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PRIMARY RESEARCH PAPER

Population dynamics of free-swimming Annelida in four Dutch wastewater treatment plants in relation to process characteristics

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Abstract Free-swimming Annelida occasionally occur in very high densities in WWTPs (WasteWater Treatment Plants) and are nowadays applied for waste sludge reduction, but their growth is uncontrollable. In order to get more insight in the population dynamics of these free-swimming Annelida, and relate their presence to process characteristics, nine ATs (Aeration Tanks) of four Dutch WWTPs were regularly sampled over a 2.5-year period. For each species, peak periods in worm population growth were defined and population doubling times and half-lives calculated. Peak periods and doubling times were compared to those in natural systems. Process characteristics were obtained from the plant operators and related to the worm populations by multivariate analysis for the first time in large-scale WWTPs. The species composition in the WWTPs was limited and the most abundant free-swimming Annelida were in decreasing order *Nais* spp., *Aeolosoma hemprichi, Pristina aequiseta, Aeolosoma variegatum, Chaetogaster diastrophus*, and *Aeolosoma tenebrarum*.This latter species had never been found before in WWTPs. Worm absence sometimes coincided with the presence of anoxic zones, but this was possibly overcome by higher temperatures in the WWTPs. Worms were present all year round, even in winter, but no yearly recurrences of population peaks were observed, probably as a result of stable food supply and temperature, and the lack of predation in the WWTPs. Peak periods were similar between the ATs of each WWTP. The duration of the peak periods was on average 2–3 months for each species and the population doubling times in the peak periods were short (on average 2–6 days), which also corresponds to a stable favorable environment. The disappearance of worm populations from the WWTPs was presumably caused by declining asexual reproduction and subsequent removal with the waste sludge. Multivariate analysis indicated that 36% of the variability in worm populations was due to spatial and temporal patterns only. In addition, no more than 4% of the variability in worm populations was related to variations in process characteristics only and worm presence was usually associated with better sludge settleability. In conclusion, our data from large-scale

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WWTPs suggest that growth of free-swimming Annelida still seems uncontrollable and that their effects on treatment processes are unclear, which makes stable application in wastewater treatment for sludge reduction difficult.

Keywords Free-swimming aquatic worms · Annelida · Wastewater treatment · Sludge · Population dynamics · Process characteristics

Introduction

Several Annelida, e.g., Tubificidae including the subfamily Naidinae, are associated with polluted environments characterized by organic enrichment and oxygen depletion (Aston, 1973; Schönborn, 1985). Their species distribution is regarded as an indicator of environmental quality (Chapman et al., 1982). Specific examples of these extreme environments are aerobic WWTPs (WasteWater Treatment Plants). The main processes in these plants are aerobic conversion of organic components from industrial or domestic wastewater into biomass (activated sludge), water and CO_2, and nutrient removal. Protozoa are the most common and well-described bacterivorous grazers in WWTPs (Curds, 1975; Ratsak, 1994) and several authors described their role as indicators of plant performance and their influence on process characteristics in these WWTPs (e.g., Madoni et al., 1993; Martín-Cereceda et al., 1996; Lee et al., 2004). However, the presence and role of metazoan organisms (Annelida, but also Nematoda and Rotifera) in several types of WWTPs were only occasionally studied in the past. It is not exactly known how Annelida end up in WWTPs, but it is likely that they originate from surrounding water bodies or are transported by birds into the ATs (Aeration Tanks) (Milbrink & Timm, 2001).

Annelida in WWTPs can be classified as "free-swimming" (in the sludge) or "sessile" (on surfaces in the plants, e.g., the reactor walls or carrier materials). They often belong to the class Aphanoneura (Aeolosomatidae) or to the class Oligochaeta (Tubificidae (including the Naidinae), Enchytraeidae, Lumbriculidae, and Lumbricidae) (Solbé, 1975). Aeolosomatidae and Naidinae are mostly free-swimming species, while the other species are more sessile. Annelida feed on organic material in the activated sludge, but some species like *Chaetogaster diastrophus* feed mainly on Protozoa (Schönborn, 1984). Research on Annelida in large-scale WWTPs has mainly focused on biofilter systems or activated sludge systems.

For biofilter systems (e.g., filter beds), the occurrence of Enchytraeidae was studied in many field and laboratory studies. The life cycles of *Lumbricillus lineatus* and *Enchytraeus albidus* were extensively described by Reynoldson (1939 & 1948). Constant humidity, high temperatures, good food supply, and lack of predators supported large year-round populations. The worms prevented the filter beds from clogging with suspended solids, thus maintaining their treatment efficiency. He also mentioned the presence of *Aeolosoma* sp. and *Pristina* sp. in the beds. Williams et al. (1968) described the ecology and seasonal patterns of two Enchytraeidae species (*Lumbricillus rivalis and Enchytraeus coronatus*) in a biofilter system. According to Hawkes & Shephard (1972), the worm populations in these filters were only influenced by seasonal fluctuations. Learner (1979) mentioned the presence of several Naidinae (*Aulophorus furcatus*, *Chaetogaster diaphanus*, *C. langi*, *Nais barbata*, *N. elinguis*, *N. variabilis*, *Pristina aequiseta*, *P. foreli*, and *P. idrensis*) in sewage filter-beds throughout Britain. Learner & Chawner (1998) described a study in which the macro-invertebrate fauna of 67 biofilters in 48 WWTPs in Britain was sampled once or twice in the 1960s and related to the physico-chemical characteristics (such as F/M: Food to Microorganism ratio, temperature, geographic location, and pH) of the biofilter environment. *Nais* spp. and/or *Pristina* spp. were found in more than 40% of the surveyed biofilters and their occurrence was positively correlated to certain geographical locations and low F/M ratios. *Aeolosoma* spp., *Chaetogaster* spp. and Tubificidae other than Naidinae were only found occasionally.

