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1	Sealworm (Pseudoterranova decipiens) infection in grey seals (Halichoerus grypus), cod (Gadus
2	morhua) and shorthorn sculpin (Myoxocephalus scorpius) in the Baltic Sea
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10	
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12	
13	Abstract
14	The anisakid nematode Pseudoterranova decipiens, known as the sealworm or cod worm, can
15	infect the flesh of several fish species. The parasite causes cosmetic problems for the fish

17 studies on the abundance or distribution of the sealworm in fish and seals in the Baltic Sea. To

16

industry and can cause abdominal discomfort if consumed by humans. There are only scattered

18 remedy this situation, the extent of sealworm infection was investigated in cod (*Gadus morhua*)

19 and shorthorn sculpin (*Myoxocephalus scorpius*) collected along the Swedish coast. A relative

20 presence of the sealworm was also investigated in samples from grey seal (Halichoerus grypus)

stomachs. Up to 100% of the fish were infected in some of the areas. Sculpin were generally

22 worse infected than cod, both in abundance and prevalence of parasites. General linear models

23 showed a significant correlation between the number of seals in an area and the prevalence of

24 sealworms in cod. There was a sharp decrease of infected fish in areas with salinity lower than 7 ‰. Even though the northern Baltic proper and the southern Bothnian Sea have a high number of 25 grey seals, only one sealworm was found in a sculpin in that region, and none in cod. In grey seal 26 stomachs the sealworm was only found in samples from the central Baltic proper; further north 27 all anisakid nematodes identified in seals were Contracaecum osculatum. The results indicate 28 29 that seal presence drives the distribution in the southern parts of the Baltic and that low salinity, or some other variable which correlates with salinity, limits the distribution in the northern part. 30 Keywords: Baltic Sea, cod, grey seal, *Pseudoterranova*, sealworm, sculpin. 31

32

33 Introduction

Infection by the larval stages of the parasitic sealworm, *Pseudoterranova sp.*, in commercial fish 34 35 species has been of great concern in the North Atlantic fisheries (McClelland 2002). So far it has not been raised as an urgent issue in the Baltic Sea area except in the southern parts (Myjak et 36 al., 1994, Szostakowska et al., 2005, Buchmann and Kania, 2012, Nadolna and Podolska, 2014). 37 However, with an increasing population of seals, the parasite's final hosts, there are concerns of 38 an increasing problem. Data on the population of grey seals in the Baltic suggest an annual rate 39 40 of increase of 7.5% since 1990; (Harding et al. 2007) and in 2013 the counted population on shore was 28,000 (www.rktl.fi). The highest density of grey seals in the Baltic is found between 41 latitudes 58° and 61°, which correspond with ICES subdivision 27 in the north to subdivision 31 42 43 in the south (Figure 1). There are also scattered colonies along the Swedish coast in the southern Baltic (Harding et al. 2007). In addition, there is a population of a few thousand harbour seals in 44 subdivisions 24 and 25 (Härkönen & Isakson 2010; Olsen et al. 2010). With a fishing industry in 45

the Baltic depending on cod (*Gadus morhua*), which is a common intermediate host for theparasite, the increasing seal population is a problem.

Pseudoterranova sp. are intestinal roundworms or nematodes belonging to the family Anisakidae 48 and can be considered a cosmopolitan genus, with a confusing taxonomy (Paggi et al. 2000). 49 Genetic studies by Buchman and Kania (2012) determined that the nematodes found in cod flesh 50 51 from the southern Baltic are *Pseudoterranova decipiens*. The sealworm has a complex life cycle, with a free-living stage and three obligate hosts required for the parasite to complete its life-52 cycle. The eggs are excreted in the faeces of a seal and sink to the sea floor where they hatch into 53 54 free-living larvae. A benthic invertebrate ingests the larvae. After the infected invertebrate is eaten by a fish, the larvae migrate from the stomach of the fish into the muscle tissue. It is at this 55 stage that the 2-3cm long larvae become clearly visible in the fillets of the fish and create a 56 problem for the fishing industry if the host is a commercially important species. When the fish is 57 ingested by a seal, their definitive host, the parasites continues to moult into an adult stage whose 58 eggs are then released with the seal faeces, and thus the life cycle is completed (McClelland 59 2001). 60

