Journal of Sea Research xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Sea Research

journal homepage: www.elsevier.com/locate/seares

Seasonal variation in parasite infection patterns of marine fish species from the Northern Wadden Sea in relation to interannual temperature fluctuations

Franziska M. Schade [a,*], Michael J. Raupach [b], K. Mathias Wegner [a]

[a] Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Hafenstrasse 43, 25992 List/Sylt, Germany
[b] German Center for Marine Biodiversity Research, Senckenberg am Meer, Südstrand 44, 26382 Wilhelmshaven, Germany

ARTICLE INFO

Article history:
Received 27 February 2015
Received in revised form 2 August 2015
Accepted 11 September 2015
Available online xxxx

Keywords:
Climate change
Heat waves
Thermal stress
Pathogen resistance
Emerging diseases
Vibrios
Molecular species identification

ABSTRACT

Marine environmental conditions are naturally changing throughout the year, affecting life cycles of hosts as well as parasites. In particular, water temperature is positively correlated with the development of many parasites and pathogenic bacteria, increasing the risk of infection and diseases during summer. Interannual temperature fluctuations are likely to alter host–parasite interactions, which may result in profound impacts on sensitive ecosystems.

In this context we investigated the parasite and bacterial Vibrionaceae communities of four common small fish species (three-spined stickleback *Gasterosteus aculeatus*, Atlantic herring *Clupea harengus*, European sprat *Sprattus sprattus* and lesser sand eel *Ammodytes tobianus*) in the Northern Wadden Sea over a period of two years. Overall, we found significantly increased relative diversities of infectious species at higher temperature differentials. On the taxon-specific level some macroparasite species (trematodes, nematodes) showed a shift in infection peaks that followed the water temperatures of preceding months, whereas other parasite groups showed no effects of temperature differentials on infection parameters.

Our results show that even subtle changes in seasonal temperatures may shift and modify the phenology of parasites as well as opportunistic pathogens that can have far reaching consequences for sensitive ecosystems.

1. Introduction

Parasites play an intrinsic but hidden role in marine ecosystems (Mouritsen and Poulin, 2002). However, little is known about how parasites are influenced by changing environments. As environmental conditions alter, shifts in host–parasite interactions are likely to occur. These changes could affect parasites in a direct or indirect way by influencing their free-living infective stages or their host range. Hence, environmental changes can result in gain or loss of parasite species as well as in increased or decreased host susceptibility (Lafferty, 1997; Lafferty et al., 2004). In particular, temperature will drive future ecological changes in marine ecosystems (Harley et al., 2006). The mean water temperature and extreme events, e.g. heat waves, are predicted to increase in the nearer future (Schär et al., 2004; Murray and Ebi, 2012) and are supposed to affect the temperature-dependent development of many parasites and pathogenic bacteria (Harvell et al., 2002). Especially many bacterial disease agents thrive in warmer waters by speeding up their biochemical reactions, which result in faster development and increased transmission stages per generation (Pounds et al., 2006; Lafferty, 2009). This can lead to higher parasite fitness and increased disease outbreaks during summer months (Karvonen et al., 2010). In the case of macroparasites, such as trematodes, it has been shown that elevated water temperatures may be beneficial, e.g. by extending the transmission periods or enhancing host penetration (Sankurathri and Holmes, 1976; Paull et al., 2012). On the other hand a subset of pathogenic bacteria and parasites might decline with warming, depending on their own thermal tolerance (Karvonen et al., 2010). Unfortunately, long-term developments of parasite fauna are largely unexplored in changing coastal ecosystems.

A highly dynamic coastal ecosystem of global importance is the European Wadden Sea (Vanbeek et al., 1989). As an essential nursery ground for many fish species, altered host–parasite-interactions may have profound consequences for this sensitive ecosystem. In the past, most parasitological studies in the Wadden Sea focussed on invertebrates (Lauckner, 1971; Thieltges, 2006; Thieltges et al., 2013) or on commercially important fish species like flatfishes or gadoids (Hilger et al., 1991; Moller and Anders, 1992). In contrast, macroparasite communities of small-sized fish were only rarely considered (Groenewold et al., 1996; Zander, 2005). Nevertheless, macroparasites often utilize small fish as intermediate host and these fishes play an important role

* Corresponding author.
E-mail address: Franziska.Schade@awi.de (F.M. Schade).

http://dx.doi.org/10.1016/j.seares.2015.09.002
1385-1101/

in the food web of the Wadden Sea by transmitting parasite to predacious fish and seabirds (Kühl and Kuipers, 1983).

In order to deepen our understanding of seasonal infection patterns of macroparasites, we investigated the parasite community of four common small fish species (three-spined stickleback *Gasterosteus aculeatus*, Atlantic herring *Clupea harengus*, European sprat *Sprattus sprattus* and lesser sand eel *Ammodytes tobianus*) in the Northern Wadden Sea over a period of two years and related our findings to water temperature, as a potential driver of infection patterns (Fig. 1). Knowledge on potential bacterial pathogens (microparasites) in Wadden Sea fish is even scarcer (Wegner et al., 2012; Thieltges et al., 2013). Therefore, we also included potentially pathogenic bacteria of the family Vibrionaceae into our survey. Vibrionaceae include many facultative symbionts and pathogenic strains (Austin and Austin, 2007) that are strongly associated with warmer water temperatures and immune-compromised hosts (Vezzulli et al., 2010; Baker-Austin et al., 2013).

The combination of parasite prevalence, mean intensity and diversity of macro- and potential microparasitic infections in relation to water temperature, as one of the main drivers of epidemiological patterns in aquatic habitats, will help to clarify how environmental change might impact host–parasite interactions in the Wadden Sea ecosystem.

2. Material and methods

2.1. Sampling and macroparasite screening

Four common fish species (three-spined stickleback *Gasterosteus aculeatus*, Atlantic herring *Clupea harengus*, European sprat *Sprattus sprattus* and lesser sand eel *Ammodytes tobianus*) from the Sylt-Rømø-Bight, North Sea, Germany were chosen as representatives to investigate the parasite community of small fish in the Northern Wadden Sea, Germany. Using a seasonal sampling scheme, 12 fish per species ($n_{total} = 308$) were caught during eight different cruises with the RV "Mya" between 2011 and 2012 (Table 1). After sampling, Atlantic herring and European sprat were investigated immediately. Three-spined stickleback and lesser sand eel were kept separately in 20l aquaria at a water temperature corresponding to prevalent sampling conditions for maximum 48 h before investigation. This short fish keeping period might have led to the transmission or loss of ectoparasites or bacteria that could potentially bias our estimates of prevalence and diversity. This will however only affect a few species of macroparasites and is unlikely to influence our estimates of bacterial diversity that did not rely on quantitative assessment.

For parasite screening narcotized fish were measured (total length 0.1 cm) and weighed (0.01 g) and body, fins and gills were screened for ectoparasites. The fish was killed by an overdose of MS-222 and afterwards dissected and screened for endoparasites under a stereomicroscope. A trichinae press was used to detect cysts and parasites in heart, liver, intestines and gills. Macroparasites were photographed under a stereomicroscope for morphological identification and stored in ethanol for molecular species identification.

2.2. Vibrionaceae isolation and identification

To isolate Vibrionaceae species, fish liver, intestine, gills and blood were plated out on selective medium (TCBS agar, Sigma-Aldrich, Steinheim) and incubated at 25 °C for 24 h. A subsample of bacterial colonies was suspended in 2 ml growth medium and incubated at 25 °C and with 300 U/min for 24 h. Vibrionaceae identification was performed by amplifying the 16S rRNA region using PCR universal eubacterial primers 16S-27f and 16S-1392r (Lane, 1991). The 20 μl PCR mixture consisted of 10.75 μl ddH_2O, 4 μl 5× Flexi Buffer, 1.2 μl $MgCl_2$ (25 mM), 1 μl dNTP (10 mM), 1 μl each forward and reverse primers (50 μM), 0.05 μl GoTaq DNA Polymerase (5 U/μl, all PCR chemicals from Promega, Mannheim) and 1 μl template DNA (bacterial liquid culture was heated to 95 °C for 5 min, concentrations approximately 10–20 ng/μl). Thermal cycling included an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of 30 s at 95 °C, 30 s at 55 °C and 90 s at 72 °C and a final extension step at 72 °C for 10 min. Sequencing was performed by the institute of clinical molecular biology (IKMB University hospital Kiel).

2.3. Macroparasite identification

DNA from macroparasites was extracted using the DNAeasy Blood and Tissue Kit (Qiagen, Hilden) according to the manufacturer's protocol. DNA suspension was kept at −20 °C until use. Molecular parasite identification was performed by amplifying the D2–D3 fragment of the 28S rDNA region of nematodes and trematodes using PCR primers D2A and D3B (De Ley et al., 2005) as well as the complete 18S rDNA of cestodes using PCR primers 18A1 and 1800 (Wollscheid and Wägele, 1999).

The 28S PCR reaction was conducted in a final reaction volume of 20 μl containing 10 μl ddH_2O, 2 μl 10× Buffer Y, 4 μl 5× Enhancer solution P (both PCR chemicals from Peqlab), 2 μl dNTP (10 mM), 0.2 μl each forward and reverse primers (20 μM), 0.1 μl Taq DNA Polymerase (5 U/μl, Peqlab) and 1.5 μl template DNA (approximately 5–10 ng/μl) using a thermocycler (Biometra) under the following conditions: initial denaturation at 95 °C for 5 min, followed by 40 cycles of 30 s at 94 °C, 1 min at 55 °C and 2 min at 72 °C and a final extension at 72 °C for 10 min.

The 18S PCR reaction was conducted in a final reaction volume of 40 μl containing 25.5 μl ddH_2O, 4 μl 10× Buffer, 4 μl 5× Enhancer solution P (both PCR chemicals from Peqlab), 4 μl dNTP (10mM), 0.2 μl each forward and reverse primers (50 μM), 0.1 μl DNA Polymerase (5 U/μl, Peqlab) and 2 μl template DNA (approximately 5–10 ng/μl) using a thermocycler (Biometra, Göttingen, Germany) under the following conditions: initial denaturation at 94 °C for 5 min, followed by 36 cycles of 45 s at 94 °C, 50 s at 50 °C and 200 s at 72 °C and a final extension at 72 °C for 10 min. Sequencing was performed by the institute of clinical molecular biology (IKMB University hospital Kiel).