For activated sludge systems, only a few studies have been done on the occurrence of Annelida. Poole & Fry (1980) found stable populations of Annelida (Aeolosomatidae and Tubificidae other than Naidinae) in three ATs during 2 months. They concluded that high TSS (Total Suspended Solids) concentrations were beneficial to the Aeolosomatidae. As high TSS concentrations usually coincide with higher sludge ages (i.e., the average residence times of the sludge in the ATs), their results suggest a positive effect of high sludge ages on worm densities. Inamori et al. (1983) mentioned the presence of large quantities of *Aeolosoma* sp., *Pristina* sp., *Nais* sp., *Dero* sp., and *Chaetogaster* sp. in the ATs of WWTPs.

In the Netherlands, most (366 out of 375) of WWTPs are nowadays activated sludge systems (Statistics Netherlands (CBS), 2007). Up to date, there has been only one long-term (1.5 years) monitoring of an annelid species (*Nais elinguis*) in one of these WWTPs (Ratsak, 2001). She concluded that high densities of this worm resulted in decreases of the SVI (Sludge Volume Index), the energy consumption for oxygen supply and, depending on the temperature, the waste sludge production. She also found that the density of worms varied both per season (high densities were both found in warm and cold periods) and per AT for unknown reasons. Population densities displayed peaks (also called “worm blooms”), which were invariably followed by a sudden disappearance of the population, a well-known phenomenon in WWTPs.

In 2001, a telephone survey of 23 WWTPs in the Netherlands (Janssen et al., 2002) showed that several worm species were often found in the consulted WWTPs. Tubificidae other than Naidinae were found all year round, but *Aeolosoma* spp. and *Nais* spp. were usually found in summer or at high temperatures. These latter species were sometimes present in such high densities, that the sludge in the ATs had a reddish color. In half of the plants surveyed, worm presence was assumed to coincide with decreases in waste sludge production and/or better sludge settling characteristics. In recent years, increasingly more researchers focused on applying Annelida (mostly *Aeolosoma hemprichi*, *Nais* spp. and Tubificidae other than Naidinae) for this purpose in laboratory set-ups (e.g., Wei et al., 2003a; Wei & Liu, 2005; Liang et al., 2006). The largest problems in this research field were and are (next to highly variable results) the before-mentioned uncontrollable population dynamics of especially free-swimming worms (Wei et al., 2003a; Ratsak & Verkuijlen, 2006).

A long-term study of worm population dynamics and possible interactions in and between large-scale WWTPs, between process characteristics and worm species that are used for sludge reduction research could provide useful insight for maintaining stable worm populations and sludge reduction rates. Therefore, free-swimming Annelida in the ATs of four Dutch WWTPs (one including a biofilter) were regularly sampled over a 2.5-year period. In order to explain the population dynamics in these artificial highly enriched ecosystems, peak periods and population doubling times were compared to those found in natural water bodies. In addition, the half-lives were calculated. Data of the process characteristics from that period were obtained from the plant operators. Process characteristics that were expected to have an influence on (e.g., sludge loading rate, sludge age) or to be influenced by the occurrence of worms (e.g., waste sludge production, TSS concentration and nutrient concentrations) were selected. For the first time, a multivariate analysis was performed to quantify possible relationships between long-term worm population growth and process characteristics in large-scale WWTPs.

Materials and methods

WWTPs

The WWTPs were chosen based on their worm diversity found in the telephone inquiry (Janssen et al., 2002). WWTPs Drachten (one AT), Nijmegen (three ATs), Renkum (three ATs), and Zwolle (two of four ATs) in the eastern and northern part of the Netherlands were sampled frequently (in total about 75 times per AT, which equals about once every 2 weeks). Plants were sampled from July 2000 through December 2002, but the sampling of Renkum was terminated in June 2002, due to plant alterations. The plants were all activated sludge systems with a low F/M ratio (<0.2 kg BOD (Biological Oxygen Demand)/kg TSS/d), with parallel ATs acting as separate systems with return sludge going to its original AT. The wastewater was routed through a series of channels constructed in the AT. Wastewater was first treated in a presettler to remove particles by sedimentation but in Drachten wastewater was first treated in a trickling filter (biofilter) where soluble and particulate BOD was removed and partly converted into bacteria. Furthermore, in Nijmegen, the presettled influent was heated with cooling water from the nearby waste incinerator from November 2001 onward and the average temperature in these ATs was therefore 22°C instead of 16°C in the other plants. Table 1 shows differences in P (Phosphorus) and N (Nitrogen) removal among the plants.

