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ORIGINAL PAPER

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Seasonal dynamics of *Trichodina* spp. on whiting (*Merlangius merlangus*) in relation to organic pollution on the eastern Black Sea coast of Turkey

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Abstract It was determined that there is a relationship between prevalence and mean densities of *Trichodina* spp. on *Merlangius merlangus* and organic pollution, measured as levels of nitrite, nitrate and phosphate in the surrounding environment. Monthly, two left anterior gill arcs of 60 *M. merlangus* (unless otherwise stated) captured with hook and line were fixed in 10% formalin. The number of *Trichodina* spp. was determined by counting all of the cells with a grid slide. The two right anterior gill arcs were used to prepare dry smears to picture the morphology of the trichodinids and to determine species composition. High levels of prevalence and densities of the protozoan were observed during the late fall, winter and early spring months. Then, prevalence decreased to lower levels but never below 60%, an indication of the important role of *M. merlangus* for *Trichodina* spp. in the studied region. A multivariate analysis revealed that the magnitude of prevalence was related to the level of all three parameters: nitrite, nitrate and phosphate ($r^2=0.59$). However, a much stronger relationship between prevalence and nitrate, phosphate, oxygen and temperature ($r^2=0.89$) was detected. Consequently, the seasonal parasite prevalence and density were affected by organic pollution. The timing for a high prevalence and mean densities of this parasite also suggests that primary production may be responsible for the observed seasonal variation in prevalence and mean densities. By using the model describing the relationship between the water quality parameters and the prevalence data of *Trichodina* spp. recalculated values and observed field data corresponded closely.

Keywords Trichodina · Organic pollution · Indicator · *Merlangius merlangus* · Seasonality

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Introduction

Monitoring pollution in seawater is expensive, and provides little information about bio-availability and effects of pollution at the biological level when chemical and physiological changes or responses are not considered (McVicar 1997). A bio-transformation would be required to exert their toxic effects. Rather than monitoring certain parameters in water, e.g. heavy metals and petroleum, other indices such as metabolic, pathologic or parasitic indicators could be used as an useful, economical and reliable indicator to determine the effects of pollutants on the ecosystem (Marcogliese and Cone 1997; Broeg et al. 1999; Overstreet 1997), knowing the fact that parasite communities are affected directly by pollution or indirectly by the effects of pollution on their intermediate and definitive hosts (Möller 1987). Thus, investigation of key parasites on key hosts present in an ecosystem can provide invaluable information about the health of the surrounding environment in terms of biodiversity and the species habitat. Selection of key parasite species and key host species in an ecosystem are vital in this process (Overstreet 1997). Various criteria involved to select suitable host/parasite systems for biomonitoring have been reevaluated by Williams and MacKenzie (2002). A parasite selected should have the ability to respond environmental fluctuations rapidly, be available throughout the study period, and be identifiable easily (also see Palm 2004). Therefore, short living parasites with high reproduction rates (mostly ectoparasites) are necessary characteristics for a parasite species to be used as a pollution indicator.

There are many studies suggesting that *Trichodina* spp. could be an appropriate epibiont as a biological indicator of pollution. Voigt (1993) used mobiline peritrichous ciliates experimentally as biological indicators of water quality. Of 33 parasite species observed, *Trichodina* spp. reflected best the pollution gradient observed (Broeg et al. 1999). Palm and Dobberstein (1999), moreover, suggested the possibility to use prevalence and density data of *Trichodina* spp. as a biological indicator to compare polluted and unpolluted areas. They suggested the trichodinid prevalence and density being related with the bacterial biomass in the environment. Recent studies indicate that trichodinids can also function as an indicator not only in boreal but also in tropical waters (Slade 2001, additional data by second author). The selected fish species, on the other hand, being utilized as ''key species'', should be local and not a migratory species, and should interact with as many levels of the food chain in the surrounding environment as possible. Therefore, Palm and Dobberstein (1999) used a flatfish and the gadiform *Gadus morhua* L. as biological indicators in the Kiel Bay and Bight, Western Baltic Sea.