Along the Swedish west coast the sealworm is common in both harbour seals (Lunneryd 1991) 61 62 and in fish (Lunneryd et al. 2001) but in the Baltic Sea there have only been a few reports of sealworms so far. The first major study of the occurrence of anisakid nematodes in Baltic cod, 63 conducted in 1976, showed no records of the sealworm. However, at that time there were very 64 65 few seals in the southern Baltic, so it was not surprising that there were no sealworms (Grabda 1976). Later studies (Myjak et al. 1994; Szostakowska et al. 2005; Buchmann and Kania 2012) 66 revealed that the sealworm was present in cod caught in ICES subdivision 25 and 26 southern 67 68 Baltic. In addition, sealworms were also found in low numbers in cod stomachs collected in 2002

69 and 2003 in the southern Baltic proper and in subdivision 27 (Perdiguero-Alonso 2008). Thulin (1989) did a study of parasites and fish diseases in Swedish waters and found sealworms only in 70 cod caught off the Swedish west coast and not at all in the Baltic Sea, despite extensive 71 sampling. (Buchmann and Kania, 2012) compared the sealworm infection levels in cod collected 72 in 1982-83 from the Bornholm Basin with that in cod collected in 2011, and noted that no 73 74 nematodes were found during 1982-83 while at least 2% of the samples surveyed in 2011 were infected with 1 to 4 sealworms. The presence of the nematodes in Baltic cod in recent times has 75 been well known among fishermen (several personal communications) but has not been public 76 77 knowledge in e.g. Sweden until very recently. It is therefore important to collect knowledge about the biology and distribution of this parasite. Factors discussed as determinants of the 78 79 distribution of sealworms are seal distribution, intermediate host distribution and environmental factors such as temperature and salinity (Measures 1996; Marcogliese 2001a; Hauksson 2011). 80 The spatial distribution in the Baltic Sea is of special interest because of its uniqueness in being a 81 82 brackish sea. Salinity has been shown to limit the survival of the sealworm larvae (Measures, 1996). As regards the survival and hatching rates of sealworm eggs in different salinity 83 environments, studies have shown no difference between sea water (35 ‰ salinity), brackish 84 85 water (17 ‰ salinity) and fresh water, but the larvae were much more sensitive: those hatched in water with higher salinity survived about 10 times as long as those hatched in freshwater 86 (Measures 1996). In the Swedish coastal areas of the Baltic Sea there is a gradient of salinity 87 88 from around 9‰ in the southern Baltic proper to about 5‰ in the southern Bothnian Sea. In this lower range of salinity in the Baltic Sea, it is not known how well the sealworms survive. 89 The aim of this study was to examine the relative presence and distribution of sealworms in the 90 91 middle Baltic Sea. The presence of the sealworm was investigated in cod, a commercially

important species, and in shorthorn sculpin (*Myoxocephalus scorpius*), a relatively sedentary
species chosen in order to reflect the accumulation of the sealworm population in local waters
(Aspholm et al.1995; Midtgaard et al. 2003). Sealworm presence was also examined in seal
stomachs collected along the Swedish coast. Variables such as fish length, salinity and seal
population density were examined in order to understand how they might influence the
distribution of the sealworm.

98

99 Materials and Methods

100 Fish and parasite sampling

Fish were collected along the east coast of Sweden, using different types of gear, such as gill nets, long lines, pots and trawls. Fish lengths were measured to the nearest centimetre. The cod were gutted by the fishermen and either examined fresh or stored on ice before being frozen for later examination. Shorthorn sculpin were all deep frozen for later investigation. No attention was paid to nematodes observed on or in the intestines or livers.

Fish were filleted and skinned with their pectoral and pelvic fins removed. The backbones, the 106 flesh around the fins and the skin were carefully inspected visually for nematodes, using a light 107 108 table. The fillets of sculpin were sliced thinly for examination on the light table because their musculature is not as transparent as cod musculature. It is important to note that this method does 109 not reveal all nematodes in the flesh (McClelland & Martell 2001; Llarena-Reino 2012), but if 110 111 applied consistently it can give an accurate relative estimate of their abundance and prevalence. In order to support this consistency, the same light table was used throughout the study and 112 samples were always examined by the same two researchers. There is a probability of of 113 114 identification error by counting Anisakis simplex larvae in the flesh instead of Pseudoterranova

115 *decipiens*. However, these larvae are commonly found in the intestines of cod in the southern

116 Baltic (Szostakowska et al. 2005; Nadolna and Podolska 2014). Because Anisakis simplex mostly

117 occur in the visceral cavity (ICES 2012 b) and *Pseudoterranova decipiens* in the flesh the chance

118 of mistake is minimized. In cases of uncertainty, nematodes were mounted on slides and, with

119 glycerol as a clearing agent, examined under the microscope.