Table 1 P (Phosphorus) and N (Nitrogen) removal in the sampled WWTPs

WWTP	P-removal (chemical)	N-removal
Drachten	From January 2002 on	Only nitrification
Nijmegen	Yes	Anoxic pre-denitrification[a]
Renkum	No	Anoxic pre-denitrification[b]
Zwolle	Yes	Anoxic pre-denitrification[c]

[a] All ATs contained anoxic zones

[b] Only ATI in WWTP Renkum contained an anoxic zone for denitrification (non-aerated; nitrate > 0 mg l^{-1})

[c] Anoxic zones were established in all ATs of WWTP Zwolle in the following periods: August–October 2001 and April–October 2002

Worm sampling

At the sampling dates, one sludge sample from each AT was taken in the middle of the mixed liquor phase of the aerated zone with a plastic sampling container tied to a long pole. The containers were sent by regular mail (which took 2 (±2) days) to our lab, except for the samples from WWTP Renkum, which were analyzed the same day. The containers from WWTPs Drachten, Nijmegen and Zwolle were about half-filled with 100–250 ml of the sludge sample ensuring the presence of oxygen. This was regularly checked after arrival of the samples with a WTW Oximeter 330 and DO (Dissolved Oxygen) concentrations were on average 2.3 (±1.6) mg O_2/l.

Two or three subsamples of 1 ml each were taken by Pasteur pipette with a cut off tip from the well-mixed sludge sample in the containers, divided into droplets and diluted with dechlorinated tap water on a Petri dish. Live specimens were counted under an Olympus SZ40 stereomicroscope with a Clay Adams laboratory counter and identified to genus level (*Nais* spp.) or species level (all other frequent species) according to Brinkhurst (1971), Brinkhurst & Jamieson (1971) and Timm (1999). Identification was regularly confirmed after live mounting in polyvinyl lactophenol using an Olympus BHT microscope. Population densities were calculated as the average of the two or three subsamples.

Process characteristics

Process characteristics in the WWTPs during the sampling period were obtained from the plant operators. Characteristics from the ATs and effluent and miscellaneous characteristics that were expected to have an influence on or to be influenced by the occurrence of worms were selected and are shown in Electronic supplementary material—Appendix 1. As characteristics were not always analyzed at the same days as the worm densities, values within 5 days before and 5 days after (when available) were averaged.

Data analysis

Population dynamics (peak periods, population doubling times and half-lives)

For each species and AT worm densities that exceeded the average accumulated density for all samples plus two standard deviations were considered to represent peaks periods. The densities outside the peak periods were averaged and this was the background density. The start of a peak period was defined as the date at which the density became higher than this background density and the end of a peak period as the date at which the density became lower. For species that never exceeded 15 specimens/ml (e.g., *C. diastrophus*) no peak periods were calculated. In addition, peak periods that lay partially outside the sampling period were excluded from calculations on duration of the peak periods. A peak period could contain several (up to three) peaks, when the worm densities did not become lower than the background densities before the start of a new peak.

Population doubling times (t_d) and half-lives (t_h) in population peaks were calculated based on exponential functions (e.g., Nandini & Sarma, 2004). The worm population densities in WWTPs are, however, not only dependent on natural population growth and decay like in a stagnant system, but also on mechanical loss via waste sludge. This loss is dependent on the sludge age during the peak periods, which was therefore incorporated in the equations. For Renkum and Zwolle, the sludge ages were obtained from the plant operators and ranged from 9 up to 48 days. For Nijmegen, the sludge age was assumed to be the average sludge age in the other WWTPs, 18 days. Furthermore, the supply of worms to the ATs with the influent and removal of worms from the plant with the effluent were both negligible, as we had observed in our pilot plants representative of large-scale WWTPs. The worm population growth rates in the downward phases of the peaks were zero, because dividing worms were hardly encountered in these phases. The population decay rates of worms were the same in the upward and downward phases of the peaks, because there was no indication for increased mortality in the downward phases. From the observed population growth and decay rates (μ_{obs} and r_{obs}) the real population growth and decay rates (μ and r) were calculated according to equations (1) and (2):

$$r_{\mathrm{obs}} = \mathrm{r} + 1/\theta = \ln(X_0/X_\mathrm{t})/(t - t_0) \quad (\text{in day}^{-1}) \tag{1}$$

where r = real population decay rate (day^{-1}), $1/\theta$ = loss rate via waste sludge (day^{-1}), θ = sludge age (days), X_t = total density of worms at time t (specimens/ml), X_0 = total density of worms at t_0 (specimens/ml), t = time at end of peak, t_0 = time at top of peak

$$\mu_{\mathrm{obs}} = \mu - r - 1/\theta = \ln(X_\mathrm{t}/X_0)/(t - t_0) \quad (\text{in day}^{-1}) \tag{2}$$

where μ = real population growth rate (day^{-1}), t = time at top of peak, t_0 = time at start of peak

Population doubling times t_d and half-lives t_h were calculated as ln(2)/μ and ln(2)/r, respectively.

