Phylogeographic structure and population connectivity of a small benthic fish (*Tripterygion tripteronotum*) in the Adriatic Sea

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Abstract

Aim: Population connectivity of benthic marine organisms depends strongly on planktonic larval dispersal and is controlled by geographic distance and oceanographic structure. We examine isolation by distance versus resistance to barriers (ocean current boundaries) against a background of post-glacial habitat expansion in a small benthic fish of the Adriatic Sea.

Location: Adriatic Sea, Eastern Mediterranean.

Taxon: *Tripterygion tripteronotum*.

Methods: We performed population genetic analyses using mitochondrial control region sequences of 550 individuals from 25 locations sampled along the Eastern Adriatic coast. Investigations of population structure included differentiation tests, cluster analyses and distance-based redundancy analysis. We then ran Lagrangian simulations of passive larval drift to examine correlations among population structure, geographic distance and the Adriatic gyre system. To test for signatures of a post-glacial range expansion, we modelled the demographic history of the populations and examined the geographic distribution of genetic diversity.

Results: Genetic population structure corresponded to the Adriatic gyres without additional effect of geographic distance. Inference of northward-biased gene flow between the northern and the Istrian gyre was consistent with simulated trajectories of passive drift, whereas the phylogeographic break coinciding with the boundary between the Central and the Northern Adriatic gyre was stronger than predicted by drift simulations. Genetic connectivity of populations within gyres was high. Genetic signatures of population expansion were consistent with a rapid post-glacial recolonization of the northern Adriatic.

Main conclusions: The combination of dense sampling and passive drift simulation allowed us to distinguish among effects of geographic distance, oceanographic features and palaeoenvironmental changes on current population structure. Comparisons...
between realized and potential connectivity illustrate the value of integrating different data sources to understand population structure and inform conservation planning.

**KEYWORDS**

Adriatic Sea, genetic differentiation, isolation by resistance, Lagrangian simulation, marine population structure, Mediterranean, ocean currents, phylogeography, seascape genetics, triplefin blenny

## 1 | INTRODUCTION

The apparent continuity of the marine environment suggests virtually unrestrained dispersal and large-scale connectivity within species, but this superficial view is contradicted by genetic studies (Hellberg, 2009; Palumbi, 2003). Although many large-bodied marine fish species are highly mobile, their generational dispersal seems to be restricted and does not preclude the evolution of strongly structured populations (e.g. Castro et al., 2007; Graves, 1998). Similarly, planktonic larval dispersal—mainly if not only manner of dispersal for sessile and small benthic species—is not at all boundless. Numerous factors, including egg type, pelagic larval duration and swimming behaviour, have been found to influence the extent of planktonic larval dispersal (Nanninga & Manica, 2018; Selkoe & Toonen, 2011). Moreover, rather than following a simple pattern of isolation by distance, planktonic dispersers often show ‘isolation by resistance’, with population connectivity affected by oceanographic features (Abrahamson, Eubanks, Blair, & Whipple, 2001; Banks et al., 2007; Benestan et al., 2016; Galarza et al., 2009; Gilg & Hilbish, 2003; Riginos, Douglas, Jin, Shanahan, & Tremi, 2011; Schunter et al., 2011; Thomas et al., 2015; Truelove et al., 2017; White et al., 2010; Xuereb et al., 2018). As a consequence, adjacent populations can become differentiated across short geographic distances while exchanging genes with more distant populations (Gilg & Hilbish, 2003; Xuereb et al., 2018). By identifying spatial patterns of connectivity and isolation, genetic data provide valuable information for the design and improvement of conservation and management strategies (Giakoumi et al., 2013; Jahnke et al., 2017; Rilov et al., 2019).

The Adriatic Sea is a biogeographically distinct part of the Mediterranean Sea (Bianchi, 2007; Spalding et al., 2007; examples: Koblmüller, Steinwender, Weiß, & Sefc, 2015; Maltagliati, Di Giuseppe, Barbieri, Castelli, & Dini, 2010; Souche et al., 2015; Wagner et al., 2019), to which it is connected by the narrow Otranto Strait. The 800-km long body of water is enclosed by a regular, sandy and gently sloping coast to the west and a steep, rocky coast along with numerous coastal islands to the east. It is divided into three sub-basins—the northern, central and southern Adriatic (Russo & Artagiani, 1996; Figure 1), which also correspond to recognized biogeographic sectors (Bianchi et al., 2012) with variation in depth, temperature and the distribution of boreal and thermophilic fauna (Bianchi & Morri, 2000; Jardas, 1996). In addition to the basin-wide surface circulation, which runs along the eastern and western Adriatic coast (the East and West Adriatic currents, respectively), recirculation gyres form in each of the three sub-basins. The northern transversal edge of the Southern Adriatic gyre approximately matches the boundary between the central and southern Adriatic sub-basins, the northern transversal edge of the Central Adriatic gyre approximately matches the boundary between the northern and central Adriatic sub-basins and a fourth cyclonic gyre exists in the very northern part of the Adriatic between the Istrian peninsula and the Po river delta (Pouline, 2001; Figure 1). Surface circulation is influenced by winds and is seasonally variable (Pouline, 2001; Russo & Artagiani, 1996; Ursella, Pouline, & Signell, 2007). Consequently, passive transport in the water currents underlies seasonal variation and population connectivity in species with planktonic larval dispersal will to some degree depend on the time of spawning (Melìà et al., 2016). In spring and summer, for instance, the East Adriatic Current, which runs northwards along the Eastern coast, is weak, whereas the recirculation cells in the northern and central sub-basins as well as the cyclonic gyre along Istria are well developed (Pouline, 2001; Ursella et al., 2007).