Chromatograms of the 28S and 18S rDNA sequences were edited and assembled using the Sequencher 4.8 software. To identify macroparasite species, we compared edited sequences against published sequences in NCBI GenBank using the nucleotide BLASTn algorithm (Altschul et al., 1990). A sequence similarity of 97% was selected as threshold for species identification. Parasites with no representative sequence in GenBank were morphologically identified. All sequences were deposited at GenBank with accession numbers KT767117-KT767180 (28S) and KT767111-KT767116 (18S).

2.4. Phylogenetic reconstruction and analyses

All chromatograms of the bacterial 16S rRNA sequences were edited and assembled using Sequencher 4.8 software. To identify closely phylogenetic relatives of isolated Vibrionaceae, we compared edited sequences against published sequences in NCBI GenBank using the BLASTn algorithm. We also included 23 reference strains that were accessed from NCBI GenBank (see Appendix A). Edited sequences and reference strains were aligned using the MUSCLE software (Edgar, 2004) and adjusted manually in AliView, version 1.15 (Larsson, 2014). A phylogenetic maximum likelihood tree was constructed using MEGA, version 6.06 (Tamura et al., 2013), based on distance matrices using the General Time Reversal model with γ-distributed rates and invariant sites (GTR + G + I) (Rodriguez et al., 1990), following the best model fit suggested by jModelTest (Posada, 2008). Statistical significance was accessed from 1000 non-parametric bootstrap replicates (Felsenstein, 1985) and tree topology was estimated using the most likely tree from maximum likelihood analysis. A radial cladogram was drawn using the interactive Tree of Life web service (http://itol.embl.de, Letunic and Bork, 2011).

All sequences were deposited at GenBank with accession numbers KT582790-KT583112 (16S).

Please cite this article as: Schade, F.M., et al., Seasonal variation in parasite infection patterns of marine fish species from the Northern Wadden Sea in relation to interannual t..., J. Sea Res. (2015), http://dx.doi.org/10.1016/j.seares.2015.09.002

2.5. Data analyses

Prevalence (percentage of infected individuals in total sample) and mean intensity (mean number of parasites per infected hosts) of macroparasites from each fish species as well as for fish species separated by sampling season and year were calculated (Bush et al., 1997). Differences in infection parameters between sampling periods were analyzed by generalized linear models (GLMs) using prevalence (family = binomial) and mean intensity (family = negative binomial) as dependent variables and fish species, sampling season and year as independent predictors as well as fish length and weight as covariates to control the influence of host size. We used individual fish as statistical units and applied multiple post-hoc tests to identify pairwise differences of significant seasonal and annual effects on infection parameters. Family wise error rates were controlled by Bonferroni correction (Miller, 1981), adjusting the statistical significance level at $P = 0.008$ for seasonal effects (six multiple tests) and at $P = 0.0125$ for annual effects (four multiple tests).

Furthermore, we determined diversity indices (Holmes and Price, 1986) including total number of macroparasite species, parasitic burden, the Shannon-Wiener index (Hs) and species evenness (E) for each fish species as well as for fish species separated by sampling season and year. To describe the diversity of the Vibrionaceae community, we clustered all sequences into operational taxonomic units (OTU), based on 97% sequence similarity criteria, using uclust and calculated diversity indices analogous to macroparasite communities. OTUs were then transformed into a distance matrix based on unifrac distances (Lozupone and Knight, 2005), incorporating phylogenetic and abundance information of Vibrionaceae OTUs. Community structure was analyzed by permutational multivariate analysis of variances (PERMANOVA) using the Adonis function of the vegan package (Oksanen et al., 2013), partitioning variance between host species, sampling season and year.

To eliminate seasonal effects and relate year wise differences of the diversity of Vibrionaceae and macroparasite communities to different temperature regimes, we calculated temperature and diversity differentials among the years (2012–2011) and analyzed them by using GLMs (family = Gaussian) with relative Hs as a measure of diversity and with relative water temperature, fish species and parasite group as independent predictors. To account for different sampling dates between the years that potentially confound temperature differences with time, we repeated the statistical analyses for single sampling time points by using the differences of temperatures summed up over the 1–16 temperature measurements preceding the actual date of sampling (see Fig. 1). For each of these 16 temperature sums we calculated the same statistical model and evaluated over which time span integration gave the maximal explanatory value. Additionally, we calculated and analyzed prevalence and mean intensity differentials of macroparasite groups and investigated year wise differences (2012–2011) of infection parameters to different temperature regimes analogous to diversity differentials.

All GLM model effects were tested by analysis of deviance and we assumed deviance change to be approximately χ^2 distributed. All statistical analyses were carried out using R statistical package (R Core Team, 2014).

Table 1
Numbers of sampled fish and identified bacteria representing sample sizes for three-spined stickleback *Gasterosteus aculeatus* (=GA), Atlantic herring *Clupea harengus* (=CH), European sprat *Sprattus sprattus* (=SS) and lesser sand eel *Ammodytes tobianus* (=AT), respectively. Terms "2011" and "2012" were used to differentiate between sampling years. T = temperature in °C, Sal = salinity in ‰.

	Date	T	Sal	pH	Fish sampled				Bacteria identified			
					GA	CH	SS	AT	GA	CH	SS	AT
2011	08.03.11	0.8	25.6	8.13	12	12	4	1	22	8	6	1
	13.07.11	17.3	29.2	8.25	12	12	10	12	4	20	15	13
	26.10.11	8.6	27.2	8.13	12	12	12	12	21	17	3	4
	16.01.12	4.2	28.0	8.20	12	12	12	6	10	19	16	9
2012	26.03.12	7.3	27.3	8.23	12	12	12	11	20	24	20	12
	16.07.12	17.1	29.9	8.17	0	12	0	12	0	22	0	6
	24.10.12	11.1	27.4	8.03	12	12	12	10	6	8	9	5
	05.02.13	2.0	28.0	8.12	12	12	0	2	13	8	0	0

3. Results

3.1. Macroparasite community

In total, we detected 16 different macroparasite species from six phyla covering nematodes, acanthocephalans, monogeneans, digeneans, cestodes and parasitic copepods (Table 2). Some parasite species only

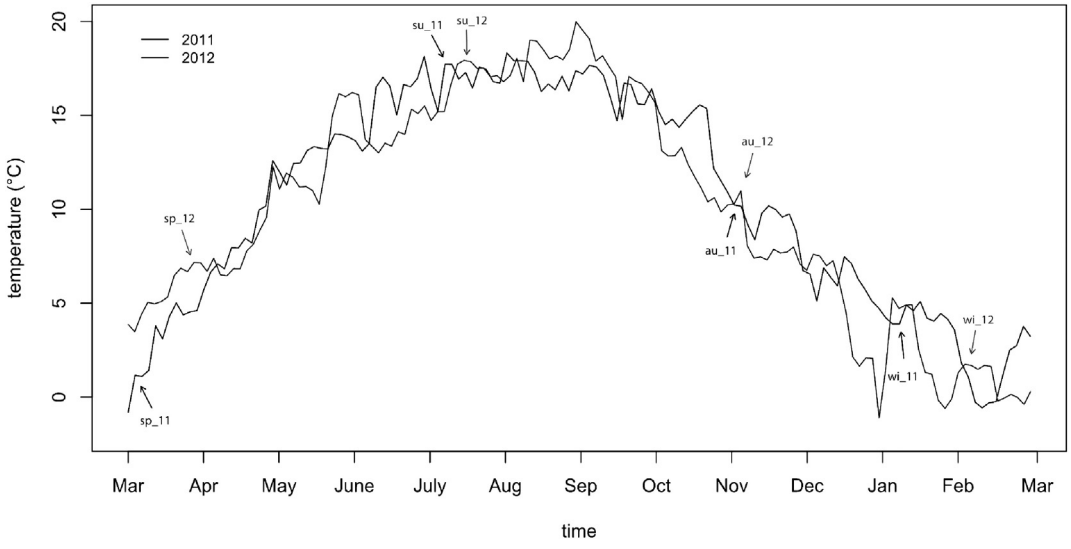

Fig. 1. Water temperature in the Sylt-Rømø-Bight, North Sea, in 2011 and 2012. Arrows show sampling days. Data provided from weekly sampling taken by Tatyana Romanova. With sp = spring, su = summer, au = autumn, wi = winter.

Please cite this article as: Schade, F.M., et al., Seasonal variation in parasite infection patterns of marine fish species from the Northern Wadden Sea in relation to interannual t..., J. Sea Res. (2015), http://dx.doi.org/10.1016/j.seares.2015.09.002

Table 2
Prevalence (upper value, in %) and mean intensity ± standard deviation (lower value) of macroparasites of three-spined stickleback (=GA), Atlantic herring (=CH), European sprat (=SS) and lesser sand eel (=AT) and sequence similarity using NCBI BLASTn algorithm. Results combine data from 2011 and 2012. Morph = Morphological identification. *Molecular identification: *Lecithocladium excisum* with 89–90% sequence similarity in GenBank, morphological identification: *Brachyphallus crenatus* (no representative sequence in GenBank). Letters in brackets give information about the organ with the highest parasite prevalence (microhabitat). B = Body Cavity, E = Eyes, F = Fins, G = Gills, I = Intestines/Intestinal wall, S = Skin, St. = Stomach.