Multivariate analysis

Multivariate analyses are suitable to recognize latent patterns in large datasets (Jongman et al., 1987). These complex datasets can be graphically summarized in low dimensions using these techniques. For a proper use, it is important to choose the appropriate response model (linear or unimodal). A preliminary DEtrended CORrespondence ANAlysis (DECORANA) was performed with log transformed abundance data of the worms and invoking the option "down-weighting of rare species." The length of the gradients as calculated by DECORANA was larger than 3.5 and thus the unimodal response model was assumed to be appropriate for this dataset (ter Braak, 1986). To analyze the importance of different abiotic conditions (WWTP, sampling year, month, and process characteristics in the WWTPs) on the worm composition in the ATs, direct ordination analysis was performed (ter Braak, 1986). The contribution of the different variables was quantified using the variance partitioning method as proposed by Borcard et al. (1992), which was also successfully applied to quantify the effects of contaminants in aquatic ecosystems (e.g., Peeters et al., 2001). The method was applied to the dataset containing the information of all four WWTPs as well as for the separate WWTPs, excluding Drachten due to the low number of samples. All ordination analyses were performed using the software program CANOCO (CANOnical COrrespondence analysis) (ter Braak & Smilauer, 1998). The statistical significance of the effect of each set of explanatory variables was tested by a Monte Carlo Permutation test (ter Braak, 1990). CANOCO extracts four axes and calculates scores for samples, species, and variables. The sequence of the extracted axes is determined by the amount of information they contain. Ordination diagrams visualize the main structure in the multivariate dataset, usually in two dimensions. The diagrams were created by using the calculated scores. Samples and species are positioned as points in the diagram while arrows represent variables. The length of the arrow is a measure of the importance of the variable, while the arrowhead points in the direction of increasing influence.

Results

General

Annelida were found in all ATs and almost all species belonged to the Aeolosomatidae (*Aeolosoma hemprichi*, *A. variegatum*and *A. tenebrarum*) or Naidinae (Tubificidae): (*Nais* spp., *Pristina aequiseta*and *Chaetogaster diastrophus*). All these species reproduce mainly asexually (Loden, 1981; Christensen, 1984; Bell, 1984) and worms with reproductive organs were indeed rarely found. Table 2 gives an overview of average densities, standard deviations, maximum densities and frequencies for the six main species. Unidentified worms or worms belonging to the genus *Dero* or to the family Tubificidae (other than the Naidinae) were rarely found and these counts were not further analyzed.

Nais spp. was the most common species (59% of all samples) and was followed in decreasing order by *A. hemprichi*(37%), *P. aequiseta*(19%), *A. variegatum*(17%), *C. diastrophus* (12%) and *A. tenebrarum*(5%). To our knowledge, this latter species was never found before in WWTPs. WWTP Nijmegen

Table 2 Overview of free-swimming Annelida in nine ATs of four Dutch WWTPs (average worm densities in specimens per ml in bold with standard deviations in italic, maximum worm densities in specimens per ml, and frequencies (%) of occurrence in all the samples of the indicated AT (or ATs) between brackets). Minimum worm densities were always zero in all ATs. Abbreviations used: D = WWTP Drachten, N = WWTP Nijmegen, R = WWTP Renkum, Z = WWTP Zwolle, numbers 1–4 indicate ATs, *n* = sample size

AT	*Naidinae*			*Aeolosomatidae*			Total
	Nais spp.	*Pristina aequiseta*	*Chaetogaster diastrophus*	*Aeolosoma hemprichi*	*Aeolosoma variegatum*	*Aeolosoma tenebrarum*	
D	**0** ± 0			**0** ± *35*	**1** ± *12*		**1** ± *46*
n = 84	3 (14)			178 (15)	79 (11)		259 (27)
N1	**5** ± *8*		**0** ± *1*	**8** ± *18*	**0** ± *0*	**0** ± *1*	**14** ± *21*
n = 72	55 (72)		3 (17)	103 (51)	3 (3)	5 (3)	115 (83)
N2	**6** ± *5*		**1** ± *2*	**11** ± *23*	**0** ± *1*	**0** ± *1*	**18** ± *24*
n = 67	19 (88)		8 (30)	132 (56)	5 (2)	3 (11)	141 (97)
N3	**5** ± *6*	**0** ± *0*	**0** ± *2*	**10** ± *21*	**0** ± *0*	**3** ± *13*	**18** ± *32*
n = 74	29 (78)	2 (1)	12 (23)	110 (54)	1 (4)	79 (27)	194 (89)
N1+2+3	**16** ± *16*		**1** ± *2*	**19** ± *34*	**0** ± *1*	**3** ± *13*	**48** ± *61*
n = 65	87 (94)		9 (48)	146 (62)	6 (5)	79 (22)	250 (97)
R1	**0** ± *1*			**0** ± *0*	**0** ± *0*		**0** ± *1*
n = 45	4 (4)			1 (4)	2 (2)		7 (7)
R2	**13** ± *19*		**0** ± *1*	**11** ± *21*	**17** ± 36		**41** ± *46*
n = 73	88 (84)		6 (19)	96 (49)	158 (56)		168 (88)
R3	**9** ± *10*		**0** ± *0*	**13** ± *29*	**55** ± *81*		**76** ± *81*
n = 72	40 (81)		2 (8)	121 (37)	297 (66)		298 (86)
R1+2+3	**22** ± *25*		**1** ± *1*	**23** ± *33*	**67** ± *88*		**113** ± *103*
n = 69	113 (87)		6 (23)	122 (64)	301 (70)		348 (88)
Z3	**2** ± *4*	**4** ± *5*		**2** ± *6*			**8** ± *10*
n = 80	29 (56)	25 (75)		34 (29)			50 (83)
Z4	**2** ± *6*	**8** ± *9*	**0** ± *1*	**5** ± *14*	**0** ± *1*		**16** ± *21*
n = 81	30 (46)	46 (79)	5 (12)	66 (31)	9 (9)		115 (81)
Z3+4	**4** ± *8*	**12** ± *12*	**0** ± *1*	**7** ± *18*	**0** ± *1*		**24** ± *28*
n = 79	35 (66)	52 (80)	5 (13)	100 (39)	9 (8)		133 (86)