There is no study evaluating synergic effects of organic pollution on the level of prevalence and mean densities on any host. Seasonal effects of organic pollution (synergic or not) on the level of trichodinid variation on a given host would be an important information in differentiating the level coming from any pollutant and organic pollution. The purpose of this study was to determine the relationship between organic pollution, measured as the quantity of nitrate, nitrite and phosphate, and density of *Trichodina* spp. present on the gadiform whiting (*Merlangius merlangus* L.) from two stations, which presumably have different levels of organic pollution along the Black Sea coast of Turkey, where whiting is restricted to a very narrow foraging habitat on the steep continental shelf. Our hypothesis was that seasonal occurrence of trichodinids on whiting depends on fluctuations in the level of organic pollution. Since there is no report of any trichodinids from the area, this annual survey also serves as the first report of trichodinid epibionts of whiting living in the area.

Materials and methods

Merlangius merlangus selected as the key host species for the coastal ecosystem of the Eastern Black Sea area of Turkey, was sampled monthly from July 2003 to 2004 from two stations. The first station is in front of a river discharge (The Sogutlu River; 41°02′06″N and 39°35′45″E) and close to the city (Trabzon), and the other is away from any heavy anthropogenic effect (Akcaabat; 41°05′04″N and 39°05′04″E). Thirty fish (unless otherwise stated) from each station were collected with hook and line on the same day. A total of 716 fish, between 8.6 cm and 25.6 cm total length, was sampled and examined from both stations during the study period. Two left anterior gill arks of each fish, immediately after capture, were removed carefully and fixed in formalin containing 1 ppt oxytetracycline to prevent any bacterial activity. Caudal, dorsal and pectoral fins were also preserved. The other two arks from the left side were used for preparing dry smears. The gill arks were soaked with 150 µl distilled water, spread over an area of 4 cm^2 on a slide, air-dried and fixed 5 min with ethyl alcohol on the site. The fixed smears were then impregnated with silver-nitrate ($AgNO_3$, 4%) for 15 min, exposed to UV light for 30 min (Modified from Lom 1958) and examined under a compound microscope (Nikon Eclipse 600). Twenty pictures of trichodinid specimens from each sample were collected randomly and measurements defined by Lom (1958) were carried out using a software (Photoshop 7.0, Adobe Inc.). The prevalence (ratio of infested fish) and density (mean number of parasites on 4 cm^2 of the infected gill of the host) of *Trichodinid* spp. were determined by counting the number of trichodinids in 1.5 ml fixative. The fixed gill arcs were further examined under the microscope for any remnants of the ciliates.

The level of organic pollution was determined by measuring nitrite (Wood et al. 1967), nitrate (Strickland and Parsons 1968) and phosphate (Murphy and Riley 1962). Salinity, dissolved oxygen and temperature were also measured at depths where the fish were captured during the sampling.

Statistical analysis

The terms prevalence and mean density were used as defined by (Bush et al. 1997). Differences among monthly mean densities or differences between mean densities recorded at two stations were tested by using the Tukey–Kramer multiple comparison test (Rózsa et al. 2000). A multiple linear regression analysis was conducted to determine whether there are any synergic or antagonistic effects of the organic pollution parameters, nitrate, nitrite and phosphate on the prevalence of *Trichodina* spp. In all tests, performed by using statistica 6.0 (Statsoft Inc., Cary, NC, USA), *P*-values less than 0.05 were considered significant.

Results

A single species of *Trichodina* sp. was dominant (98.1%) in the samples taken from the gills of *M. merlangus*. The species description will be the matter of another communication. *Trichodina puytoraci* (Lom 1962) was the second most prevalent species (1.9%) (Lom 1962). Only three specimens of *Trichodina claviformis* (Dobberstein and Palm 2000) were also recorded from all silver stained samples examined (Dobberstein and Palm 2000).

Water quality parameters during the survey are given in Table 1.

Seasonal trends in prevalence, mean density of *Trichodinid* spp. on whiting and water quality parameters were similar at both stations (Table 2, Fig. 1). There was no difference in the level of prevalence (*t* test, $P > 0.05$) and mean densities (*t* test, $P > 0.05$) at both stations. Thus, both stations were treated as the same. Prevalence of *Trichodina* spp. on the gills of *M. merlangus* peaked in March (93%), then gradually decreased until June (60%). During the summer months, the level of prevalence stayed low. Starting from November, the prevalence again jumped to 90% and stayed above that level until April.