	GA	CH	SS	AT	Similarity
Number of fish examined	84	96	62	66	
Mean total length (cm)	5.63 ± 1.1	9.69 ± 1.5	9.14 ± 1.4	11.88 ± 1.9	
Mean total weight (g)	1.76 ± 0.9	5.74 ± 2.3	5.06 ± 2.6	5.11 ± 2.2	
NEMATODA					
Contracaecum osculatum (I, BC)	1.2 1 ± 0			1.5 1 ± 0	99%
Contracaecum rudolphi (I, BC)	3.6 1.7 ± 1.2				99–100%
Contracaecum septentrionale (I, BC)			1.6 2 ± 0		99%
Hysterothylacium auctum (I, BC)	13.1 2.4 ± 1.9	8.3 3.9 ± 6.6	43.5 5.1 ± 5.3	3.0 1 ± 0	99–100%
ACANTHOCEPHALA					
Acanthocephalus lucii (I)		3.1 1 ± 0			Morph.
MONOGENEA					
Gyrodactylus sp. (F)	11.9 1.5 ± 0.8				Morph.
Mazocraes alosae (G)	6.0 1.4 ± 0.9	14.6 1.4 ± 0.6	16.1 1.8 ± 1.9	4.5 1 ± 0	90–95% + Morph.
DIGENEA					
Labratrema minimus (I)			1.6 1 ± 0	1.5 1 ± 0	Morph.
Brachyphallus crenatus (St)	17.9 4.2 ± 4.4	22.9 2.7 ± 3.9	25.8 6.4 ± 10.3	65.2 5.4 ± 5.4	89–90%* + Morph.
CESTODA					
Scolex pleuronectis (I)	2.4 1 ± 0				98%
Bothriocephalus scorpii (I)	2.4 5 ± 1.4				98–99%
Diphyllobothrium dentriticum (I)	3.6 1.7 ± 1.2				99%
Triaenophorus lucii (I)	1.2 1 ± 0				Morph.
PARASITIC COPEPODA					
Caligus elongatus (F, S)	28.6 1.3 ± 1	5.2 1 ± 0	1.6 1 ± 0	1.5 1 ± 0	Morph.
Lernaeenicus sprattae (E)			19.4 1 ± 0		Morph.
Thersitina gasterostei (G)	3.6 1 ± 0				Morph.

occurred in a single host species, e.g. the copepod *Lernaeenicus sprattae* in European sprat or the acanthocephalan *Acanthocephalus lucii* in Atlantic herring, whereas others were present in several hosts. The digenean *Brachyphallus crenatus*, the nematode *Hysterothylacium auctum* and the copepode *Caligus elongatus* were the dominant parasites, reaching highest prevalence in sand eel (65.2%), sprat (43.5%) and stickleback (28.6%), respectively.

The highest diversity was found in the parasite community of the three-spined stickleback consisting of 12 different macroparasite species, with all four cestode species occurring exclusively in this host (Tables 2, 3). The diversity of the parasite communities from Atlantic herring, European sprat and lesser sand eel ranged from five to seven different parasite species and showed diversity indices in the range from 1.46 to 1.67 (Table 3).

Table 3
Diversity indices of the macroparasite communities of three-spined stickleback, (=GA), Atlantic herring (=CH), European sprat (=SS) and lesser sand eel (=AT) Hs = Shannon-Wiener index, E = evenness.

	GA	CH	SS	AT
Number of macroparasite species	*12*	*5*	*7*	*6*
Parasitic burden	23.2	10.0	18.3	10.4
Hs	2.31	1.46	1.67	1.47
E	0.93	0.90	0.86	0.90

Beside significant effects of host species on parasite prevalence (Fig. 2, Table 4) and mean intensity (Fig. 3, Table 5), we found strong influences of sampling season on trematode and nematode infection parameters.

Trematodes followed the seasonal temperature course and occurred significantly more often in spring (55.3%) and summer (54.2%) than in autumn (29.8%) and winter (30.4%) (sp vs. au $\chi^2_1 = 2.73$, $P < 0.001$, sp vs. wi $\chi^2_1 = 2.23$, $P = 0.002$, su vs. au $\chi^2_1 = 2.42$, $P = 0.001$, su vs. wi $\chi^2_1 = 1.98$, $P = 0.004$). Highest mean intensity was detected in winter (4.8 ± 1.8), but significantly different mean intensities were only found between spring (3.8 ± 0.8) and autumn (2.5 ± 0.3) (sp vs. au $\chi^2_1 = 13.38$, $P < 0.001$) and between summer (4.6 ± 0.9) and autumn (su vs. au $\chi^2_1 = 14.82$, $P < 0.001$). Additionally, we found significant differences between sampling years on infection parameters of trematodes with increased prevalences in spring and autumn 2012 (Fig. 2, sp_11 vs. sp_12 $\chi^2_1 = 5.61$, $P < 0.001$, au_11 vs. au_12 $\chi^2_1 = 2.27$, $P < 0.001$) and increased mean intensities in spring 2012 (Fig. 3, sp_11 vs. sp_12 $\chi^2_1 = 30.38$, $P < 0.001$) compared to 2011.

Please cite this article as: Schade, F.M., et al., Seasonal variation in parasite infection patterns of marine fish species from the Northern Wadden Sea in relation to interannual t..., J. Sea Res. (2015), http://dx.doi.org/10.1016/j.seares.2015.09.002

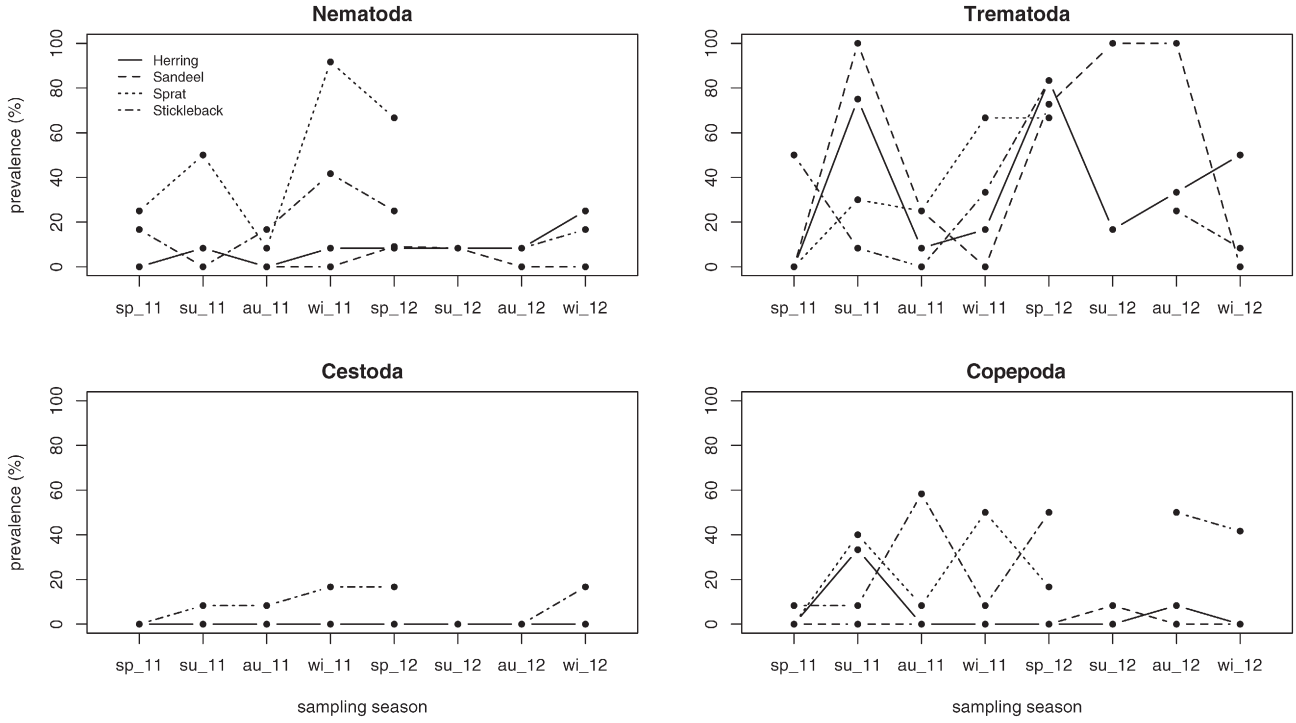

Fig. 2. Prevalence of macroparasite phyla separated by fish species and sampling period. Gaps represent sampling events with missing fish species.

In contrast to trematodes, nematodes were found significantly more often in winter (31.9%) compared to summer (12.5%) and autumn (6.4%) (wi vs. su $\chi^2_1 = 1.32$, $P = 0.005$, wi vs. au $\chi^2_1 = 2.58$, $P < 0.001$), whereas intensity was significantly higher in spring (5.4 ± 1.4) and winter (3.8 ± 1.0) compared to autumn (2.0 ± 0.7) (sp vs. au $\chi^2_1 = 12.20$, $P < 0.001$, wi vs. au $\chi^2_1 = 20.81$, $P < 0.001$). Nematode prevalence was also significantly affected by fish weight. Lighter fish, after controlling for species affiliation, were significantly more often infected with nematodes (Table 4).

Cestodes and parasitic copepods were comparatively rare (Table 2) and we could not detect any seasonal or annual pattern, probably a result of low prevalence.

3.2. Vibrionaceae community

We found 20 OTUs representing 13 clearly identified species from the family Vibrionaceae in the organs of all four marine fish species (Fig. 4). Seven species belonged to the genus *Vibrio*, three species to

Table 4
Summary of seasonal and annual effects on macroparasite prevalence. Generalized linear models containing prevalence of macroparasite phyla as dependent variables and fish species, year, season, fish length and fish weight as independent predictors. Significant effects are shown in bold. Family = binomial.