Apart from current seascape structure, a major impact on population structure of aquatic biota in the Adriatic was produced by sea level changes during the glacial cycles. During the Last Glacial Maximum (24,000–18,000 years BP), the sea level was more than 120 m lower than today (Lambeck, Rouby, Purcell, & Sambridge, 2014), leaving the northern Adriatic desiccated. With rising sea levels, this area became available for re-colonization by marine organisms and the present coastline was established about 7,000 years BP (Fleming et al., 1998; Lambeck, Antonioli, Purcell, & Silenzi, 2004). Population structure in the northern Adriatic is therefore of recent origin and possibly affected by bottlenecks, founder events and population expansion (Koblmüller et al., 2015; Ledoux et al., 2018; Maltagliati et al., 2010).

Despite the surging interest in seascape genetics in general and the oceanographic appeal of the Adriatic Sea, relatively few studies have addressed population structure in the Adriatic. Among those that did, some found that genetic clusters within species reflected the Adriatic sub-basins (Carreras et al., 2017; Ledoux et al., 2018; Schiavina, Marino, Zane, & Melià, 2014), whereas in others, there was only weak or no differentiation by
FIGURE 1  (a) Male (left) and female Tripterygion tripteronotum. Photographs by Maximilian Wagner. (b) Sampling sites and recirculation gyres in the Adriatic Sea. The broken white line (labelled LGM) indicates the sea level during the last glacial maximum following Amorosi, Maselli, and Trincardi (2016) [Colour figure can be viewed at wileyonlinelibrary.com]
TABLE 1  Triperygion tripteronotum population samples from the Adriatic and Mediterranean locations and population genetic diversity. For sampling locations on islands, the name of the island is given in parentheses. Locations are assigned to Adriatic gyres and sub-basins. The last column indicates whether the location was included as point of particle release in the Lagrangian drift simulations. N, sample size; $H_E$, genetic diversity; $h$, number of different haplotypes; HR, haplotype richness.

| Location number | Location (island) | Latitude (N) | Longitude (E) | Gyre | Sub-basin | Sampling year | N  | $H_E$ | $h$ | HR | Lagrangian simulation |
|-----------------|-------------------|--------------|---------------|------|-----------|---------------|----|-------|-----|----|------------------------|
| 1               | Rovinj            | 45.090       | 13.640        | Istrian | Northern | 2017          | 19 | 0.842 | 9   | 7.061 | Yes                     |
| 2               | Valsaline         | 44.850       | 13.837        | Istrian | Northern | 2008 (n = 35), 2017 (n = 15) | 50 | 0.664 | 10  | 5.066 | Yes                     |
| 3               | Sarkodana         | 44.845       | 13.837        | Istrian | Northern | 2008          | 24 | 0.783 | 10  | 6.785 | Yes                     |
| 4               | Beli (Cres)       | 45.109       | 14.362        | Northern | Northern | 2006          | 16 | 0.750 | 7   | 6.188 | Yes                     |
| 5               | Glavotok (Krk)    | 45.093       | 14.441        | Northern | Northern | 2006          | 13 | 0.718 | 6   | 6.000 | Yes                     |
| 6               | Baška (Krk)       | 44.958       | 14.766        | Northern | Northern | 2017          | 28 | 0.783 | 13  | 7.217 | Yes                     |
| 7               | Klenovica         | 45.098       | 14.846        | Northern | Northern | 2006          | 17 | 0.625 | 5   | 4.313 | Yes                     |
| 8               | Pudarica (Rab)    | 44.712       | 14.828        | Northern | Northern | 2007          | 13 | 0.885 | 8   | 8.000 | Yes                     |
| 9               | Prizna            | 44.601       | 14.970        | Northern | Northern | 2007          | 13 | 0.718 | 6   | 6.000 | Yes                     |
| 10              | South of Potočnica (Pag) | 44.597 | 14.833        | Northern | Northern | 2006          | 18 | 0.837 | 7   | 6.089 | Yes                     |
| 11              | Dubrava 1 (Pag)   | 44.475       | 15.008        | Northern | Northern | 2007          | 19 | 0.386 | 5   | 3.717 | No                      |
| 12              | Dubrava 2 (Pag)   | 44.494       | 14.987        | Northern | Northern | 2017          | 15 | 0.476 | 5   | 4.485 | No                      |
| 13              | Božava (Dugi Otok) | 44.141 | 14.906        | Northern | Northern | 2006          | 27 | 0.675 | 7   | 4.399 | No                      |
| 14              | Sušica (Ugljan)   | 44.144       | 15.087        | Northern | Northern | 2006          | 37 | 0.824 | 13  | 6.592 | Yes                     |
| 15              | Vitan (Pašman)    | 43.897       | 15.413        | NA    | NA        | 2006          | 6  | 1.000 | 6   | NA   | Yes                     |
| 16              | Grebaštica        | 43.630       | 15.936        | Central | Central | 2014          | 24 | 0.667 | 10  | 5.033 | Yes                     |
| 17              | Okrug Gornji (Čiovo) | 43.494 | 16.244        | Central | Central | 2014          | 33 | 0.928 | 18  | 8.827 | No                      |
| 18              | Sutivan (Brač)    | 43.385       | 16.501        | Central | Central | 2014          | 35 | 0.874 | 15  | 7.442 | Yes                     |
| 19              | Povlja (Brač)     | 43.340       | 16.839        | Central | Central | 2014          | 21 | 0.776 | 5   | 4.576 | Yes                     |
| 20              | Bol (Brač)        | 43.263       | 16.609        | Central | Central | 2014          | 19 | 0.731 | 9   | 6.167 | Yes                     |
| 21              | West of Mlaska (Hvar) | 43.134 | 17.105        | Central | Central | 2012          | 16 | 0.442 | 4   | 3.581 | Yes                     |
| 22              | Mlaska (Hvar)     | 43.139       | 17.142        | Central | Central | 2012          | 27 | 0.613 | 11  | 6.206 | Yes                     |
| 23              | South of Bogomolje (Hvar) | 43.118 | 16.994        | Central | Central | 2012          | 18 | 0.490 | 6   | 5.092 | Yes                     |
| 24              | Ropa (Mljet)      | 42.752       | 17.459        | Southern | Southern | 2007          | 25 | 0.663 | 6   | 4.653 | Yes                     |
| 25              | Molunat           | 42.454       | 18.434        | Southern | Southern | 2007          | 17 | 0.427 | 5   | 4.088 | Yes                     |
| 26              | Ag, Georgios Pagon (Corfu) | 39.699 | 19.677        | NA    | NA        | 2018          | 18 | 0.837 | 9   | NA   | No                      |
| 27              | Plakias (Crete)   | 35.192       | 24.373        | NA    | NA        | 2017          | 15 | 0.848 | 9   | NA   | No                      |
| 28              | Petres (Crete)    | 35.358       | 24.369        | NA    | NA        | 2017          | 2  | 1.000 | 2   | NA   | No                      |
| 29              | Anavyssos         | 37.685       | 23.938        | NA    | NA        | 2017          | 4  | 0.500 | 2   | NA   | No                      |
sub-basins (Boissin et al., 2016; Garoia, Guarniero, Piccinetti, & Tinti, 2004; Koblmüller et al., 2015; López-Márquez et al., 2019; Maltagliati et al., 2010; Papetti et al., 2013; Souche et al., 2015). In addition to the division into large sub-basins, the ragged shoreline and wealth of near-shore islands along the eastern Adriatic coast provide a richly structured environment, which could further shape population differentiation of littoral and benthic species on a more local scale (Ledoux et al., 2018). The analysis of patterns of genetic differentiation within the Adriatic sub-basins requires a much denser sampling regimen than was performed in most previous studies.