High numbers of the protozoan (approximately 50 cells per host) were observed during winter and spring months (Fig. 1). There was a gradual decrease starting from May to December. Mean densities of the parasite in January and May were significantly higher than during months of summer and fall in the same year (Fisher LSD test, $P < 0.05$).

A multiple linear regression analysis was conducted to determine which parameters contributed to the level of prevalence (Table 3). It was found that all three parameters contributed to the level of the observed prevalence (adjusted $r^2 = 0.59$). The model obtained was:

$$\text{Prevalence}(\%) = 0.821 + \text{Nitrite} \times 0.12 + 0.01 \times \text{Nitrate} - 0.08 \times \text{Phosphate}$$

Inclusion of oxygen into the model significantly improved adjusted r^2 to 67% ($P = 0.03$). The new model was:

$$\text{Prevalence} = 0.618 - 0.081 \times \text{Phosphate} + 0.89 \times \text{Nitrite} + 0.015 \times \text{Nitrate} + 0.022 \times \text{Oxygen}$$

Phosphate ($P = 0.007$) and temperature ($P = 0.038$) were the most effective parameters connected to the level prevalence.

The best model describing 89% (adjusted r^2) of the variation (non-adjusted r^2 was 95%) was obtained when phosphate, nitrate, oxygen and temperature (T) were included altogether in the model (Fig. 1).

$$\text{Prevalence} = 0.394 + 0.023 \times \text{Nitrate} - 0.079 \times \text{Phosphate} + 0.022 \times \text{Oxygen} + 0.022 \times T$$

Mean condition factors of the fish from two stations were approximately 0.79 ± 0.13 and were significantly lower ($P < 0.05$) in March than during the other months of the survey. Mean length of fish was 13.79 ± 0.082 (Table 1).

There was a major change in water quality parameters in December 15. Water temperature dropped to 8°C from 14°C of the previous month, indicating a seasonal

Table 1 Prevalence, mean density of *Trichodina* sp. and weight and length of its host

Months	Number of host examined	Prevalence	Mean density	Weight (g)	Length (cm)
January	42	0.88	61.9 ± 13.6	29.3 ± 2.36	15.1 ± 0.3
February	60	0.88	48.0 ± 6.37	16.0 ± 0.96	12.9 ± 0.3
March	59	0.93	53.8 ± 8.51	22.4 ± 2.05	14.1 ± 0.34
April		ND			
May	55	0.76	70.4 ± 21.9	20.3 ± 0.94	13.8 ± 0.18
June	57	0.60	60.5 ± 10.4	22.9 ± 0.97	14.5 ± 0.19
July	60	0.62	50.7 ± 1.29	21.6 ± 1.31	13.9 ± 0.27
August	120	0.75	43.5 ± 4.78	13.7 ± 0.57	12.3 ± 0.15
September	117	0.68	31.4 ± 3.76	19.2 ± 1.14	13.4 ± 0.22
October	56	0.61	31.5 ± 5.13	32.7 ± 2.28	15.5 ± 0.34
November	30	0.90	24.8 ± 5.03	26.7 ± 3.34	14.7 ± 0.47
December	60	0.87	25.2 ± 3.37	20.9 ± 0.73	14 ± 0.15

ND not done

Table 2 Water quality parameters during survey

Months	NO_3 (μa/l)	NO_2 (μa/l)	PO_3 (μa/l)	O_2	Temperature (°C)
January	1.43	0.72	1.08	9.50	12.875
February	2.26	0.59	0.00	10.25	12.000
March	0.61	0.03	0.00	10.00	13.500
April	0.89	0.09	0.11	9.60	12.250
May	0.12	0.13	0.28	9.10	8.100
June	0.24	0.11	2.50	8.70	9.500
July	1.53	0.12	1.72	10.05	7.900
August	4.32	0.03	1.71	9.40	7.050
September	2.16	0.01	3.19	11.35	11.000
October	2.66	0.01	2.19	5.05	10.400
November				6.00	13.000
December				4.66	17.500