Abundance is defined as the mean number of sealworms found per fish examined (i.e. uninfectedfish are included) and prevalence as the proportion of fish infected (Margolis et al. 1982).

122

123 Seals and parasite sampling

Nematode samples consisting of 2,195 specimens were collected from seal stomachs during dietary investigations of by-caught and culled seals (Lundström et al. 2010). The stomachs were deep frozen before the investigation. Samples of up to 50 randomly picked nematodes were taken from each stomach and stored in a mixture of 70 % ethanol and 10-20 % glycerol. The nematodes were mounted on slides for microscopic examination and glycerol was used as a clearing agent to make the nematode cuticle more transparent. The nematode species and life stage were identified by internal characteristics (ICES 1984, 2012a, b).

131

Models for spatial distribution and infection presence of *Pseudoterranova sp.* in fish
To understand the influence of different variables on the spatial distribution and infection
patterns of the sealworm in cod and shorthorn sculpin, we used a generalized additive model
(GAM), with a backward stepwise approach. This method was chosen as it compensates for the
unbalanced sampling design between different seasons and collection areas (Hastie & Tibshirani
1990). The explanatory variables used were year, length of the fish, gear type (net, hook, pot),

salinity and a seal density index. The seal density was derived from a function of the number of 138 hauled out seals in nearby colonies and the distance between sampling areas and haul-outs. The 139 distance was scaled between 1 (0 km) and 0 (100 km) using a cosine function: 140 seal density index= relative haul out size×cosine (distance× $\pi/(\max distance/2))$). 141 Only haul outs closer than a max distance of 100 km to the sampling areas were used as this 142 143 distance was assumed to cover the absolute majority of grey seal movements from a haul out (Sjöberg & Ball 2000). The seal density index was also calculated with the assumption that a 144 single grey seal is twice as important a vector for the transmission of the nematodes as a harbour 145 146 seal where the two species occur in the same area (Brattey 1990). The estimate of haul out size was an average of the maximum number of counted seals during the moult period for the years 147 2006 to 2010. The index was log-transformed before used it in the analyses. 148 First we used a quasi-Poisson distribution with the log transformed abundance, i.e. sealworms 149 per individual fish, used as the nominal response variable. A quasi-Poisson model is more 150 suitable than the classical Poisson distribution when dealing with over-dispersed data (Wood, 151 2006). Secondly we used a zero-inflated negative binomial distribution (ZINB) with the 152 abundance of sealworms per individual fish used as the nominal response variable; this differs 153 154 from the quasi-Poisson model in that all zero values are removed. The models were fitted with the function 'gam' in the 'mgvc' package in R-project (R-project 2011). 155 The full models for cod and shorthorn sculpin were formulated thus: 156 157 Response variable = Year + s(Length) + (Geartype) + s(Salinity) + s(Seal Density Index)where s is an isotropic smoothing function (thin plate regression spline). In order to simplify the 158 interpretation of the results, the maximum degrees of freedom (measured as number of knots, k) 159 160 allowed to the smoothing functions were limited for the variables length, salinity, seal density

index (k=4). Gear code was treated as a factor. Gear type was excluded from the ZINB model for
shorthorn sculpin as the majority of the shorthorn sculpin were caught in nets and there was not
enough replication for the other gear types.

164 **Results**

165 Parasites in fish and seals

166 In total 1,043 cod and 665 shorthorn sculpin were examined for the larval stage of sealworm (Table 1). Two of the cod samples, X1 and X2 (in table 1 and figure 1), were collected offshore 167 and ended up as outliers and were therefore excluded from further analyses. 966 cod were thus 168 169 included in the analyses. All samples were collected along the Swedish coastline, from the southern Baltic up to the southern part of the Bothnian Sea, between 2004 and 2011. A total of 170 2,029 nematodes were found in the musculature and all nematodes examined were sealworms. 171 A total of 2,169 anisakid nematodes were identified in subsamples from 82 grey seal stomachs, 172 however only six samples contained sealworms. Of the 20 sealworms found, two were in life 173 stage L3, two in L4 and 16 in the adult stage. The sealworms were found in seals collected in 174 ICES subdivision 25 and 27 (Table 2). 175