In the present study, we performed dense sampling of the triplefin blenny Tripterygion tripteronotum (Tripterygiidae; Figure 1) along the eastern Adriatic shoreline and investigated population structure in relation to the Adriatic sub-basins and recirculation gyres. The small (<7 cm) fish are restricted to the shallow (0–6 m) rocky littoral (de Jonge & Videler, 1989; Wirtz, 1978). Their distribution therefore follows a narrow band along the rocky coast of the eastern Adriatic and is interrupted between islands and along stretches of sandy coast, which rarely exceed a few hundred metres in length along this part of the Adriatic. As adult triplefins are non-migratory, highly territorial, epibenthic to cryptobenthic fish with relatively sedentary lifestyle (Schunter, Pascual, Garza, Raventós, & Macpherson, 2014; Wirtz, 1978; own observations), dispersal over any significant distance would necessarily occur during the larval phase. Tripterygion tripteronotum attach their eggs to algal aufwuchs in the males’ territories (de Jonge & Videler, 1989). After hatching, larvae disperse into the inshore waters for 2–3 weeks (Macpherson & Zika, 1999; Raventos & Macpherson, 2001), but apparently remain close to the shore as no larvae were found in hauls >2.5 km from the shore (Sabates, Zabala, & García-Rubies, 2003). Spawning occurs from early spring to late summer (de Jonge & Videler, 1989; Macpherson & Zika, 1999; Wirtz, 1978), and accordingly, Tripterygion larvae were abundant in plankton samples collected at depths of 2–3 m near the Spanish coast from April to September (Sabates et al., 2003). In the related species T. delaisi, which has a similar planktonic larval duration and distribution as T. tripteronotum, the estimated percentage of self-recruitment to a study population in north-eastern Spain was as low as 6.5% (Schunter et al., 2014), which, however, did not prevent population differentiation to occur on a geographic scale of >100 km in the same area (Carreras et al., 2017; Carreras-Carbonell, Macpherson, & Pascual, 2006, 2007). We hypothesized that planktonic dispersal of T. tripteronotum larvae may likewise be restricted, and its scale and direction determined by larval behaviour, oceanic currents and the topography of the eastern Adriatic coast. Differentiating between effects of geographic distance and effects of potential dispersal barriers called for a dense sampling of locations both within and across recirculation gyres. To assess the role of passive larval dispersal following the Adriatic currents in shaping population connectivity, we compared genetic population structure with potential connectivity estimated from simulations of particle drift.

2 | MATERIALS AND METHODS

2.1 | Sampling and sequencing of the mitochondrial control region

Tripterygion tripteronotum were sampled from 25 locations along the Croatian coast of the eastern Adriatic Sea (n = 550; Figure 1), and from four Eastern Mediterranean locations (n = 39). Table 1 reports sample sizes and year of sampling (2006–2017) per location. Fish were captured at depths of 0–2 m with hand nets while snorkelling. Fin clips were taken from the caudal fins and preserved in 96% ethanol. Fish were released immediately and were frequently observed to return to their territories. Whole genomic DNA was extracted following a rapid Chelex protocol (Richlen & Barber, 2005), and a part of the mitochondrial control region was PCR amplified with primers L-Pro-F, Tropheus (Koblmüller et al., 2011) and TDK-D (Lee, Conroy, Huntting Howard, & Kocher, 1995) as described previously (Koblmüller et al., 2011, 2015). We expected the mtDNA locus to be a suitable marker for population structure, as mitochondrial sequence diversity in vertebrates is generally in accordance with expectations for neutral evolution (Karl, Toonen, Grant, & Bowen, 2012; McCusker & Bentzen, 2010) and sex-specific restrictions of larval dispersal are unlikely. Chain termination sequencing was carried out with primer L-Pro-F, Tropheus and complemented with the other primer when necessary for unambiguous, full-length base calling. Sequencing reactions were purified with Sephadex G-50 (GE Healthcare, Vienna, Austria) and sequence electropherograms were obtained and visualized on an ABI 3130xl capillary sequencer (Applied Biosystems, Vienna, Austria). Sequences were aligned manually (only few gaps were introduced into the alignment) in MEGA X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018). The total length of the sequence alignment was 392 bp. Although short, the fragment comprised 67 variable sites, 31 of which were singleton sites. Sequences are deposited in GenBank under accession numbers MT656680–MT657268.