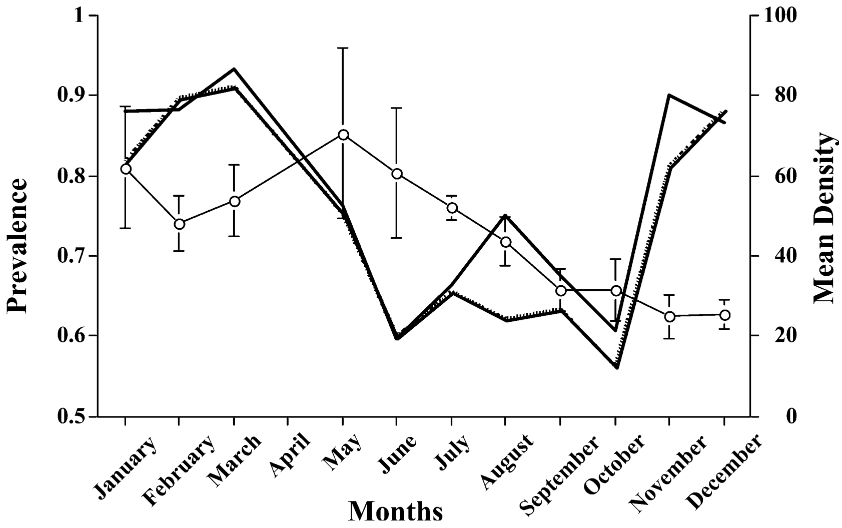

Fig. 1 Monthly observed prevalence (*solid line*), expected prevalence (*dotted line*) and mean densities (*solid line with circle*) of *Trichodina* spp. on *Merlangus merlangius* off the eastern coast of the Black Sea. Each data point is the mean values of prevalence and density of trichodinids on *M. merlangus* captured in two different locations. Expected prevalence is produced from the model; prevalence (%) = 0.394 + $0.023 \times NO_3 - 0.079 \times PO_4 + 0.022 \times O_2 + 0.022 \times T$, where T is the temperature

turnover of the water. We failed to get sample from station A, even tried three times with a 1-day interval.

Discussion

The relationship between a healthy ecosystem and parasites on hosts inhabiting that ecosystem draws considerable attention in recent years. In organically polluted areas, numbers of bacteria and algae, food of protozoans, increases (La Rosa et al. 2001). Thus, some ectoparasites of fish would benefit from this and increase their numbers on their host significantly. As a result, it is reasonable to suggest that a host could be under chronic environmental stress resulting in impaired immunity or immuno-tolerance (Khan and Thulin 1991). Moreover, parasite species diversity in healthy ecosystems, where most hosts and vectors are present, is richer than in polluted ecosystems. Some parasites requiring complex life history patterns will be absent along with pollution related disappearance of their vector hosts. Generally, only the parasites/epibionts needing a single host will be present in such systems (Yeomans et al. 1997; Kuperman 1992; McVicar 1997).

Table 3 Results of regression analysis

Parameters versus prevalence (M)	F-test	r^2	Probability (P)
Nitrate versus M	0.05	0	0.82
Nitrite versus M	3.29	0.22	0.11
Phosphate versus M	14.47	0.63	0.007
O, T versus M	3.07	0.35	0.07
Nitrate, Nitrite, Phosphate versus M	4.86	0.59	0.06
Nitrate, Nitrite, Phosphate, O versus M	5.01	0.67	0.07
Nitrate, Nitrite, Phoshate, T versus M	6.06	0.72	0.05
Nitrate, Nitrite, Phosphate, O,T versus M	10.38	0.85	0.04
Nitrate, Phosphate, O, T versus M	17.15	0.89	0.009

The observed level of prevalence and mean densities of infestation were higher during the late fall to early spring months than during the rest of the year suggesting that primary production, peaking around February (Sorohin 1983), is an important factor in prevalence spread of *Trichodinid* spp. on *M. merlangus*. Moreover, we detected a synergic effect of nitrate, nitrite and phosphate on the prevalence and densities of the protozoan by multivariate analysis. However, this relationship could only explain 59% of the observed variation. There are some other factors responsible for the rest of the variation. When we excluded nitrite and included oxygen and temperature, the representativeness of the model increased to 89%. Palm and Dobberstein (1999) stated that abundance of *Trichodina* spp. is an indicator of host health and the level of eutrophication of the surrounding environment. Similarly they found that there was an increase in winter months in the abundance of these protozoans. Ozer (2003), on the other hand, found that prevalence and mean densities of *T. domerguei* and *T. tenuidens* increased gradually with the seasonal increase in temperature. This phenomenon was probably due to the fact that, as Palm and Dobberstein (1999) suggested, seasonal eutrophication increased in their study site, which was a river opening to the Black Sea.