displayed the most diverse worm population. *A. hemprichi*, *A. variegatum* and *Nais* spp. were found in all WWTPs, but not in all ATs. *C. diastrophus* was found in all WWTPs except Drachten, *A. tenebrarum* only in Nijmegen and *P. aequiseta* mostly in Zwolle (except for one sample of Nijmegen AT3).

Population dynamics

For each species, season and AT, peak periods were calculated (Table 3).

Most importantly, no yearly patterns in the peak periods were observed. In addition, the mere presence of a worm species did not always result in peak periods of this species. In WWTP Drachten, peak periods (Table 3) were found in the first month of the sampling period but thereafter no worm densities higher than 1 specimen/ml were found. The three ATs of WWTP Nijmegen had similar peak periods of *A. hemprichi* and *Nais* spp. In addition, only in AT3 a peak period of *A. tenebrarum* was found. In Renkum ATI, no peak periods were observed because of very low worm densities (Table 3) and the ATs differed in peak periods, although AT2 and AT3 showed similarities in species and densities. Zwolle AT3 and AT4

Table 3 Peak periods for each worm species, season (in which the highest worm density was reached) and AT. Abbreviations used: D = WWTP Drachten, N = WWTP Nijmegen, R = WWTP Renkum, Z = WWTP Zwolle, numbers 1–4 indicate ATs, Ah = *A. hemprichi*, Av = *A. variegatum*, At = *A. tenebrarum*, Na = *Nais* spp., Pa = *P. aequiseta*

	2000		2001				2002	
	Sum	Aut	Win	Spr	Sum	Aut	Win	Spr
D	Ah, Av							
N1	Na							Ah
N2	Na						Ah	
N3	Na, Ah		Na					Ah, At
R1								
R2	Av			Ah, Na				
R3				Av		Ah, Na		
Z3	Pa			Ah, Na, Pa				Ah
Z4			Ah	Ah, Na, Pa				Ah

showed similar peak periods. The average durations of the peak periods for *A. hemprichi*, *A. tenebrarum*, *A. variegatum*, *Nais* spp. and *P. aequiseta* were 91 (±50), 84, 64, 96 (±37), and 58 (±21) days, respectively.

In Table 4 an overview of calculated average (plus standard deviations), minimum and maximum population doubling times, and minimum and maximum population half-lives in days for each species in the exponential phases of the peaks is shown.

The population doubling times for the Naidinae (on average 5–6 days) were somewhat longer than for the Aeolosomatidae (on average 2–4 days). Infinitely long population half-lives are biologically impossible, but were calculated when the observed population decay rates were only slightly higher than the inverse of the sludge age. This resulted in very low real population decay rates (Eq. 1) and subsequently, in infinitely long population half-lives. These values thus signify a large influence of sludge age (and not worm death) on the disappearance of the worm populations. It seems that worm population growth thus simply stops after a certain time and populations are removed with the waste sludge.

Table 4 Average population doubling times (t_d) in bold plus standard deviations, minimum and maximum population doubling times between brackets, minimum and maximum population half-lives (t_h) in days for each species in the exponential phases of the peaks

Species	t_d	t_h
Nais spp.	**5** ± 3 (1–12)	2–∞
P. aequiseta	**6** ± 1 (5–6)	∞
A. hemprichi	**4** ± 1 (2–6)	1–∞
A. variegatum	**3** ± 2 (1–6)	1–∞
A. tenebrarum	**2** ± 1 (1–3)	2–8

Multivariate analysis

Figure 1 shows the results of an analysis in which the worm species composition was directly related to the abiotic variables.

Approximately 57% of the variation in the species composition was explained by the variables included in the analysis. Sampled WWTP, sampling date as well as characteristics of the WWTP and AT all affected the worm species composition. Figure 1a clearly shows that the samples from Zwolle were positioned apart from the samples of the other WWTPs mainly related to the much higher abundances of *P. aequiseta*. The samples of Nijmegen are situated in the upper part of the diagram corresponding with higher abundances of *A. tenebrarum*. The samples of Renkum are mostly situated in left lower corner of the diagram coinciding with higher abundances of *A. variegatum*. The partitioning of the variance indicated that WWTP explained most of the variance (Table 5) followed by sampling date.