Response	Nematoda prevalence					Trematoda prevalence				
	Df	Dev	ResDf	ResDev	P(>Chi)	Df	Dev	ResDf	ResDev	P(>Chi)
Null			307	282.9				307	419.5	
Fish species (F)	3	39.6	304	243.2	**<0.001**	3	25.5	304	394.0	**<0.001**
Year (Y)	1	0.03	303	243.2	0.861	1	15.9	303	378.1	**<0.001**
Season (S)	3	26.5	300	216.7	**<0.001**	3	20.1	300	358.0	**<0.001**
Fish length	1	1.8	299	214.9	0.180	1	3.1	299	354.9	0.079
Fish weight	1	7.5	298	207.4	**0.006**	1	1.6	298	353.4	0.211
F*Y	3	2.7	295	204.7	0.443	3	2.1	295	351.3	0.555
F*S	9	14.6	286	190.1	0.102	9	55.8	286	295.5	**<0.001**
Response	Cestoda prevalence					Copepoda prevalence				
	Df	Dev	ResDf	ResDev	P(>Chi)	Df	Dev	ResDf	ResDev	P(>Chi)
Null			307	74.2				307	259.7	
Fish species (F)	3	21.4	304	52.8	**<0.001**	3	40.9	304	218.8	**<0.001**
Year (Y)	1	0.2	303	52.7	0.669	1	0.2	303	218.6	0.637
Season (S)	3	2.2	300	50.4	0.527	3	3.2	300	215.4	0.363
Fish length	1	0.3	299	50.1	0.568	1	3.0	299	212.4	0.082
Fish weight	1	0.4	298	49.7	0.509	1	1.2	298	211.2	0.272
F*Y	3	0	295	49.7	1.000	3	11.9	295	199.3	**0.008**
F*S	9	0	286	49.7	1.000	9	23.6	286	175.8	**0.005**

Please cite this article as: Schade, F.M., et al., Seasonal variation in parasite infection patterns of marine fish species from the Northern Wadden Sea in relation to interannual t..., J. Sea Res. (2015), http://dx.doi.org/10.1016/j.seares.2015.09.002

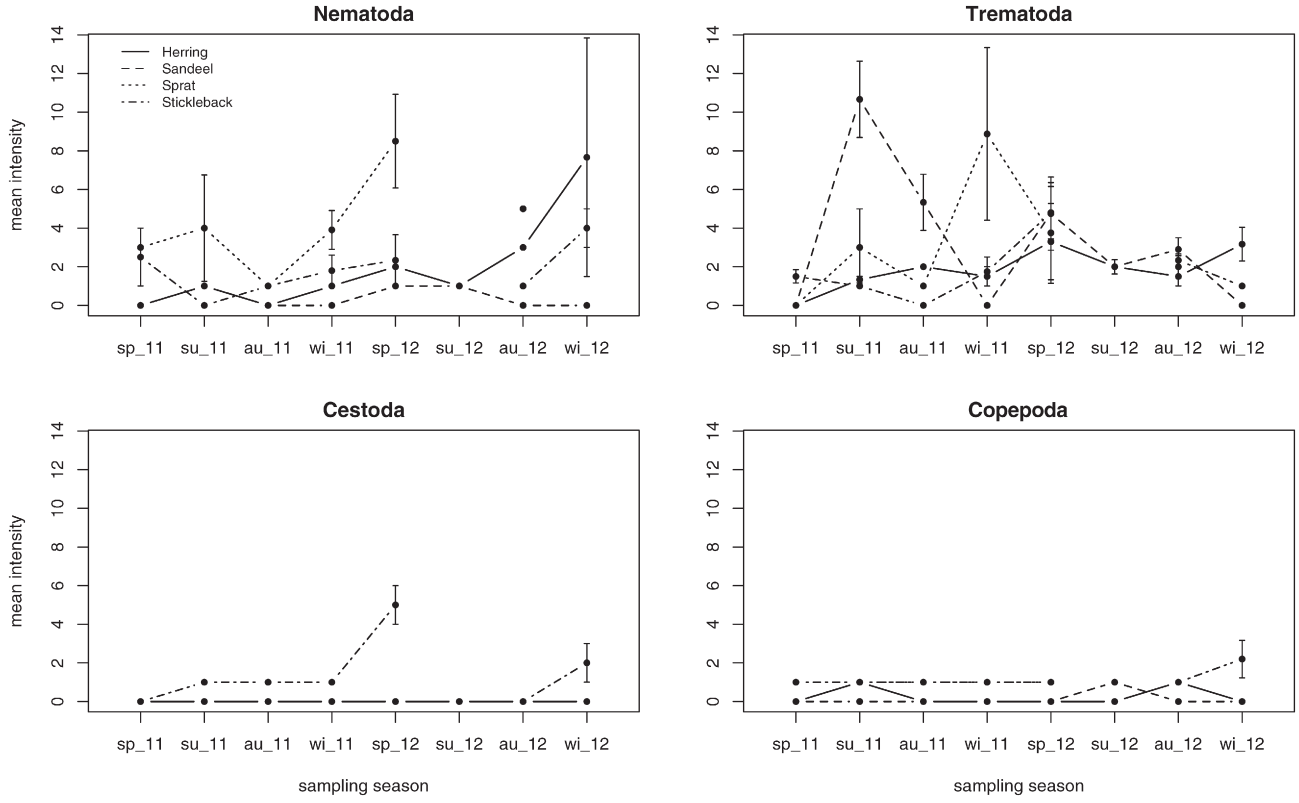

Fig. 3. Mean intensity of macroparasite infections. Phyla were separated by fish species and sampling period. Gaps represent sampling events with missing fish species.

Aliivibrio, one species each to *Listonella*, *Enterovibrio* and *Photobacterium*. Due to the low phylogenetic resolution of the 16S rRNA gene marker it was not possible to identify closely related species within the *Splendidus* clade. Accordingly, most strains were assigned to the *Splendidus* clade (44.6%), followed by *A. logei* (13.6%) and *V. tapetis* with 9.9%, which were found in all four or at least three of the investigated fish species. The Vibrionaceae diversity differed significantly between fish species (Table 6). The highest diversity was found in Atlantic herring, comprising all 13 species and showing a diversity index (Hs) of 1.54, whereas the lowest diversity could be found in lesser sand eel with only 5 species and an Hs value of 1.23.

In addition to differences between host species, we also found seasonal variation in the Vibrionaceae communities with the highest diversity in summer (Hs = 1.53), followed by autumn (Hs = 1.49), spring (Hs = 1.38) and winter (Hs = 1.16). Some Vibrionaceae species (*V. breoganii*, *V. inusitatus* and *V. penaeicida*) were exclusively detected in summer. Furthermore, the Vibrionaceae communities differed significantly between sampling years (Table 6).

Table 5
Summary of seasonal and annual effects on mean macroparasite intensity. Generalized linear models containing mean intensity of macroparasite taxa as dependent variables and fish species, year, season, fish length and fish weight as independent predictors. Significant effects are shown in bold. Family = negative binomial.

Response	Nematoda intensity					Trematoda intensity				
	Df	Dev	ResDf	ResDev	P(>Chi)	Df	Dev	ResDf	ResDev	P(>Chi)
Null			307	278.8				307	424.8	
Fish species (F)	3	78.5	304	200.3	<**0.001**	3	48.2	304	376.6	<**0.001**
Year (Y)	1	1.9	303	190.9	0.168	1	7.2	303	369.4	**0.007**
Season (S)	3	33.0	300	157.8	<**0.001**	3	37.2	300	332.3	<**0.001**
Fish length	1	0.7	299	157.1	0.400	1	0.03	299	332.2	0.857
Fish weight	1	0.6	298	156.5	0.450	1	2.6	298	329.6	0.107
F*Y	3	5.4	295	151.1	0.142	3	23.6	295	306.0	<**0.001**
F*S	9	17.9	286	133.2	**0.037**	9	52.9	286	253.1	<**0.001**
Response	Cestoda intensity					Copepoda intensity				
	Df	Dev	ResDf	ResDev	P(>Chi)	Df	Dev	ResDf	ResDev	P(>Chi)
Null			307	63.3				307	200.9	
Fish species (F)	3	33.2	304	30.1	**0.002**	3	43.9	304	157.0	<**0.001**
Year (Y)	1	3.5	303	26.6	0.061	1	1.6	303	155.4	0.204
Season (S)	3	1.9	300	24.7	0.583	3	5.7	300	149.7	0.126
Fish length	1	0.05	299	24.6	0.831	1	1.0	299	148.7	0.307
Fish weight	1	0	298	24.6	0.987	1	0.3	298	148.3	0.558
F*Y	3	0	295	24.6	1.000	3	11.6	295	136.7	**0.009**
F*S	9	0	286	24.6	1.000	9	17.9	286	118.8	**0.036**

Please cite this article as: Schade, F.M., et al., Seasonal variation in parasite infection patterns of marine fish species from the Northern Wadden Sea in relation to interannual t..., J. Sea Res. (2015), http://dx.doi.org/10.1016/j.seares.2015.09.002