Sampling was conducted under permits issued by the Hellenic Ministry of Environment and Energy (85404/130/9-1-2008) and by the Ministry of Environmental and Nature Protection of the Republic of Croatia (KLASA: UP/1-324-01/16-01/05; URBROJ: 525-13/0323-16-2; KLASA:UP/1-324-01/17-01/20; URBROJ: 525-13/1282-17-2; KLASA: UP/1-324-01/18-01/05; 525-13/1282-18-2). Ethical approval for the study was obtained from the ethics committee of the University of Graz (approval number 39/19/63 ex 2019/20).

2.2 | Haplotype networks

We used Fitchi (Matschiner, 2016) to construct a haplotype genealogy based on a maximum likelihood (ML) tree inferred under default settings in PhyML 3.0 (Guindon et al., 2010). The HKY+I+G model was selected as the best fitting model of evolution based on the Bayesian Information Criterion (BIC) in the ‘Smart Model Selection’ (SMS; Lefort, Longueville, & Gascuel, 2017) tool implemented in
2.3 | Population structure and genetic diversity

We used Arlequin 3.5.1.2 (Excoffier, Laval, & Schneider, 2005) to calculate estimates of hierarchical and pairwise population differentiation based on haplotype frequencies ($\theta_{ST}$) and uncorrected genetic distances between haplotypes ($\phi_{ST}$). $P$-values obtained in tests for pairwise population differentiation were adjusted for multiple testing (325 pairwise comparisons among 26 populations; locations with <13 sampled individuals were not included in differentiation testing) using the procedure of Benjamini and Hochberg (1995). Arlequin 3.5.1.2 was also used to calculate estimates of genetic diversity within populations (haplotype diversity $H_e$, number of different haplotypes $h$). In order to be able to compare haplotype numbers between populations despite different sample sizes (Table 1), we estimated haplotype richness ($HR$) by rarefaction based on $n = 13$ with a custom-made program written in Perl (available at request from KMS). Due to the small sample size ($n = 6$), location 15 (Vitane on Pašman island) was not included in any population-level analyses (here and below).

2.4 | Geographic patterns of differentiation and diversity

We conducted distance-based redundancy analysis (dbRDA; Legendre and Anderson (1999)) using the R package ‘vegan’ (Oksanen et al., 2015) to separate the effects of ocean circulation and geographic distance on genetic structure. Two separate analyses were performed using the function ‘capscale’ with either pairwise $\theta_{ST}$ or $\phi_{ST}$ values between the Adriatic population samples as dependent variables. In each of the two analyses, we included a categorical predictor variable representing assignment to one of the four recirculation gyres and a continuous predictor variable representing geographic distance between locations. The assignment of sampling locations to recirculation gyres is shown in Table 1 and was based on maps of surface flow in the Adriatic basin (Poulain, 2001; for a schematic representation, see Figure 1) and Lagrangian simulations of particle drift (see below). Geographic distances between sampling locations were calculated from their longitudinal and latitudinal coordinates (Table 1; for the geographic distance matrix, see Appendix S1) with the R package ‘fields’ using the function ‘r.dist.earth’ (Fields Development Team, 2006; Fields: tools for spatial data, see http://www.cgd.ucar.edu/Software/Fields). Then, principal coordinates of the geographic distance matrix were calculated in ‘vegan’ with the function ‘pcmn’ (truncation threshold > maximum distance). The first axis captured 98% of the variation in the geographic distance matrix and was used to represent spatial variation in the dbRDA models. The model using $\theta_{ST}$ as dependent variable was conditioned on haplotype diversity ($H_e$) in order to account for the dependence of $\theta_{ST}$ on genetic variation (Hedrick, 2005). Significance of predictor effects was assessed with 9,999 permutations using the function ‘ANOVA.cca’.

We used non-metric multidimensional scaling of the population-level haplotype frequencies (function ‘metaMDS’ in the package ‘vegan’) to visualize distances between populations and used the function ‘envfit’ to fit a variable representing the four recirculation gyres onto the ordination (significance estimate based on 9,999 permutations).

Bayesian phylogeographic and ecological clustering (BPEC; Manolopoulou, Legarreta, Emerson, Brooks, & Tavaré, 2011) was used to identify genetically distinct geographic population clusters among the Adriatic samples. Preliminary runs of 1 million iterations each, in which we used different prior settings for maxMig (maxMig = 1–4) and hence the maximum number of clusters (maxMig + 1), converged on a three-cluster model (posterior probability of 1.0 for three clusters in runs with maxMig ≥ 2). We then conducted a final analysis with maxMig = 2 and without relaxing the parsimony criterion ($ds = 0$). The analysis was run for 10 million iterations, with 10,000 posterior samples saved.

To test for a latitudinal gradient in genetic diversity, we calculated Spearman correlation coefficients between the latitudes and either $H_e$ or $HR$ of the sampled populations. Additionally, we tested for differences in $H_e$ and $HR$ between populations in the northern and the central sub-basin by fitting two generalized linear models (GLMs) with ‘sub-basin’ as predictor and either $H_e$ or $HR$ as response variables, assuming quasi-binomial and quasi-Poisson error distributions respectively.

2.5 | Coalescent modelling of divergence, migration and demographic history

To estimate rates of gene flow between the Istrian and Northern Adriatic gyre, we applied the isolation-with-migration coalescent model implemented in IMa2 (Hey, 2010). The model employed the HKY model of sequence evolution (Hasegawa, Kishino, & Yano, 1985). The analysis was run for 30 million generations, sampling every 1,000th genealogy with the first 100,000 steps discarded as burn-in, and replicated three times with different random number seeds. Effective sample sizes (ESS) for all parameters were >50, indicating a good mixing of MCMC chains and good coverage of parameter space. To translate parameter estimates into absolute values, we assumed an average generation time of 1 year (de Jonge & Videler, 1989) and alternative substitution rates of 1.11%, 2.61%, 3.61% and 6.77% per site per MY, which correspond to the range of substitution rates inferred for T. delaisi (Koblmüller et al., 2015), a species closely related to T. tripteronomatum (Carreras-Carbonell, Macpherson, & Pascual, 2005).