The process characteristics explained approximately 4% of the variation in the species data and this contribution is significant. An analysis with the process characteristics as explanatory and WWTP and sampling date as covariables showed that SVI was most important, since this variable has the longest arrow (Fig. 2).

Especially the species *A. variegatum* was associated with higher SVI values. *C. diastrophus* seems to be associated with lower denitrification efficiencies (higher values of N in the effluent) whereas *A. tenebrarum* was associated with higher BOD concentration in the effluent. In addition, Table 6 indicates that SVI was associated with worm species composition in all three WWTPs.

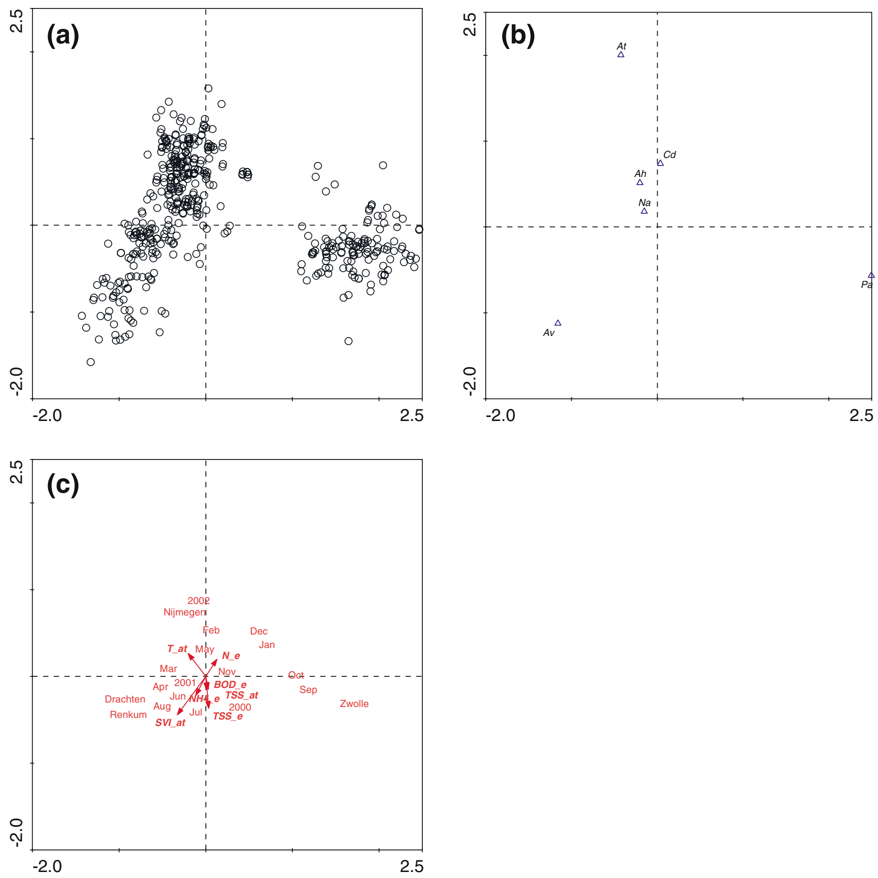

Fig. 1 Graphical presentation of the direct ordination of Annelida species composition related to abiotic conditions. The distribution for the first two axes is given for a) samples, b) species, and c) abiotic variables. Abbreviations used: b) *Ah* = *A. hemprichi*, *Av* = *A. variegatum*, *At* = *A. tenebrarum*, *Na* = *Nais* spp., *Pa* = *P. aequiseta, Cd= C. diastrophus*.c) Suffix "_at" indicates characteristics in the ATs, suffix "_e" indicates characteristics in the effluent, TSS = Total Suspended Solids concentration, SVI = Sludge Volume Index, T = Temperature, BOD = Biological Oxygen Demand concentration, NH4 = NH_4^+ concentration as indication of nitrification efficiency, N = sum of NH_4^+, NO_2^- and NO_3^- concentrations as indication of nitrification efficiency

Discussion and conclusions

General

During the 2.5-year sampling period, almost exclusively Naidinae and Aeolosomatidae were found in the WWTPs, as is also observed by other authors (e.g., Solbé, 1975). Relatively few species were found, possibly due to the extreme conditions in the plants: high organic pollution levels and turbulence in the ATs. In Drachten, Renkum AT1 and Zwolle worms were (temporarily) completely absent during the sampling period. The worm absence may have been related to the presence of toxic compounds (e.g., un-ionized ammonia) or to other unknown factors. In addition, the absence from Renkum AT1 and Zwolle may have been due to the presence of anoxic zones, through which the sludge and worms were partly recirculated (Table 1). However, Nijmegen also contained anoxic zones but this did not prevent worm population growth. We hypothesize that the positive effect of the higher influent temperatures in this WWTP on worm population growth enabled them to cope with the local anoxic conditions. Several authors found that population growth rates on sterilized activated sludge were positively correlated with temperature with optima of 25–35°C for the species that were encountered in our research (Inamori et al., 1983; Kuniyasu et al., 1997).