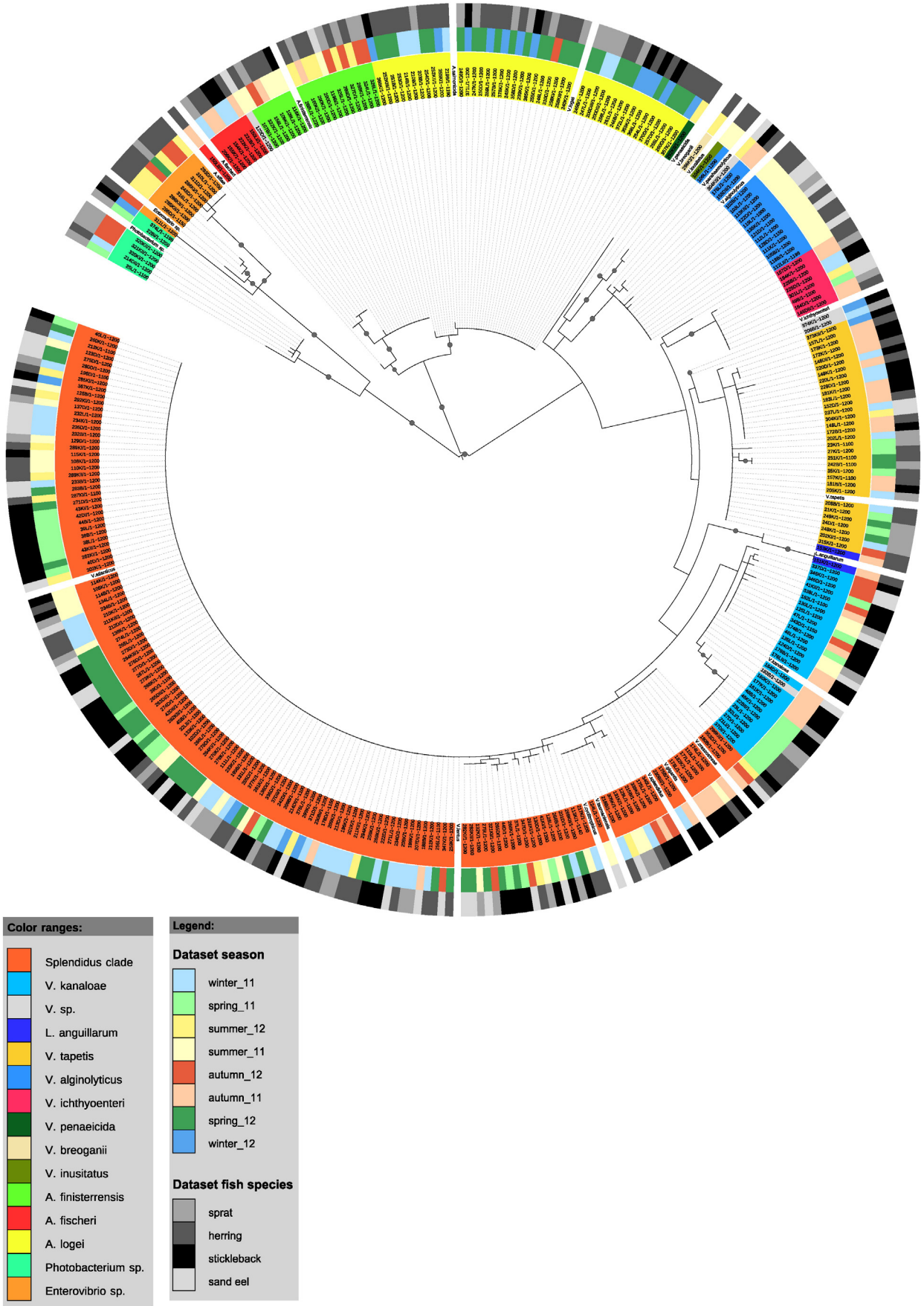

Fig. 4. Phylogenetic maximum likelihood tree based on distance matrices (GTR + G + I) using partial 16S rRNA sequences (n = 323) of Vibrionaceae isolates from four marine fish species. Inner colour circle represents species/group affiliation of different Vibrionaceae strains, whereas middle circle and outer circle show different sampling periods and different fish species. White slots represent reference strains accessed from NCBI GenBank. Gray dots at the nodes display bootstrap values of >80%.

Please cite this article as: Schade, F.M., et al., Seasonal variation in parasite infection patterns of marine fish species from the Northern Wadden Sea in relation to interannual t..., J. Sea Res. (2015), http://dx.doi.org/10.1016/j.seares.2015.09.002

Table 6
Summary of seasonal and annual effects on the variance of Vibrionaceae communities. Permutational multivariate analysis of variances of distance matrices for Vibrionaceae (unifrac distance), including independent predictors fish species, sampling year and season. Significant effects are shown in bold.

Response	Variance of Vibrionaceae communities					
	Df	sumSq	meanSq	F	R2	Pr(>F)
Fish species (F)	3	0.007	0.002	4.39	0.28	**0.011**
Year (Y)	1	0.003	0.003	5.24	0.11	**0.009**
Season (S)	3	0.002	0.001	1.28	0.08	0.293
F*Y	3	0.003	0.001	2.33	0.15	0.057
F*S	9	0.005	0.001	1.14	0.22	0.382
Residuals	8	0.004	0.000		0.17	
Total	27	0.024			1.00	

Table 7
Summary of relative temperature effects on relative diversity. Generalized linear models containing relative diversity as dependent variable and relative temperature, fish species and parasite group (macroparasite and Vibrionaceae pooled) as independent predictors. Significant effects are shown in bold. Family = Gaussian.

Response	Relative diversity				
	Df	Dev	ResDf	ResDev	P(>Chi)
Null			31	12.26	
Relative temperature (T)	1	3.52	30	8.74	<**0.001**
Fish species (F)	3	2.32	27	6.42	**0.003**
Parasite group (P)	1	0.30	26	6.12	0.175
T*F	3	1.07	23	5.05	0.087
T*P	1	1.47	22	3.59	**0.003**

3.3. Comparison of communities

In our two-year comparison we observed year-wise differences of water temperature and species diversity with significantly increased diversity differentials of infectious species at higher temperature differentials (Fig. 5, Table 7). Furthermore, the diversity differential differed significantly between fish species (Table 7). In conclusion, the highest temperature and diversity difference could be seen in spring with an increased water temperature of 6.5 °C corresponding to an increased macroparasite diversity of 1.29 in Atlantic herring in spring 2012 (Fig. 5). By integrating over summed temperatures preceding the sampling dates we observed similar correlation coefficients (range: 0.403–0.487) as for the analysis only taking temperature at the day of sampling ($r^2 = 0.452$), indicating that the observed patterns hold when longer time periods were considered.

Next to increased diversity differentials we found increased prevalence differentials of some parasite groups at higher temperature differentials (Fig. 6, Table 8). The relative prevalence of nematodes ($\chi^2_1 = 460.2$, $P = 0.005$) and trematodes ($\chi^2_1 = 10052.0$, $P = 0.026$) followed the temperature differential between the years with highest prevalence differences in spring (Fig. 6).

Even if we could not find overall relative temperature effects on parasite intensity differentials (Table 8), post-hoc tests detected also increased relative intensities of trematodes ($\chi^2_1 = 21.3$, $P = 0.039$) at relative higher temperatures (Fig. 7).

4. Discussion

In this study we detected interannual variation in micro- and macroparasite communities that were correlating to short periods of increased water temperatures between years (Fig. 1). Next to increased diversities of infectious species, we also observed a forward shift and extension in peak prevalence and mean intensity of macroparasites (trematodes) following warmer spring seasons. For nematodes, highest prevalence differences could be also found in spring, whereas other parasite groups showed no such correlation between temperatures and infection parameters.

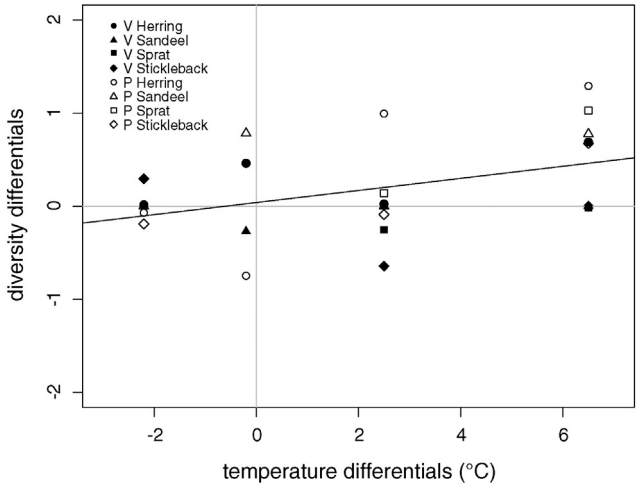

Fig. 5. Diversity and temperature differentials of Vibrionaceae and macroparasites separated by fish species. V = Vibrionaceae, P = macroparasites.

Our results as well as previous studies (Groenewold et al., 1996; Zander, 2005) show that parasitism is common in the analyzed four small fish species of the Wadden Sea. In total, we detected 16 different macroparasite species from six different phyla. The highest diversity of macroparasites could be found in the three-spined stickleback, which stems from a mixture of marine and anadromous populations. Owing to its greater mobility in fresh- and seawater habitats, the parasite richness and the infection risk seems to be higher for anadromous species than for pure marine fish species.

The diversity of the parasite communities from Atlantic herring, European sprat and lesser sand eel ranged from five to seven different parasite species and showed diversity indices in the range from 1.46 to 1.67, which is considerably higher than previously reported diversities from the German Wadden Sea of 0.95 for European sprat and 0.20 for lesser sand eel (Groenewold et al., 1996). In the past, many parasitological studies were exclusively based on morphological identification, but particularly nematodes or larval stages of parasites are difficult to identify at the species-level (Bhadury and Austen, 2010; Locke et al., 2010). Molecular techniques, such as DNA barcoding (Hebert et al., 2003a; Hebert et al., 2003b), can be powerful tools to distinguish between morphologically similar parasites at any stage in their live cycle (Moszczynska et al., 2009). Next to potential underestimation of diversity in early parasitological studies, higher diversities may be attributable to changed environmental conditions in the Wadden Sea, such as increased air and water temperatures or invasive species co-introducing new parasites and diseases to local populations (Reise and van Beusekom, 2008).

We revealed significant correlations between temperature and trematode as well as nematode infection parameters. Trematodes showed increased prevalences and mean intensities in spring and summer, coinciding with previous results (Mouritsen, 2002; Poulin, 2006). More interestingly, we detected increased prevalences in spring and autumn 2012 as well as increased mean intensities in spring 2012 in comparison with sampling in 2011. With a temperature increase of 6.5 °C in spring and 2.5 °C in autumn, both seasons were much warmer in 2012 (Fig. 1). Particularly, warmer spring seasons may not only increase infectivity of trematodes, but also shift and extend the length of transmission periods (Sankurathri and Holmes, 1976; Thieltges and Rick, 2006). On the other hand nematodes showed significantly decreased infection parameters in warmer seasons (summer, autumn), reflecting different phenologies in comparison to trematodes. Depending on their thermal tolerance some life cycles stages might be more susceptible to temperature changes than others (Harley et al., 2006; Lafferty, 2009; Karvonen et al., 2010), indicating that effects of changing