176

177 Spatial distribution and infection patterns

The final models for sealworm abundance in shorthorn sculpin and cod are presented in Tables 3 and 4. All the effects attained in the final models are highly significant. For the quasi-Poisson models for both shorthorn sculpin and cod, the variables year, length, salinity and seal density index all had a significant effect on the predicted abundance. For shorthorn sculpin the final model explained 76.9 % and for cod 35.5 % of the abundance. In the zero inflated negative binomial models, the variables length, salinity and seal density index had a significant effect on 184 the predicted abundance for both species. For shorthorn sculpin the final model explained 71.4 % and for cod 27.1 % of the abundance. Because shorthorn sculpin is a more sedentary species than 185 cod, it is more relevant to show the effects of the significant univariate predictors included in the 186 final version of the quasi Poisson model, where zero values were included (Figure 2). For cod we 187 present the predictors for the zero inflated negative binomial distribution, excluding the zero 188 189 values (Figure 3). For both species there is a trend of more nematodes with longer fish length, with higher salinity and (for the southern part of the Baltic only) with higher seal density index. 190 An obvious trend further north with decreasing salinity is lower infection rate despite high seal 191 192 density index. In the south the pattern is more complex but the infection rates increase with higher seal density. Analysis of the residuals did not reveal any major departure from the main 193 model assumptions of normality and homogeneity of variance. 194

195

196 Discussion

This investigation of anisakid nematodes in grey seals in the Baltic Sea confirms that the species 197 is a final host for sealworms in the area. This picture is confirmed by a Polish study of 9 seals 198 stranded and by-caught in Poland. The most common species was C. osculateum, 59.3% of all 199 200 specimens, sealworm contributed with 31% while the number of A. simplex was less than 1% (Skrzypczak et al., 2014). In grey seals from Finnish waters where all identified nematodes were 201 Contracaecum osculatum (Valtonen et al. 1988). C. osculatum was the dominant species in this 202 203 study as well and constituted 97 % of all identified nematodes. Anisakis simplex was found in one seal from the southern Baltic. Sealworms were not found at all in grey seals from the 204 northern parts of the study area but in subdivision 27 they were found in 14% of the samples (in 205 206 37 seals).

For the two fish species investigated, the most important factors for the abundance of the 207 nematodes were salinity, seal density and fish length. The models for sculpin explained more of 208 the variation of abundance of nematodes than the models for cod. A reason for this may be that 209 sculpins are more sedentary (sculpins lack a swimbladder), whereas cod can and do migrate long 210 distances (Otterlind 1985). In any given sampling area the cod caught may be from genetically 211 212 different stocks with different migration patterns (Ovegård et al. 2012). This means that different individuals found in one area may have been subjected to different infection rates. Some 213 individuals may have spent most of their lives in areas with little or no infection risk. Fish that 214 215 are more sedentary near a coastline with a higher seal density have a higher likelihood of infection (Haukson 2002; 2011). An example of this is the two samples, X1 and X2, which were 216 217 caught with a trawl far from the mainland (30 km and 70 km respectively) and which showed a low infection rate (Table 1). 218

The most obvious parameter driving the distribution of the sealworms was salinity. At around 219 7‰, in areas north of Öland, there is an apparent decrease in the abundance of nematodes. There 220 are many variables both biotic and physiological variables that correlate with the salinity 221 gradient in the Baltic Sea; hence salinity itself may not be the main reason for the limit in 222 223 distribution. It seems reasonable to look for a biological factor, such as the distribution of the parasites' intermediate hosts, whose distribution in turn is linked to salinity. The number of 224 invertebrate species declines in the Baltic with declining salinity (Bonnsdorf 2006) and it could 225 226 be lack of suitable hosts with lower salinity which is important. The sealworm uses both benthic meiofauna hosts and epibenthic copepods in the first larval stage. Later it uses macro-227 invertebrate hosts, such as amphipods, mysids etc. for the second larval stage (McClelland 228

2002). Different macro invertebrates have different importance in transmitting the sealworm to
fish, e.g. mysids have been shown to be more important than amphipods (Margoglise 2001).
No sealworms were found in cod north of latitude 59°, but one was found in a sculpin from the
Bothnian Sea, in subdivision 30. This means that the parasite occurs in the area but it is rare. This
observation was verified by local fishermen who on rare occasions have noticed the sealworm in
cod fillets. The higher latitudes showed the lowest abundance of sealworm despite the highest
seal density index (Table 2).