Past population size changes were inferred separately for the two phylogeographic groups (Istrian and Northern Adriatic gyre; Central and Southern Adriatic gyre) by means of a Bayesian...
coalescent approach (Bayesian skyline tree prior) as implemented in BEAST 1.10.4 (Suchard et al., 2018). We employed the same model of molecular evolution as for ML-tree inference (see above) and assumed a strict molecular clock and alternative substitution rates of 1.11%, 2.61%, 3.61% and 6.77% per site per MY (Koblmüller et al., 2015). For both datasets, two independent runs of 100 million generations each were conducted, sampling every 1,000th step, with a burn-in of the first 10% of sampled generations. Tracer 1.5 (Rambaut & Drummond, 2009) was used to check for run convergence and sufficiently large effective sample sizes (ESS > 200; Kuhner, 2009). Runs were combined using LogCombiner (part of the BEAST package) and Bayesian skyline plots (BSPs) were visualized in Tracer.

2.6 | Lagrangian modelling of passive dispersal

Lagrangian simulations were performed using the Parcels toolbox v2.0.0beta2 (Delandmeter & van Sebille, 2019; Lange & van Sebille, 2017) based on daily mean circulation fields produced by the CMCC (Euro-Mediterranean Center on Climate Change) for the Adriatic Forecasting System (AFS, http://oceanlab.cmcc.it/afs/; last accessed 16 November 2019). Current velocity, wind stress, water temperature and salinity fields produced by the AFS are based on the AREG model (Adriatic Regional Model; Oddo, Pinardi, & Zavatarelli, 2005; Oddo, Pinardi, Zavatarelli, & Coluccelli, 2006), an implementation of the Princeton Ocean Model for the Adriatic Sea nested into the general circulation model of the Mediterranean Sea. Surface fluxes are interactively computed using model predicted sea surface temperature and realistic atmospheric data provided by the European Centre for Medium Range Weather Forecast (ECMWF) with a frequency of 6 hr and a resolution of 0.25° (2004–2015) or 0.125° (2015–2018). To allow full reproducibility of the simulations we created a Docker image (chrishah/parcels-jupyter-notebook:2.0.0beta2, available from https://hub.docker.com/; last accessed 16 November 2019) fully contained to use the Parcels toolbox v2.0.0beta2 Python API inside a Jupyter Notebook server. The code for running the simulations and visualization of the results is deposited (https://github.com/chrishah/trippart) and permanently archived with Zenodo (https://doi.org/10.5281/zenodo.3906970).

To assess potential patterns of passive larval dispersal in the study area and connectivity between the sampled populations, Lagrangian particles were advected in the AREG surface flow fields. Particles were released at the study locations (see Table 1) and one additional, unsampled location (southern Kornati archipelago, 15.515°N, 43.675°E) every 12 hr in the period from February to September throughout the years 2004–2018, i.e. all full years for which the AREG data were available at the time of performing the simulations (6,668 particles per location). Simulations failed due to lack of AREG data for four study locations (number 11, 12, 13 and 17). We used a fourth-order Runge–Kutta integration scheme with a time step interval of 5 min and particles were allowed to drift for 20 days. The biological components of the model, particle release period (February to September) and drift duration (20 days) are based on previously reported life history traits of T. tripterontonotum (de Jonge & Videler, 1989; Macpherson & Zika, 1999; Raventos & Macpherson, 2001; Sabates et al., 2003; Wirtz, 1978). Data visualization was performed in R v3.3.2 (R Core Team, 2013).

3 | RESULTS

3.1 | A phylogenetic break at the boundary between the central and the northern sub-basin

The ML-tree–based haplotype network (Figure 2) revealed two groups of haplotypes, one dominated by samples from the northern sub-basin (i.e. the Istrian and Northern Adriatic gyres) and the other dominated by samples from the central and the southern sub-basin. The individuals from location 15 (Vitane on Pašman island), which is located at the boundary between the central and the northern sub-basin, were distributed in both haplotype groups (three in the Istrian/northern and three in the central/southern haplotype group). Additionally, 16 Istrian/northern-group haplotypes were detected in several central and southern locations (locations 17, 18, 20, 22, 23 and 24; locations shown in Figure 1), and similarly, 12 central/southern-group haplotypes were found dispersed all over the northern basin (locations 3, 4, 6, 7, 8, 10, 13 and 14). All pairwise population differentiation estimates between Istrian/northern and central/southern locations were statistically significant (Appendix S2).

3.2 | Population structure is associated with the recirculation gyres

We conducted distance-based redundancy analysis (dbRDA) to evaluate isolation by gyres and isolation by geographic distance, and found a significant association of genetic structure with gyres ($df = 3; F = 5.65, p = 0.002$ for $\theta_{ST}$ values; $F = 11.69, p = 0.0002$ for $\Phi_{ST}$ values), but not with geographic distance ($df = 1; F = 1.84, p = 0.17$ for $\theta_{ST}$ values; $F = 1.57, p = 0.22$ for $\Phi_{ST}$ values). Controlling for geographic distance in the estimation of the gyre effect accounts for a gap in sampling density at the boundary between the Northern and the Central Adriatic gyre (between locations 14 and 16) and implies that geographic distance cannot be responsible for the observed phylogeographic structure. We also performed non-metric multidimensional scaling of the population-level haplotype frequency matrix and again detected a significant association between haplotype frequencies and gyres ($r^2 = 0.85, p = 0.0001$). The first ordination axis reflected the phylogeographic break between the central/southern and the Istrian/northern groups (Figure 3a). Differentiation between the Istrian and the Northern Adriatic gyre was evident along the second ordination axis, whereas there was no clear separation between populations bordering the Southern and the Central Adriatic gyre. In concordance with these results, the BPEC analysis revealed three DNA sequence clusters corresponding
to the Istrian gyre, the Northern Adriatic gyre and the pool of Central and Southern Adriatic gyre populations (Figure 3b).