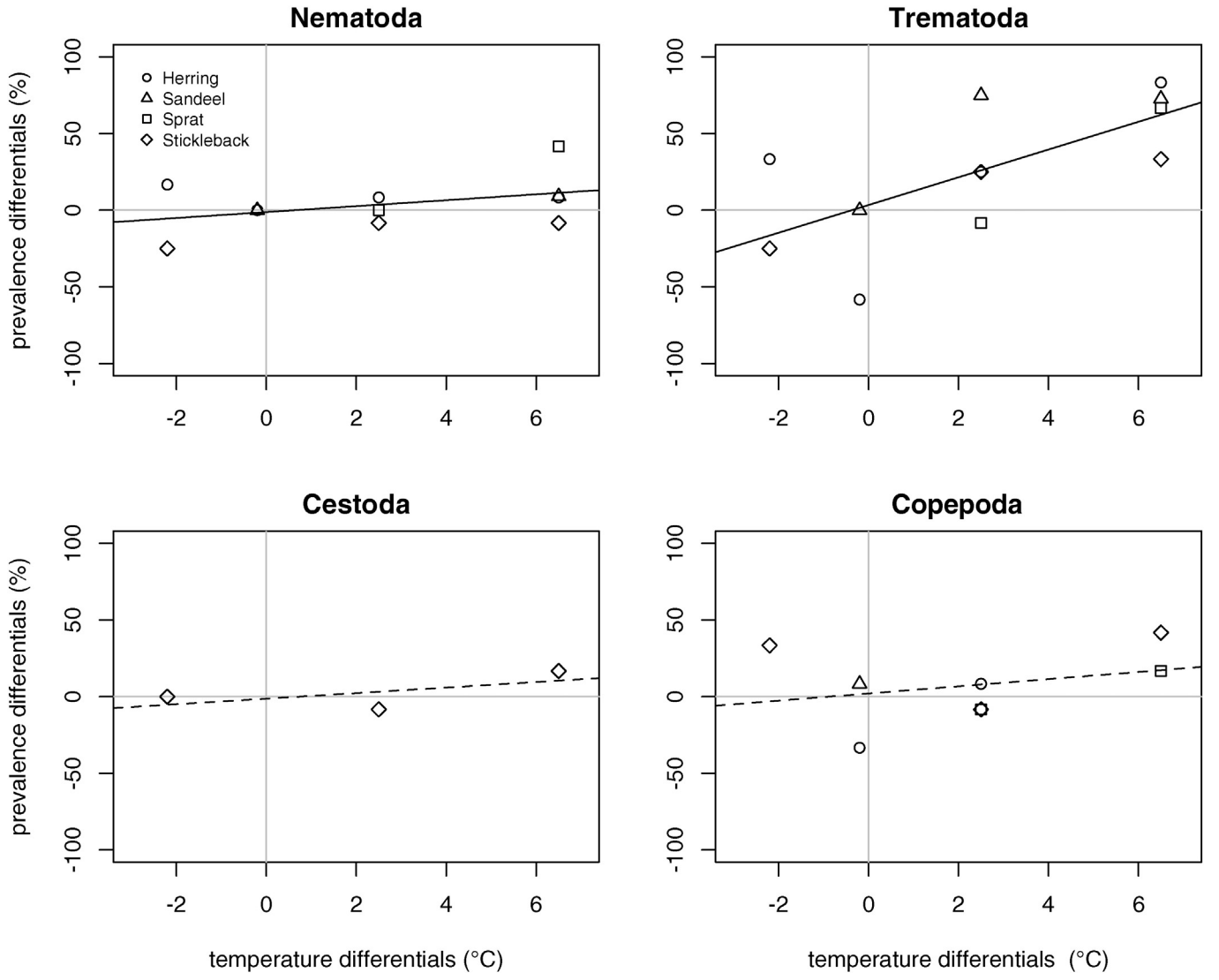

Fig. 6. Prevalence and temperature differentials of macroparasites separated by fish species and parasite groups. Solid lines represent significant relative temperature effect on relative parasite prevalence, whereas dashed lines represent non-significant relative temperature effect on relative parasite prevalence. Statistical significance level at $P = 0.05$.

temperatures have to be investigated in a group or even in a species specific fashion.

Additionally, we found a significant interaction of lower fish weight and increased parasite prevalence. Comparatively lighter fish were significantly more often infected with nematodes, reflecting previous results of a common carp fingerling (Boerlage et al., 2012). This pattern might suggest that either the infection itself caused the weight loss by depleting energy resources of the host or smaller hosts were already immune-compromised leading to higher infections intensities.

Bacterial studies in the Wadden Sea have been mainly concentrated on oysters and mussels (Lhafi and Kuhne, 2007; Schets et al., 2010; Wendling et al., 2014) and to our knowledge only one study has yet been conducted on the bacterial symbionts of flatfish including potential microparasites (Wegner et al., 2012). In our study, the Vibrionaceae communities of small-sized fish species were comparatively diverse with 20 different OTUs. The highest diversity of Vibrionaceae was found in Atlantic herring, suggesting that filter feeding is an effective way to accumulate Vibrionaceae strains from the water column in the

Table 8
Summary of relative temperature effects on relative infection parameters. Generalized linear models containing relative prevalence and intensity as dependent variables and relative temperature, fish species and parasite groups (Nematoda, Trematoda, Cestoda, and Copepoda were pooled) as independent predictors. Significant effects are shown in bold. Family = Gaussian.

Response	Relative prevalence					Relative intensity				
	Df	Dev	ResDf	ResDev	P(>Chi)	Df	Dev	ResDf	ResDev	P(>Chi)
Null			33	32,249				33	259.8	
Relative temperature (T)	1	7273	32	24,975	**0.002**	1	10.0	32	249.8	0.204
Fish species (F)	3	1870	29	23,105	0.474	3	33.4	29	216.4	0.144
Parasite group (P)	3	2936	26	20,168	0.268	3	32.6	26	184.8	0.164
T*F	3	1804	23	18,364	0.490	3	32.4	23	152.5	0.155
T*P	3	3453	20	14,910	0.201	3	29.0	20	123.5	0.195

Please cite this article as: Schade, F.M., et al., Seasonal variation in parasite infection patterns of marine fish species from the Northern Wadden Sea in relation to interannual t..., J. Sea Res. (2015), http://dx.doi.org/10.1016/j.seares.2015.09.002

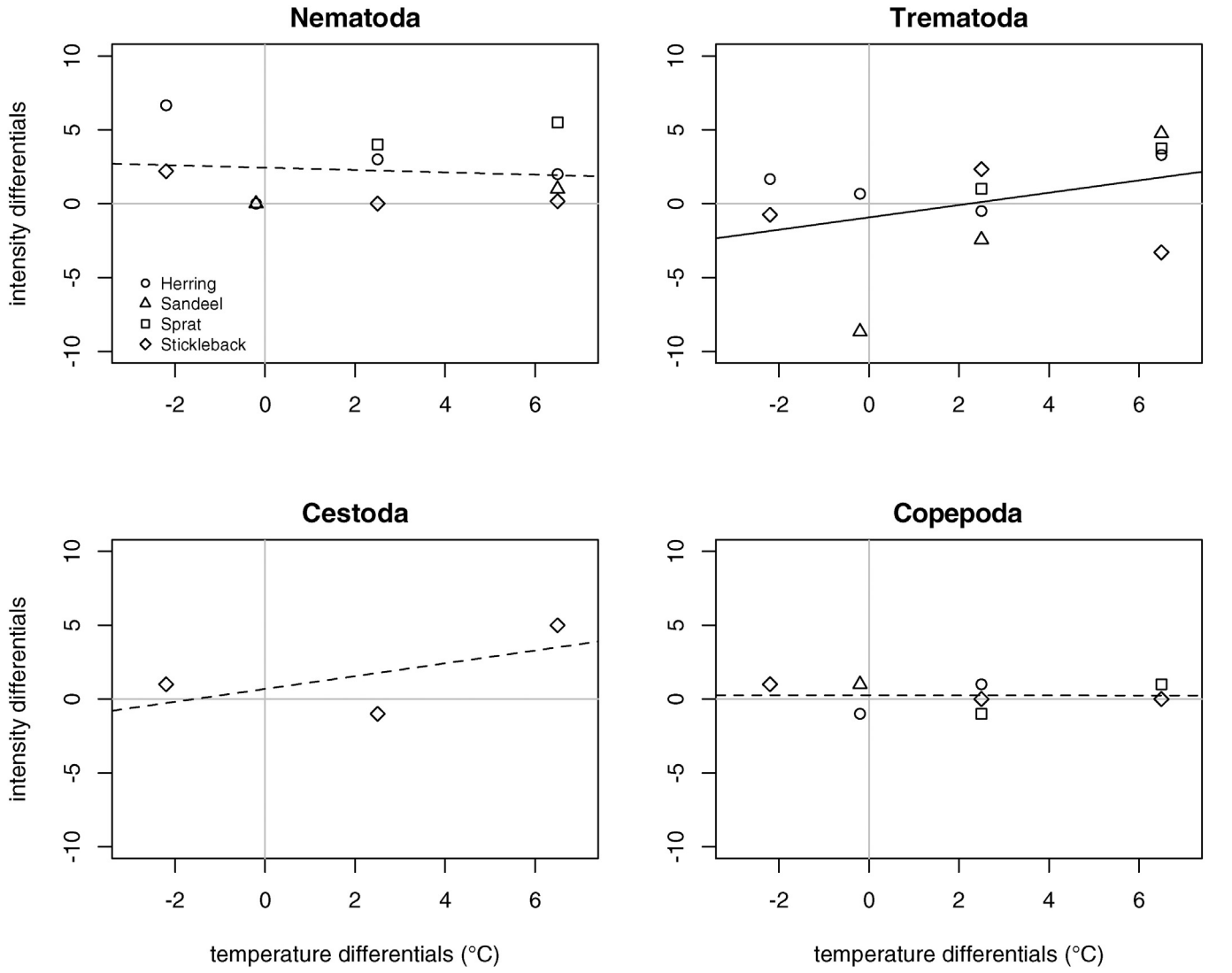

Fig. 7. Intensity and temperature differentials of macroparasites separated by fish species and parasite groups. Solid lines represent significant relative temperature effect on relative parasite intensity, whereas dashed lines represent non-significant relative temperature effect on relative parasite intensity. Statistical significance level at $P = 0.05$.

body. In total, the Vibrionaceae communities were mostly dominated by species from the *Splendidus* clade. Particularly some strains of *Vibrio splendidus* are known to cause vibriosis in fish species, resulting in acute mortalities, particularly in summer months (Diggles et al., 2000; Jensen et al., 2003). The occurrence of potentially pathogenic strains from the family Vibrionaceae is highly correlated with temperature, leading to increased virulence and the occurrence of emerging agents in warmer waters (Thompson et al., 2004; Vezzulli et al., 2010). Beside *Vibrio splendidus*, we detected other strains of potential pathogens of fish (*Listonella anguillarum*), crustaceans (*V. penaeicida*) and bivalves (*Vibrio tapetis*), which were associated with mass mortality events in some cases (Allam et al., 2002; Aguirre-Guzman et al., 2003; Planas et al., 2005; Pernet et al., 2012). Optimal growth temperature is not known for most of the bacterial species here, but vibriosis in fish tends to occur at water temperatures above 15 °C (Frans et al., 2011).

