transported to the Laboratory of Chair of Aquaculture, Estonian University of Life Sciences. During tissue sampling, fish were thawed in +4°C and any additional phenotypic information (i.e. if the fish showed darkened coloration indicative of post-spawning stage) was recorded. Collection of anterior and posterior kidney tissues were carried out in the laboratory as described earlier. All fish were caught following regulations established by Fishing Act passed by Parliament of Estonia (https://www.riigiteataja.ee/en/eli/ee/513062016002/consolide/current).

The minimum legal size limit for sea trout in Estonia is 50 cm (https://www.riigiteataja.ee/en/eli/ee/513062016002/consolide/current). To include fish under legal size limit, special fishing permit was issued to the authors by Ministry of the Environment of Estonia (licence no. 2/2017). Sea trout (no. of sampled fish, $n = 20$) were caught with gillnets and euthanized by sharp blow to the head according to the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes (https://eur-lex.europa.eu/eli/dir/2010/63/oj). After which fish were transported on ice to the Laboratory of Chair of Aquaculture, Estonian University of Life Sciences and sampled as described earlier.

APPENDIX B

SAMPLED RIVERS, JUVENILE FISH AND ESTIMATED PARASITE PREVALENCE

River/ Stream	Dash & Vasemägi, 2014 *n*	Bruneaux et al., 2017 *n*	Lauringson et al., 2021 *n*	Current study *n*	Total *n*	River mouth coordinates (°N, °E)	*Tetracapsuloides Bryosalmonae* infection status Neg.	Pos	Prev.
Altja	44				44	59°35′09.9″, 26°07′06.4″	24	20	0.45
Höbringi				20	20	59°07′17.5″, 23°32′55.6″	20	0	0
Jämaja				13	13	58°00′19.4″, 22°02′27.1″	13	0	0
Kaberla				20	20	59°30′40.3″, 25°16′24.0″	20	0	0
Keila	43			26	69	59°24′16.4″, 24°16′42.9″	16	53	0.77
Kiruma				14	14	58°29′33.8″, 22°17′56.0″	14	0	0
Kloostri				20	20	59°16′04.5″, 24°04′52.3″	20	0	0
Kunda	61			99	160	59°31′12.1″, 26°31′57.8″	133	27	0.17
Kuusalu	13				13	59°29′26.4″, 25°25′38.7″	10	3	0.23
Ligeoja				20	20	58°25′27.6″, 22°07′03.2″	20	0	0
Loo oja				20	20	59°29′50.5″, 25°27′19.4″	19	1	0.05
Loobu	34			18	52	59°34′13.9″, 25°47′50.0″	23	29	0.56
Männiku	32			20	52	58°15′44.0″, 24°03′48.5″	52	0	0
Mustoja	269	16	59	80	424	59°35′05.2″, 26°10′04.0″	92	332	0.78
Nõva				24	24	59°14′09.6″, 23°40′22.2″	24	0	0
Nuutri				5	5	59°00′22.6″, 22°44′28.0″	5	0	0
Pada	54			20	74	59°29′58.4″, 26°46′05.3″	74	0	0
Pidula				13	13	58°25′21.5″, 22°06′59.1″	13	0	0
Pudisoo				20	20	59°31′28.4″, 25°32′26.7″	2	18	0.90
Pühajõgi	9				9	59°25′38.1″, 27°32′03.6″	9	0	0
Punabe				20	20	58°35′06.7″, 22°29′10.3″	20	0	0
Selja				20	20	59°32′56.1″, 26°24′16.9″	1	19	0.95
Taaliku				21	21	58°36′12.1″, 22°58′33.4″	21	0	0
Timmkanal				20	20	58°07′56.6″, 24°34′19.3″	4	16	0.80
Toolse	39			20	59	59°31′42.7″, 26°28′27.3″	59	0	0
Vääna				20	20	59°25′31.9″, 24°20′30.3″	0	20	1
Vainupea	135	88		21	244	59°34′45.7″, 26°16′46.7″	27	217	0.89
Valgejõgi	21			19	40	59°34′56.6″, 25°42′21.2″	0	40	1
Valkla	9			20	29	59°29′38.0″, 25°20′16.1″	0	29	1
Vanajõgi				20	20	58°52′49.3″, 22°24′35.5″	20	0	0
Vasalemma			77	20	97	59°18′16.3″, 24°06′59.9″	81	16	0.16
Vihterpalu				20	20	59°16′13.6″, 23°53′01.7″	0	20	1
Võsu	36			20	56	59°35′04.7″, 25°58′21.4″	16	40	0.71