We conducted coalescent-based modelling of 'isolation with migration' to test for gene flow between the Istrian and the Northern Adriatic gyre. Reconstructed gene flow was highly asymmetric and occurred almost exclusively from the northern into the Istrian gyre (from Northern Adriatic into Istrian gyre: $m = 1.857 - 1.877$, 95% HPD intervals = 0.448 - 4.988; from Istrian into Northern Adriatic gyre: $m = 0.003 - 0.008$, 95% HPD intervals = 0.000 - 1.153; Figure 4). Divergence time estimates between the Istrian and Northern Adriatic gyre populations converged on ~10–60 (5–156) thousand years ago, depending on the substitution rate (Figure 4; Appendix S3). The overestimation of the divergence time, compared to the post-glacial refilling of the northern basin, may be due to the fact that past population size changes are not accounted for in the model (Liu et al., 2014).

We used hierarchical AMOVAs to partition genetic variance between and within gyres. Accounting for genetic distances between haplotypes ($\Phi_{ST}$ analysis), the largest proportion of variance was assigned between gyres (57% of total variance; $\Phi_{CT} = 0.57$, $p < 0.001$), with much less variance among populations within gyres (1.93% of total variance; $\Phi_{SC} = 0.04$, $p < 0.001$). Hierarchical AMOVA based on haplotype frequencies ($\theta_{ST}$ analysis) likewise assigned a larger proportion of variance to between-gyre structure (21% of total variance; $\theta_{CT} = 0.21$, $p < 0.001$) than within-gyre population structure (3.75% of total variance; $\theta_{SC} = 0.05$, $p < 0.001$).

### 3.3 Population differentiation within recirculation gyres

As shown above, a small but significant proportion of genetic variance was assigned to within-gyre structure by hierarchical ANOVA. Within the northern (11 populations) and the central (8 populations) gyres, global $\theta_{ST}$ and $\Phi_{ST}$ estimates were similar and significant ($\theta_{ST}$, Northern Adriatic gyre = 0.057, $p < 0.001$; Central Adriatic gyre = 0.049, $p < 0.001$; $\Phi_{ST}$, Northern Adriatic gyre = 0.053, $p < 0.001$; Central Adriatic gyre = 0.027, $p = 0.004$). Pairwise differentiation estimates between populations within gyres were low and significant results were not consistent between...
θ_{ST} and Φ_{ST} analyses (Appendix S2). Pairwise θ_{ST} values within the Northern Adriatic gyre were significantly related to geographic distance between populations (dbRDA, $F = 3.56$, $p = 0.001$) when conditioned by $H_e$ (analysis conducted as in above section, without variable ‘gyre’). In contrast, neither Φ_{ST} among populations within the Northern Adriatic gyre nor pairwise θ_{ST} and Φ_{ST} values in the Central Adriatic gyre were correlated with geographic distance (all $p > 0.5$). Cases of geographically distant, undifferentiated population pairs as well as geographically close, differentiated population pairs (Appendix S2) further supported the conclusion that geographic distance is not a main driver of population structure within gyres.

3.4 | Temporal stability

As Adriatic populations were sampled from 2006 to 2017 (Table 1), we examined whether temporal differentiation might have contributed to the observed patterns. No differentiation was found between $T. tripteronotum$ sampled in 2008 and 2017 at Valsaline ($n = 35$ and 15, respectively; θ_{ST} = 0.004, $p = 0.30$; Φ_{ST} = 0.001, $p = 0.35$). Pairwise differentiation estimates among populations of the northern sub-basin (comprising the Istrian and Northern Adriatic gyres) were not correlated with sampling time differences (in years; Mantel tests, using Valsaline samples from 2008: $r = -0.09$, $p = 0.73$ for Φ_{ST} and $r = -0.15$, $p = 0.77$ for θ_{ST}; using Valsaline samples from 2017: $r = -0.00003$, $p = 0.48$ for Φ_{ST} and $r = 0.06$, $p = 0.32$ for θ_{ST}).
The analogous test for the phylogenetic clade comprising the central and southern sub-basins is not meaningful because sampling years are correlated with geographic regions (Table 1). Overall, there is no evidence in our data that the protracted sampling period might have an effect on our analyses.

3.5 | Lagrangian simulations of passive dispersal

We used Lagrangian simulations of particle drift to examine whether genetic differentiation across recirculation gyres could be explained by retention of passively drifting larvae within each of the gyres (Figure 5, Appendix S4). Simulated particle drift reflected the recirculation gyres, but did not adhere strictly to gyre boundaries. Northward drift between gyres was observed at a higher rate than southward drift, and as expected, drift patterns showed seasonal variation across the simulated period (February to September; Appendix S4).

3.6 | No northwards decline in genetic diversity

Estimates of genetic diversity within each population are shown in Table 1. Four to 18 different haplotypes (median value = 7) were detected in the Adriatic populations, and haplotype diversity (\(H_T\)) ranged from 0.38 to 0.93 (mean ± SD = 0.69 ± 0.15). Haplotype diversity was independent of sample size, but the number of haplotypes was positively correlated with sample size. Therefore, we estimated haplotype richness (HR) based on the minimum sample size of \(n = 13\) (range: 3.58–8.83 haplotypes, mean ± SD = 5.75 ± 1.41) to allow comparisons among populations. Contrary to expectations for a step-wise northwards range expansion, neither haplotype richness nor haplotype diversity co-varied with latitude (\(H_T\): Spearman \(r = 0.35, p = 0.10\); HR: Spearman \(r = 0.24, p = 0.25\); \(n = 24\) populations) or decreased between the central and the northern sub-basin (\(H_T\): GLM with quasi-binomial error distribution, est. = −0.01, \(p = 0.91\); HR: GLM with quasi-Poisson error distribution, est. = 0.10, \(p = 0.75\)).