Here, we show that a considerable high portion of the fluctuation in the prevalence and mean densities were attributed to seasonal change in the parameters nitrite, nitrate and phosphate. If a site is polluted organically (eutrophication), it means that there are more bacteria and more trichodinids. Having high levels of prevalence fluctuation (10% to 90%) at the same site suggests that organic pollution also should be included in the analysis when any *Trichodina* sp. is supposed to be used as a pollution indicator.

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References

Broeg K, Zander S, Diamant A, Korting W, Kruner G, Paperna I, Von Westernhagen H (1999) The use of fish metabolic, pathological and parasitological indices in pollution monitoring—1. North Sea Helgol Mar Res 53:171–194

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83:575–583

Dobberstein RC, Palm HW (2000) Trichodinid Ciliates (Peritrichia : Trichodinidae) From the Bay of Kiel, with Description of *Trichodina Claviformis* Sp N. Folia Parasitol 47:81–90

Khan RA, Thulin J (1991) Influence of pollution on parasites of aquatic animals. Adv Parasitol 30:201–238

Kuperman BI (1992) Fish parasites as bioindicators of the pollution of bodies of water. Parazitologiia 26:479–482

La Rosa T, Mirto S, Marino A, Alonzo V, Maugeri TL, Mazzola A (2001) Heterotrophic bacteria community and pollution indicators of mussel-farm impact in the Gulf of Gaeta (Tyrrhenian Sea). Mar Environ Res 52(4):301–21

Lom J (1958) Contribution to the systematics and morphology of endoparasitic trichodinids from amphibians, with a proposal of uniform specific characteristics. J Protozool 5:251–263

Lom J (1962) Trichodinid ciliates of fishes from the Rumanian Black Sea Coast. Parasitology 52:49–61

Marcogliese DJ, Cone DK (1997) Parasite communities as indicators of ecosystem stress. Parassitologia 39:227–232

McVicar AH (1997) The development of marine environmental monitoring using fish diseases. Parassitologia 39:177–181

Möller H (1987) Pollution and parasitism in the aquatic environment. Int J Parasitol 17:353–361

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36

Overstreet RM (1997) Parasitological data as monitors of environmental health. Parassitologia 39:169–175

Ozer A (2003) The Occurrence of *Trichodina domerguei* Wallengren, 1897 and *Trichodina tenuidens* Fauré-Fremiet, 1944 (Peritrichia) on Three-spined Stickleback, *Gasterosteus aculeatus* L., 1758 found in a Brackish and Freshwater Environment. Acta Protozool 42:41–46

Palm HW (2004) The Trypanorhyncha Diesing, 1863. PKSPL-IPB Press, Bogor, x + 710 pp

Palm HW, Dobberstein RC (1999) Occurence of trichodinid ciliates (Peritricha: Urceolariidae) in the Kiel Fjord, Baltic Sea, and its possible use as a biological indicator. Parasitol Res 85:726–732

Rózsa L, Reiczigel J, Majoros G (2000) Quantifying parasites in samples of hosts. J Parasitol 86:228–232

Slade CL (2001) The ecology of trichodinids (Ciliophora: Peritrichida) from southwest Java, Indonesia with taxonomic descriptions. MSc Thesis, University of Bremen, p 188

Sorohin YL (1983) The Black Sea. In: Ketchum BH (ed) Ecosystem of the world estuaries and enclosed seas. Elsevier, London, pp 253–292

Strickland JDH, Parsons TR (1968) A practical handbook of seawater analysis. Bull Fish Res Board Can 167:310

Voigt MOC (1993) Protozoan ectocommensals of toadfish and soldier crab as indicators of organic pollution in the Brisbane River estuary. University of Queensland, Queensland

Williams HH, Mackenzie K (2002) Marine parasites as pollution indicators: an update. Parasitology 126:27–41

Wood ED, Armstrong FA, Richards FA (1967) Determination of nitrate in seawater by cadmium copper reduction to nitrit. J Mar Biol Assoc UK 47:23–31

Yeomans WE, Chubb JC, Sweeting RA (1997) Use of protozoan communities for pollution monitoring. Parassitologia 39:201–212