The relative diversities of both macroparasites and Vibrionaceae correlated to the relative temperature differential between the two years, showing highest diversity differentials at larger temperature differences also when temperature was integrated over longer time periods. Along with the shifts in phenology of parasite life cycles and disease occurrence, we suggest that temperature may represent an important driver of parasite population dynamics, although they are buffered from direct environment by the host physiology (Paperna and Overstreet, 1981). And even if only a subset of parasites as well as opportunistic pathogens react to small changes in seasonal temperatures, shifts in life cycles and infection parameters can potentially have severe consequences for sensitive ecosystems.

We are aware that we based our results on the direct comparison between two years only, and that many other factors could have varied between years that could also contribute to the observed results. One such potential confounding factor in our data are the different sampling times between years giving parasites more time to accumulate in 2012 due to later sampling. While integration over summed temperatures preceding sampling dates gave qualitatively similar results, we cannot completely disregard the effect of time here. However, temporal differences between sampling time points will mainly affect long-lived macroparasites that continuously infect hosts, while bacterial populations react much faster to environmental conditions. Furthermore, if only time has an effect on macroparasite accumulation we should have observed increased infection rates for all macroparasite species and not mainly for those that have been shown to adjust their life cycle to temperature (e.g. trematodes, see Sankurathri and Holmes, 1976; Mouritsen, 2002; Thieltges and Rick, 2006). As consequence, we have to be cautious in deriving generalities from these data. However, we think that the potential involvement of small and short temperature changes in shifting phenologies of host–parasite interactions deserve

more attention in the near future. In addition, comprehensive long-term studies should be designed and conducted to identify other potential drivers and the timing of parasite infection patterns in coastal ecosystems.

5. Conclusion

The results of this study imply that parasitism is a common phenomenon in three-spined stickleback, Atlantic herring, European sprat and lesser sand eel of the Northern Wadden Sea and that water temperature might potentially influence the infection parameters in parasite communities of small fish. Next to increased diversities of infectious species due to short periods of elevated water temperatures, we found that warmer spring temperatures might have resulted in a forward shift and extension in peak prevalence and mean intensity of trematodes. These results may indicate that even small changes in seasonal temperatures might affect the phenology of parasites as well as opportunistic pathogens, potentially leading to far reaching consequences that need to further investigations, especially in sensitive ecosystems like the Wadden Sea.

Acknowledgements

We would like to thank the crew from the RV "MYA" for help with fishing in the Sylt-Rømø-Bight and Harry Palm for assisting in morphological identification of macroparasites. The study was funded by DFG Emmy Noether Programme grant WE 4614/1-1.

Appendix A

Accession numbers of 16s rRNA reference strains from NCBI Genbank that were used for phylogeny.

Reference strain	Accession number
Aliivibrio finisterrensis	EU541616.1
Aliivibrio fischeri	NR_074990.1
Aliivibrio salmonicida	NR_074847.1
Aliivibrio sifiae	AB464965.1
Enterovibrio sp.	HM566000.1
Listonella anguillarum	KC210821.1
Photobacterium	AB681357.1
Vibrio alginolyticus	KF155242.1
Vibrio atlanticus	FN582272.1
Vibrio breoganii	EU931112.1
Vibrio crassostreae	KF193921.1
Vibrio cyclitrophicus	KF488567.1
Vibrio gigantis	KF155253.1
Vibrio ichthyoenteri	NR_042122.1
Vibrio inusitatus	DQ922921.1
Vibrio kanaloae	KC812974.1
Vibrio lentus	HE584786.1
Vibrio logei	AY292932.1
Vibrio parahaemolyticus	KC476545.1
Vibrio penaeicida	JF836189.1
Vibrio splendidus	KF009770.1
Vibrio tapetis	HE795157.1
Vibrio tasmaniensis	HE584774.1

References

Aguirre-Guzman, G., Labreuche, Y., Ansquer, D., Espiau, B., Levy, P., Ascencio, F., Saulnier, D., 2003. Proteinaceous exotoxins of shrimp-pathogenic isolates of *Vibrio penaeicida* and *Vibrio nigripulchritudo*. Cienc. Mar 29 (1), 77–88.

Allam, B., Paillard, C., Ford, S.E., 2002. Pathogenicity of *Vibrio tapetis*, the etiological agent of brown ring disease in clams. Dis. Aquat. Organ. 48 (3), 221–231. http://dx.doi.org/10.3354/dao048221.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.

Austin, B., Austin, D.A., 2007. Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish. Fourth edition. Springer, Heidelberg, Germany.

Baker-Austin, C., Trinanes, J.A., Taylor, N.G.H., Hartnell, R., Siitonen, A., Martinez-Urtaza, J., 2013. Emerging Vibrio risk at high latitudes in response to ocean warming. Nat. Clim. Change 3 (1), 73–77. http://dx.doi.org/10.1038/nclimate1628.

Bhadury, P., Austen, M.C., 2010. Barcoding marine nematodes: an improved set of nematode 18S rRNA primers to overcome eukaryotic co-interference. Hydrobiologia 641 (1), 245–251. http://dx.doi.org/10.1007/s10750-009-0088-z.

Boerlage, A.S., Graat, E.A.M., Verreth, J.A., de Jong, M.C.M., 2012. Higher attack rate of fish-borne trematodes (Heterophyidae) in common carp fingerlings (*Cyprinus carpio*) at lower fish weight. Parasitol. Res. 111 (2), 875–879. http://dx.doi.org/10.1007/s00436-012-2913-y.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al revisited. J. Parasitol. 83 (4), 575–583. http://dx.doi.org/10.2307/3284227 Ecological Studies 206. Springer, Heidelberg, Germany, pp. 295–307.

De Ley, P., De Ley, I.T., Morris, K., Abebe, E., Mundo-Ocampo, M., Yoder, M., Heras, J., Waumann, D., Rocha-Olivares, A., Burr, A.H.J., Baldwin, J.G., Thomas, W.K., 2005. An integrated approach to fast and informative morphological vouchering of nematodes for applications in molecular barcoding. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 360 (1462), 1945–-1958. http://dx.doi.org/10.1098/rstb.2005.1726.

Diggles, B.K., Carson, J., Hine, P.M., Hickman, R.W., Tait, M.J., 2000. Vibrio species associated with mortalities in hatchery-reared turbot (*Colistium nudipinnis*) and brill (*C. guntheri*) in New Zealand. Aquaculture 183 (1-2), 1–12. http://dx.doi.org/10.1016/s0044-8486(99)00280-x.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucl. Acids Res. 32 (5), 1792–1797. http://dx.doi.org/10.1093/nar/gkh340.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39 (4), 783–791. http://dx.doi.org/10.2307/2408678.

Frans, I., Michiels, C.W., Bossier, P., Willems, K.A., Lievens, B., Rediers, H., 2011. *Vibrio anguillarum* as a fish pathogen: virulence factors, diagnosis and prevention. J. Fish. Dis. 34 (9), 634–661. http://dx.doi.org/10.1111/j.1365-2761.2011.01279.x.

Groenewold, S., Berghahn, R., Zander, C.D., 1996. Parasite communities of four fish species in the Wadden Sea and the role of fish discarded by the shrimp fisheries in parasite transmission. Helgoland Mar. Res. 50 (1), 69–85. http://dx.doi.org/10.1007/bf02367137.

Harley, C.D.G., Hughes, A.R., Hultgren, K.M., Miner, B.G., Sorte, C.J.B., Thornber, C.S., Rodriguez, L.F., Tomanek, L., Williams, S.L., 2006. The impacts of climate change in coastal marine systems. Ecol. Lett. 9 (2), 228–241. http://dx.doi.org/10.1111/j.1461-0248.2005.00871.x.

Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S., Samuel, M.D., 2002. Ecology—climate warming and disease risks for terrestrial and marine biota. Science 296 (5576), 2158–2162. http://dx.doi.org/10.1126/science.1063699.

Hebert, P.D.N., Cywinska, A., Ball, S.L., deWaard, J.R., 2003a. Biological identifications through DNA barcodes. Proc. R. Soc. Lond. B 270, 313–321. http://dx.doi.org/10.1098/rspb.2002.2218.

Hebert, P.D.N., Ratnasingham, S., deWaard, J.R., 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc. R. Soc. Lond. B 270, 96–99. http://dx.doi.org/10.1098/rsbl.2003.0025.

Hilger, I., Ullrich, S., Anders, K., 1991. A new ulcerative flexibacteriosis-like disease (yellow pest) affecting young Atlantic cod Gadus-Morhua from the German Wadden Sea. Dis. Aquat. Organ. 11 (1), 19–29. http://dx.doi.org/10.3354/dao011019.

Holmes, J.C., Price, P.W., 1986. Communities of parasites. In: Anderson, D.J., Kikkawa, J. (Eds.), Community Ecology: Pattern and Process. Blackwell Scientific, Oxford, pp. 187–213.

Jensen, S., Samuelsen, O.B., Andersen, K., Torkildsen, L., Lambert, C., Choquet, G., Paillard, C., Bergh, O., 2003. Characterization of strains of Vibrio splendidus and V-tapetis isolated from corkwing wrasse Symphodus melops suffering vibriosis. Dis. Aquat. Organ. 53 (1), 25–31. http://dx.doi.org/10.3354/dao053025.

Karvonen, A., Rintamaki, P., Jokela, J., Valtonen, E.T., 2010. Increasing water temperature and disease risks in aquatic systems: climate change increases the risk of some, but not all, diseases. Int. J. Parasitol. 40 (13), 1483–1488. http://dx.doi.org/10.1016/j.ijpara.2010.04.015.

Kühl, H., Kuipers, B.R., 1983. Qualitative food relationships of Wadden Sea fishes. In: Wolff, W.J. (Ed.)Ecology of the Waddensea II. Balkema, Rotterdam, pp. 112–123.

Lafferty, K.D., 1997. Environmental parasitology: what can parasites tell us about human impacts on the environment? Parasitol. Today 13 (7), 251–255. http://dx.doi.org/10.1016/s0169-4758(97)01072-7.

Lafferty, K.D., 2009. The ecology of climate change and infectious diseases. Ecology 90 (4), 888–900. http://dx.doi.org/10.1890/08-0079.1.