3.7 | Recent population expansion in the Adriatic Sea

The haplotype network of the Adriatic samples comprises a small number of highly frequent haplotypes and numerous low-frequency haplotypes, a pattern that is indicative of recent population expansion (Figure 2). Coalescence models (Bayesian skyline plots) supported recent population expansion in both phylogeographic groups (Figure 6). The onset of population growth in the northern sub-basin clade was dated to >10,000 to <75,000 years ago, dependent on the

![FIGURE 5](https://wileyonlinelibrary.com)
assumed substitution rate. Consistent with the hydrologic history, population growth reconstructed for the central/southern clade was less pronounced and preceded the northward expansion.

3.8 | Relationship to Mediterranean populations

The ML-tree–based network (Figure 2) associated the Eastern Mediterranean haplotypes (Corfu, Crete, Athens) with the northern Adriatic group, and some haplotypes were even shared between Corfu and the northern Adriatic sub-basin. A statistical parsimony network suggested alternative connections, which linked some Eastern Mediterranean haplotypes to either of the two Adriatic haplotype groups (Appendix S5). In analyses of $\Theta_{ST}$ and $\Phi_{ST}$, significant differentiation was detected between populations from Corfu and Crete (the two Mediterranean locations with sufficient sample sizes for population genetic analyses) on the one hand and all of the Adriatic populations on the other hand (Appendix S2). Non-metric multidimensional scaling placed Corfu and Crete apart from the Adriatic populations along the first ordination axis, and separated from each other along the second axis (Appendix S6).

4 | DISCUSSION

4.1 | Population structure in relation to the Adriatic circulation patterns

The present study employed dense geographic sampling along the eastern Adriatic coast to differentiate between the effects of distance versus oceanic circulation patterns on marine population connectivity. We found that genetic population differentiation of T. tripteronotum corresponded to boundaries between the Adriatic recirculation gyres. This result blends in with findings in other marine species and regions, where circulation systems control gene flow and generate patterns of ‘isolation by resistance’ (Banks et al., 2007; Benestan et al., 2016; Thomas et al., 2015; White et al., 2010; Xuereb et al., 2018). However, notwithstanding the intuitive role of the Adriatic gyre systems, other factors might coincide with the gyre boundaries and function as additional or even dominant drivers of population structure. To address this possibility, we investigated whether the observed population structure could indeed be explained by the impermeability of gyre boundaries to passive larval drift. Although the drift trajectories produced by Lagrangian simulations reflected the Adriatic gyres (Figure 5), particles were not exclusively retained within their gyres of origin. Rather, a considerable proportion of particles released in locations of the Northern Adriatic gyre drifted into the Istran gyre consistent with the empirical evidence for northward-biased gene flow between the two gyres. Hence, the observed population structure in the northern Adriatic sub-basin can be explained by recent divergence in the wake of the filling of the northern basin after the last glacial maximum (Lambeck et al., 2014), and incomplete isolation between the two gyres with asymmetric gene flow mediated by northward drifting larvae.

In contrast, drift of particles from the Central Adriatic into the Northern Adriatic gyre predicted that gene flow between these two gyres, if driven by passive drift, should occur at higher rates than indicated by the observed phylogeographic break. Our simulation results concur with Lagrangian analyses by Rossi, Ser-Giacomi, Lopez, and Hernandez-Garcia (2014), who likewise identified a leaking hydrodynamical province boundary in the same region. As in our study, incongruity between potential connectivity inferred from drift simulations and the connectivity realized by presumably passive dispersal was observed in the seagrass Posidonia oceanica across the Adriatic basin (Jahnke et al., 2017). Suggested explanations for this discrepancy included clonal reproduction, low settlement success and small-scale hydrodynamics (Jahnke et al., 2017). In T. tripteronotum, mechanisms such as larval swimming behaviour or differential settlement success (Riginos et al., 2011; Selkoe & Toonen, 2011; Shanks, 2009) may contribute to the restriction of gene flow between the Central and the Northern Adriatic gyre, but it is presently unclear which oceanographic features confine the dispersal of T. tripteronotum larvae in that region.

It is noteworthy that the phylogeographic break coincides not only with the sub-basin and gyre boundaries but also with a gap in the conglomerate of nearshore islands and with a stretch of unusually steeply sloping shoreline (Figure 1). Possibly, if larvae are retained close to their

**FIGURE 6** Bayesian skyline plots depicting estimated changes in population size of Adriatic T. tripteronotum through time. Thick lines and broken lines represent the median and 95% HPD intervals respectively. The y-axis represents the population size parameter (female effective population size times the substitution rate). The x-axes are scaled by alternative substitution rates to illustrate the range of time estimates.
natal sites (Brandl et al., 2019), genes may move in a wide front along the island-rich coastline, whereas gene flow may be curbed along the narrow belt of suitable habitat in the region where we observe the phylogeographic break. Furthermore, selection could contribute to the observed break either in that fish achieve very little reproductive success after crossing between gyres or by selecting against mitochondrial introgression in the face of nuclear gene flow (Morales, Pavlova, Joseph, & Sunnucks, 2015). In both cases, an abrupt change in the selection regime between the central and the northern sub-basin would be required to counter the gene flow potential that was suggested by the drift simulations.

Based on available data, it is difficult to assess whether the phylogeographic break between approx. 43.6° and 44° northern latitude on the eastern Adriatic coast is generally shared across species. In the densely sampled octocoral *Paramuricea clavata*, a distinct genetic discontinuity was detected between the Kvarner area (represented in our sample by locations 5–12, Figure 1) and the Kornati area (Ledoux et al., 2018). The Kornati sites of Ledoux et al. (2018) are located at ~43.8° northern latitude, i.e. within the latitudinal range of the phylogeographic break in *T. tripteronotum*, such that the genetic divergences in *T. tripteronotum* and *P. clavata* may indeed coincide geographically. Other studies in the Adriatic Sea lack the necessary geographic sampling to locate genetic breaks (Carreras et al., 2017; Jahne et al., 2017; Koblmüller et al., 2015; López-Márquez et al., 2019; Schiavina et al., 2014).