Furthermore, *A. tenebrarum*, *C. diastrophus* and *P. aequiseta* were not present in all WWTPs and worm dispersal in the WWTPs may also be related to their natural occurrence in the direct surroundings of the WWTP.

The maximum worm densities for the three main species in our research (*Nais* spp., *A. hemprichi* and *P. aequiseta*) were 88, 178 and 46 specimens/ml, respectively. Maximum worm densities found by other researchers in different wastewater treatment systems were variable. In large-scale plants the maximum densities varied from 0.3 Naidinae/ml (Learner & Chawner, 1998) and 30 Aeolosomatidae/

Table 5 Partitioning of the variance in the species data in percentages obtained from partial canonical correspondence analyses

Variables	Fraction explained	
	Gross[a]	Pure[b]
WWTP	40.3	24.4
Sampling date	19.5	11.3
Year	12.7	4.8
Month	9.0	6.1
Process characteristics	16.0	4.3
Effluent (NH_4^+,N,TSS, BOD)	8.4	1.1
AT (TSS, SVI, T)	12.5	2.1
Shared by WWTP, sampling date and process characteristics		16.8
Total	56.8	56.8

[a] Calculated through a direct analysis with only the listed variables as explanatory

[b] Calculated through a direct analysis with the listed variables as explanatory and all others as covariables

All analyses were significant ($P < 0.010$) according to the Monte Carlo Permutation Test. Abbreviations used: NH_4^+—NH_4^+ concentration, N—sum of NH_4^+, NO_2^- and NO_3^- concentrations as indication of nitrification efficiency, TSS—Total Suspended Solids concentration, BOD—Biological Oxygen Demand concentration, SVI—Sludge Volume Index, T—Temperature

ml (Poole & Fry, 1980) to 160 *N. elinguis*/ml (Ratsak, 1994). In contrast, the maximum densities in lab-scale systems were much higher. Inamori et al. (1983) found maximum densities of 1000 *Aeolosoma*, 200 *Nais* and 200 *Pristina*/ml and Wei et al. (2003b) found maximum densities of around 700 and 125 specimens/ml for the former two species.

Population dynamics

The occurrence of population peaks did not follow a yearly pattern, but usually showed high similarities within each WWTP. Ratsak (1994) also found that the density of *N. elinguis* in a WWTP varied both per season and even per AT for unknown reasons. We cannot rule out longer-term patterns even though in natural populations of aquatic Annelida annual growth patterns are common. For example, during a seven-year period, Loden (1981) always found peaks in spring in field populations of Naidinae. Only *P. aequiseta* showed peaks in autumn but we did not observe this in the WWTPs either. The highest

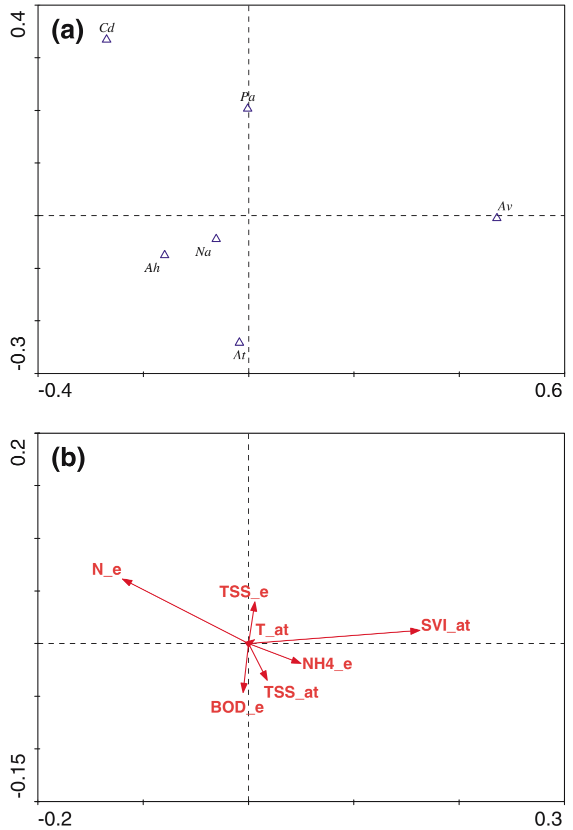

Fig. 2 Graphical presentation of a partial correspondence analysis in which the species data were constrained by the process characteristics of the WWTP after removing the effects of WWTP and sampling date. The distribution for the first two axes is given for a) species and b) process characteristics. Abbreviations used: a) *Ah* = *A. hemprichi*, *Av* = *A. variegatum*, *At* = *A. tenebrarum*, *Na* = *Nais* spp., *Pa* = *P. aequiseta*, *Cd*= *C. diastrophus*.b) Suffix "_at" indicates characteristics in the ATs, suffix "_e" indicates characteristics in the effluent, TSS = Total Suspended Solids concentration, SVI = Sludge Volume Index, T = Temperature, BOD = Biological Oxygen Demand concentration, NH4 = NH_4^+ concentration as indication of nitrification efficiency, N = sum of NH_4^+, NO_2^- and NO_3^- concentrations as indication of nitrification efficiency