Lafferty, K.D., Porter, J.W., Ford, S.E., 2004. Are diseases increasing in the ocean? Annu. Rev. Ecol. Evol. Syst. 35, 31–54. http://dx.doi.org/10.1146/annurev.ecolsys.35.021103.105704.

Lane, D.J., 1991. 16S/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M. (Eds.), Nucleic acid techniques in bacterial systematics. Wiley & Sons, Chichester, United Kingdom, pp. 115–175.

Larsson, A., 2014. AliView: a fast and lightweight alignment viewer and editor for large data sets. Bioinformatics 30 (22), 3276–-3278. http://dx.doi.org/10.1093/bioinformatics/btu531.

Lauckner, G., 1971. Zur Trematodenfauna der Herzmuscheln Cardium edule und Cardium lamarcki. Helgoland. Wiss. Meer. 22, 377–400.

Letunic, I., Bork, P., 2011. Interactive Tree Of Life v2: online annotation and display of phylogenetic trees made easy. Nucl. Acids Res. 39, 475–478. http://dx.doi.org/10.1093/nar/gkr201.

Lhafi, S.K., Kuhne, M., 2007. Occurrence of Vibrio spp. in blue mussels (*Mytilus edulis*) from the German Wadden Sea. Int. J. Food Microbiol 116 (2), 297–300. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.01.007.

Please cite this article as: Schade, F.M., et al., Seasonal variation in parasite infection patterns of marine fish species from the Northern Wadden Sea in relation to interannual t..., J. Sea Res. (2015), http://dx.doi.org/10.1016/j.seares.2015.09.002

Locke, S.A., McLaughlin, J.D., Dayanandan, S., Marcogliese, D.J., 2010. Diversity and specificity in *Diplostomum* spp. metacercariae in freshwater fishes revealed by cytochrome c oxidase I and internal transcribed spacer sequences. Int. J. Parasitol. 40 (3), 333–343. http://dx.doi.org/10.1016/j.ijpara.2009.08.012.

Lozupone, C., Knight, R., 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. 71 (12), 8228–8235. http://dx.doi.org/10.1128/aem.71.12.8228-8235.2005.

Miller, R.G., 1981. Simultaneous Statistical Inference. Second Edition. Springer, New York.

Moller, H., Anders, K., 1992. Epidemiology of fish diseases in the Wadden Sea. Ices J. Mar. Sci. 49 (2), 199–208. http://dx.doi.org/10.1093/icesjms/49.2.199.

Moszczynska, A., Locke, S.A., McLaughlin, J.D., Marcogliese, D.J., Crease, T.J., 2009. Development of primers for the mitochondrial cytochrome c oxidase I gene in digenetic trematodes (Platyhelminthes) illustrates the challenge of barcoding parasitic helminths. Mol. Ecol. Resour. 9, 75–82. http://dx.doi.org/10.1111/j.1755-0998.2009.02634.x.

Mouritsen, K.N., 2002. The *Hydrobia ulvae–Maritrema subdolum* association: influence of temperature, salinity, light, water-pressure and secondary host exudates on cercarial emergence and longevity. J. Helminthol. 76 (4), 341–347. http://dx.doi.org/10.1079/joh2002136.

Mouritsen, K.N., Poulin, R., 2002. Parasitism, community structure and biodiversity in intertidal ecosystems. Parasitology 124, S101–S117. http://dx.doi.org/10.1017/s0031182002001476.

Murray, V., Ebi, K.L., 2012. IPCC special report on managing the risks of extreme events and disasters to advance climate change adaptation (SREX). J. Epidemiol. Community Health 66 (9), 759–760. http://dx.doi.org/10.1136/jech-2012-201045.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. Vegan: Community Ecology Package. R package version 2.0-9. http://CRANR-projectorg/package=vegan.

Paperna, I., Overstreet, R.M., 1981. Parasites and diseases of mullets (Mugilidae). In: Oren, O.H. (Ed.), Aquaculture of Grey Mullets. Cambridge University Press, Cambridge, pp. 411–493.

Paull, S.H., LaFonte, B.E., Johnson, P.T.J., 2012. Temperature driven shifts in a host–parasite interaction drive nonlinear changes in diseases risk. Glob. Change Biol. 18 (12), 3558–3567. http://dx.doi.org/10.1111/gcb.12018.

Pernet, F., Barret, J., Le Gall, P., Corporeau, C., Degremont, L., Lagarde, F., Pepin, J.F., Keck, N., 2012. Mass mortalities of Pacific oysters *Crassostrea gigas* reflect infectious diseases and vary with farming practices in the Mediterranean thau lagoon, France. Aquacult. Environ. Interact. 2 (3), 215–237. http://dx.doi.org/10.3354/aei00041.

Planas, M., Perez-Lorenzo, M., Vazquez, J.A., Pintado, J., 2005. A model for experimental infections with Vibrio (Listonella) anguillarum in first feeding turbot(*Scophthalmus maximus* L.) larvae under hatchery conditions. Aquaculture 250 (1–2), 232–243. http://dx.doi.org/10.1016/j.aquaculture.2005.04.050.

Posada, D., 2008. JModelTest:phylogentic model averaging. Mol. Biol. Evol. 25 (7), 1253–1256. http://dx.doi.org/10.1093/molbev/msn083.

Poulin, R., 2006. Global warming and temperature-mediated increases in cercarial emergence in trematode parasites. Parasitology 132, 143–151. http://dx.doi.org/10.1017/s0031182005008693.

Pounds, J.A., Bustamante, M.R., Coloma, L.A., Consuegra, J.A., Fogden, M.P.L., Foster, P.N., La Marca, E., Masters, K.L., Merino-Viteri, A., Puschendorf, R., Ron, S.R., Sanchez-Azofeifa, G.A., Still, C.J., Young, B.E., 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature 439 (7073), 161–167. http://dx.doi.org/10.1038/nature04246.

R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (http://www.R-project.org/).

Reise, K., van Beusekom, J.E.E., 2008. Interactive effects of global and regional change on a coastal ecosystem. Helgol. Mar. Res. 62 (1), 85–91. http://dx.doi.org/10.1007/s10152-007-0102-7.

Rodriguez, F., Oliver, J.L., Marin, A., Medina, J.R., 1990. The general stochastic-model of nucleotide substitution. J. Theor. Biol. 142, 485–501.

Sankurathri, C.S., Holmes, J.C., 1976. Effects of thermal effluents on parasites and commensals of physa-gyrina say (mollusca-gastropoda) and their interactions at the lake Wabamun, Alberta. Can. J. Zool. 54 (10), 1742–1753. http://dx.doi.org/10.1139/z76-202.

Schär, C., Vidale, P.L., Lüthi, D., Frei, C., Häberli, C., Liniger, M.A., Appenzeller, C., 2004. The role of increasing temperature variability in European summer heatwaves. Nature 427 (6972), 332–336.

Schets, F.M., van den Berg, H., Rutjes, S.A., Husman, A.M.D., 2010. Pathogenic Vibrio species in Dutch shellfish destined for direct human consumption. J. Food Prot. 73 (4), 734–738.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729. http://dx.doi.org/10.1093/molbev/mst197.

Thieltges, D.W., 2006. Effect of infection by the metacercarial trematode *Renicola roscovita* on growth in intertidal blue mussel *Mytilus edulis*. Mar. Ecol. Prog. Ser. 319, 129–134. http://dx.doi.org/10.3354/meps319129.

Thieltges, D.W., Rick, J., 2006. Effect of temperature on emergence, survival and infectivity of cercariae of the marine trematode *Renicola roscovita* (Digenea : Renicolidae). Dis. Aquat. Org. 73 (1), 63–68.

Thieltges, D.W., Engelsma, M.Y., Wendling, C.C., Wegner, K.M., 2013. Parasites in the Wadden Sea food web. J. Sea Res. 82, 122–133. http://dx.doi.org/10.1016/j.seares.2012.06.002.

Thompson, J.R., Randa, M.A., Marcelino, L.A., Tomita-Mitchell, A., Lim, E., Polz, M.F., 2004. Diversity and dynamics of a north Atlantic coastal Vibrio community. Appl. Environ. Microbiol. 70 (7), 4103–4110. http://dx.doi.org/10.1128/aem.70.7.4103-4110.2004.

Vanbeek, F.A., Rijnsdorp, A.D., Declerck, R., 1989. Monitoring juvenile stocks of flatfish in the Wadden Sea and the coastal areas of the southeastern North Sea. Helgolander Meeresun. 43 (3–4), 461–477.

Vezzulli, L., Previati, M., Pruzzo, C., Marchese, A., Bourne, D.G., Cerrano, C., VibrioSea, C., 2010. Vibrio infections triggering mass mortality events in a warming Mediterranean Sea. Environ. Microbiol. 12 (7), 2007–2019. http://dx.doi.org/10.1111/j.1462-2920.2010.02209.x.

Wegner, K.M., Shama, L.N.S., Kellnreitner, F., Pockberger, M., 2012. Diversity of immune genes and associated gill microbes of European plaice Pleuronectes platessa. Estuar. Coast. Shelf Sci. 108, 87–96. http://dx.doi.org/10.1016/j.ecss.2011.09.001.

Wendling, C.C., Batista, F.M., Wegner, K.M., 2014. Persistence, seasonal dynamics and pathogenic potential of *Vibrio* communities from Pacific oyster hemolymph. PLoS One 9 (4), e94256. http://dx.doi.org/10.1371/journal.pone.0094256.

Wollscheid, E., Wägele, H., 1999. Initial results on the molecular phylogeny of the Nudibranchia (Gastropoda, Opisthobranchia) based on 18S rDNA data. Mol. Phylogenet. Evol. 13 (2), 215–226.

Zander, C.D., 2005. Comparative studies on goby (teleostei) parasite communities from the North and Baltic Sea. Parasitol. Res. 96 (1), 62–68. http://dx.doi.org/10.1007/s00436-005-1327-5.

Please cite this article as: Schade, F.M., et al., Seasonal variation in parasite infection patterns of marine fish species from the Northern Wadden Sea in relation to interannual t..., J. Sea Res. (2015), http://dx.doi.org/10.1016/j.seares.2015.09.002