In contrast to the situation in *T. tripteronotum*, no genetic divergence between populations from different gyres seems to exist in another triplefin species, *T. delaisi*, as specimens sampled from one location in the Central Adriatic gyre grouped among populations of the Northern Adriatic gyre (Koblmüller et al., 2015). Adult Tripterygion delaisi occur in the same regions of the Eastern Adriatic as adult *T. tripteronotum*, but in a much wider range of depth (typically 3–40 m (Wirtz, 1978), except for occasional, more shallow occurrence at very shadowy places (Koblmüller et al., 2015)). In New Zealand, triplefin species inhabiting the very shallow benthic zone are also more strongly structured than deeper-dwelling species (Hickey, Lavery, Hannan, Baker, & Clements, 2009). Hickey et al. (2009) concluded that ‘forces structuring populations, whatever these may be, decline with increasing depth’. It will be useful to complement the present study with investigations of more species, for instance, representatives of blenniid fishes, in order to identify determinants of (crypto)benthic population structure along the Adriatic coast.

### 4.2 Connectivity among populations within gyres

With benthic, territorial adults and inshore-dispersing planktonic larvae (de Jonge & Videler, 1989; Sabates et al., 2003; Wirtz, 1978), gene flow among *T. tripteronotum* populations might be limited by distance, as observed, for instance, in the octocoral *Paramuricea clavata* in the same region (Ledoux et al., 2018) or, albeit on somewhat larger geographic scales, in other species of Tripterygiidae along the coasts of New Zealand (Hickey et al., 2009) and Spain (Carreras-Carbonell, Macpherson, & Pascual, 2006). In contrast to this prediction, population structure within gyres was low and we detected no effect of geographic distance on genetic population structure after accounting for structure across gyre boundaries.

We also observed numerous disagreements between *θ*_{ST} and *Φ*_{ST}-based inferences of population differentiation within gyres, which are likely due to the different sensitivities of the two statistics to drift and mutations at low levels of differentiation (Sefc, Payne, & Sorenson, 2007). Both test statistics indicate weak differentiation of populations situated in an enclosed bay on the island of Pag (locations 11 and 12) and in the Split Channel (locations 17–19) from several other populations within the respective recirculation gyre. We conclude that connectivity within gyres is generally high, but may be reduced in the case of populations at secluded locations.

### 4.3 Post-glacial recolonization of the northern Adriatic and relationship between Adriatic and Eastern Mediterranean haplotypes

Genetic signatures of population expansion were consistent with a rapid recolonization of the northern sub-basin. A comparable signal was previously observed in the related triplefin *T. delaisi* (Koblmüller et al., 2015), whereas no evidence for rapid population expansion in the northern Adriatic was detected, for instance, in the octocoral *P. clavata* (Ledoux et al., 2018). In contrast, populations of *P. clavata* displayed the expected diversity gradient along the recolonization axis (Ledoux et al., 2018), which was absent in the present study. In *T. tripteronotum*, the absence of a northwards decline in genetic diversity may be explained by higher connectivity among populations within gyres and particularly by northward-biased gene flow, which introduces genetic variability into the northern-most, Istrian gyre.

The placement of the Eastern Mediterranean haplotypes in the phylogenetic network did not conform to a geographic cline in the distribution of Mediterranean haplotypes along the Adriatic basin. Rather, the Eastern Mediterranean shared haplotypes with the northern Adriatic populations, but not with the central and southern Adriatic populations (Figure 2). This unexpected pattern may essentially be a product of the random sorting of ancestral polymorphisms. Late Pleistocene sea level changes (Rohling et al., 2009) likely induced population shifts, local population extinctions and fluctuations in population sizes in species inhabiting the shallow littoral (Sefc et al., 2017). These events are also likely to have precipitated geographic redistributions and changes in the frequencies of mitochondrial haplotypes, culminating in the transfer of Mediterranean haplotypes into the northern sub-basin, while they declined in frequency in the central sub-basin.

### 5 Conclusions

The dense sampling employed in the present study allowed us to separate correlations of population structure with geographic
distance (isolation by distance) from correlations with oceanographic features (isolation by resistance) in the Adriatic Sea. While we document a strong relationship between population structure and the Adriatic sub-basin and gyre structure, we also demonstrate that population differentiation across one of the investigated gyre boundaries is stronger than predicted by the simulated effect of gyres on passive larval dispersal. Our data clearly support a role for ocean currents in shaping population structure and connectivity in our study species, but additional mechanisms seem to be at work to reduce gene flow between the central and the northern Adriatic sub-basin.

Biophysical modelling of potential connectivity has been recognized as a valuable tool for marine conservation planning (Coleman et al., 2017; Melià et al., 2016; Rossi et al., 2014). Our results corroborate the insight that species-specific differences in realized connectivity (Paterno et al., 2017) and discrepancies between realized and potential connectivity (Jahne et al., 2017) call for integrating oceanographic simulations with biological data collection, preferably across a range of species, in order to inform efficient conservation planning.

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DATA AVAILABILITY STATEMENT
Sequences are deposited in GenBank under accession numbers (MT656680–MT657268). The code for running the Lagrangian drift simulations and visualization of the results is deposited (https://github.com/chrisrh/trippart) and permanently archived with Zenodo (https://doi.org/10.5281/zenodo.3906970).

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BIOSKETCH
Kristina M. Sefc is broadly interested in factors shaping diversification processes. The research group studies the roles of habitat structure, geography, palaeoenvironmental fluctuations and behaviour in diversification and speciation, with a focus on aquatic systems.

Author contributions: KMS and SK conceived the research ideas; KMS, SK, MW, and LZ conducted the fieldwork with assistance from SZ and MK; MW, LZ, SW, BS, PA, TW, NB, TK and SI collected and analysed the genetic data; KMS, SK and MW finalized the population genetic analyses; CH conducted the Langrangian simulations; KMS led the writing with assistance from SK, MW, MK, SZ and CH.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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