Table 6 The importance of process characteristics according to the partitioning of the variance in the worm data per WWTP

WWTP	AT	Effluent	Variance explained (%)
Nijmegen	SVI, T	NH_4^+	5
Renkum	SVI	N	7
Zwolle	SVI, TSS		6

Abbreviations used: SVI, Sludge Volume Index; T, temperature; NH_4^+, NH_4^+ concentration; N, sum of NH_4^+; NO_2^- and NO_3^- concentrations as indication of nitrification efficiency; TSS, Total Suspended Solids concentration densities of several *Nais* species in a polluted river were also found in spring by Schönborn (1985), who concluded that this was a food issue. Therefore, the stable food supply and temperatures could explain the absence of annual growth patterns in the WWTPs. This was also supported by asexual reproduction of the species in our research, which usually indicates favourable conditions like food availability, higher temperatures and low NaCl concentrations (Learner et al., 1978; Loden, 1981). In addition, top-down predation as observed in field situations by, for example, fishes (Wallace & Webster, 1996) is virtually absent from WWTPs, which is another explanation for the seemingly random population dynamics. The absence of seasonal patterns for no apparent reason was also sometimes observed for other invertebrates like Nematoda (Michiels & Traunspurger, 2004).

The average durations of the peak periods were quite similar, all around 2–3 months, but variability within one species was high for unknown reasons. Once worm population growth was triggered, the population doubling times were short (on average 2–6 days), which also indicated favourable conditions. They were slightly longer for the Naidinae than for the Aeolosomatidae. In experiments with sterilized activated sludge (Inamori et al., 1983; Kuniyasu et al., 1997) population doubling times for *A. hemprichi*, *Pristina* sp. and *Nais* sp. were also 1–6 days depending on TSS concentration. Similar to our results, the longest population doubling times were found for *Nais* sp. Under more natural conditions (laboratory set-ups with polluted river water and detritus as food source), the population doubling times of several *Nais* species were longer (3–16 days) (Lochhead & Learner, 1983; Schönborn, 1985).

The sudden disappearance of the population peaks could be due to the observed lack of dividing worms observed in the downward phases of the peaks, which was possibly caused by senescence phenomena (Martinez & Levinton, 1992). This was illustrated by the high values for the population half-lives, which indicated a low population decay rate but a big influence of removal with waste sludge.

Multivariate analysis

The results from the multivariate analysis suggested that the variability in worm populations was mostly due to spatial and temporal differences. This was possibly caused by the before-mentioned absence of certain worm species from some plants (e.g., *A. tenebrarum* was only present in Nijmegen).

Process characteristics in the plants could be related to only 4% of the variability in worm populations. There were indications that worms were related to SVI, which was also found by other authors (e.g., Ratsak, 2001; Wei et al., 2003b). The present study indicated that *A. variegatum* was associated with higher SVI values, whereas the other taxa were associated with lower SVI values. The latter can be explained by compacting of the sludge flocs by the worms, which increased the settleability. The association of *C. diastrophus* with higher denitrification efficiencies could be due to removal of denitrifying bacteria populations, but this is not likely, since *C. diastrophus* mainly feeds on Protozoa. The association of *A. tenebrarum* with higher BOD concentrations in the effluent could be due to the release of suspended solids in the water phase because of sludge consumption. It is unknown why other species did not show these associations.

In contrast, other authors suggested much more relations between the presence of worms and several process characteristics. Ratsak (1994) concluded that high worm densities not only resulted in a low SVI but also lower energy consumption for oxygen supply and, depending on the temperature, less sludge production. The worms had no influence on the effluent quality in terms of BOD and nutrients. Wei et al. (2003b) also reported lower SVI values and less sludge production as a result of the presence of especially *Nais* sp. and to a lesser extent as a result of the presence of *A. hemprichi*. However, he reported a decrease in effluent quality in terms of TSS and BOD. In addition, growth of *A. hemprichi* was somewhat positively correlated to temperature and negatively to pH, while growth of *N. elinguis* was somewhat positively correlated to TSS, temperature and DO and negatively to F/M ratio. Inamori et al. (1987) and Zhang (1997) reported similar phenomena.

However, in these researches interactions among variable process characteristics, for example sludge age, which is inversely correlated to sludge production (van Loosdrecht & Henze, 1999), are left out of consideration. Wei & Liu (2006) for example concluded from their research that not all results from their study could be attributed to the presence of worms due to the lack of a control system. The process characteristics we obtained from the studied Dutch WWTPs could hardly be related to the worm composition and densities in the samples from those WWTPs. These data suggest that the population peaks of free-swimming Annelida are phenomena that are hard to explain and control, and confirms that their supposed effects on WWTP process performance, like waste sludge production, should be interpreted with much care. We can, however, not rule out that elaborations to materials and methods, e.g., obtaining data for other process characteristics, could have revealed new relations that were not found in the current survey.

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