In the Baltic there is an increasing conflict between seals and the inshore fishery using nets, long 236 237 lines and traps. Damage to catch and gear caused by seals in Sweden was estimated to cost the industry more than five million euro in 2004 and the majority of this was caused in the Baltic 238 (Westerberg et al. 2006). The economic impact from the sealworm problem was not included in 239 this figure. Contacts within the fishing industry are unable to say what amount of landed cod is 240 destroyed because of sealworm contamination or sold for a lower price due to extra labour costs 241 during filleting. We do know that the problem affects mainly the inshore fishery, a sector of the 242 fishing industry that already has a problem with seals, as they catch the highest amount of 243 infected fish (Westerberg et al. 2006). 244

Studies of seal diets in the Baltic Sea show that there has been a change from pelagic fish
species, especially herring and sprat, to a more benthic diet, with cod becoming an important
prey in recent years (Lundström et al. 2010). This change could influence the prevalence of the
sealworm as it increases the probability of an infected cod being predated. It is anticipated that
parasite prevalence will be given more attention in the future and that further studies are needed.
The seal population will probably continue to increase and as a consequence cause higher
sealworm infection rates in commercially valuable fish.

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260	Experiments.
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400	Legends to Figures
401	Fig. 1. Map with positions where cod and shorthorn sculpin were collected. Large bold figures
402	indicate ICES area. The details for each position are found in table 2.
403	
404	Fig. 2. Each plot represents the effects of the predictors on the abundance of sealworm in
405	shorthorn sculpin estimated in the final Quasi Poisson model, where zero values were included
406	(see table 3). The ranges of the variables are represented on the x-axis and the probability (logic
407	scale) of the abundance of sealworm is represented on the y-axis. The dotted lines represent the

408 95 % confidence intervals around the response curve.

410 Fig. 3. Each plot represents the effects of the predictors on the abundance of Sealworm in cod 411 estimated in the final Zero Inflated Negative Binominal distribution model, excluding the zero 412 values. (see Table 4). The ranges of the variables are represented on the x-axis and the 413 probability (logic scale) of the abundance of sealworm is represented on the y-axis. The dotted 414 lines represent the 95 % confidence intervals around the response curve.

416 Tables with their legends

Table 1. Sampling areas with positions and collection details. No. fish is the number of fish
examined. Prevalence is the proportion of infected fish, abundance is the mean number of
sealworms found in all fish in a sample. Salinity in the sampling area is based on data from
HELCOM. Seal density index is derived from a cosine function of the number of hauled out
seals in nearby colonies and the distance to the colony.

Fish	Ν	Subdivisio	No.	Prevalenc	Abundanc		Salinit	Seal density
species	0	n	fish	e	e	SD	у	index
						0.2		
Shorthorn	1	30	18	0.06	0.06	4	5.2	6.8
sculpin	2	29	10	0	0	0	6.4	6.8
	3	29	56	0	0	0	5.7	7.1
						0.1		
	4	27	52	0.02	0.02	4	6.4	7.8
						0.6		
	5	27	59	0.25	0.34	6	6.7	7.7
	6	28	95	0	0	0	7.7	5.8
	7	27	50	0.38	0.72	1.2	7.1	5.6
	_					1.0		
	8	27	50	0.36	0.62	5	7.4	5.3
						0.5		
	9	27	129	0.22	0.43	2	7.3	5.5
	10	27	24	0.10	0.10	6.6		- -
	10	27	34	0.12	0.18	5	7.5	5.6
	11	25	40	0.70	<i>с</i> 1	6.4 7	75	<i>C</i> A
	11	25	48	0.79	6.4	/	1.5	6.4
	10	25	10	0.92	4.25	9.5	7.0	C
	12	25	12	0.85	4.25	3 12	7.9	0
	12	24	52	1	14 17	1.5	86	6
Cod	2	24	106	1	14.17	0	6.0	7.1
Cou	5	29	100	0	0	01	0.5	/.1
	4	27	71	0.03	0.03	0.1 7	61	78
	4	27	/1	0.05	0.05	07	0.4	7.0
	7	27	50	0.34	0.5	6	7	5.6
	,	<i>~ 1</i>	50	0.34	0.5	03	/	5.0
	14	28	56	0.05	0.07	2	74	49
	1 1	20	20	0.05	0.07	0^{2}	/• 1	
	9	27	52	0.08	0.08	7	7.2	5.5

					0.4		
15	27	60	0.12	0.15	8	7.2	5.6
					2.0		
16	25	123	0.46	1.23	3	7.5	6
17	25	57	0.14	0.81	4.4	7.6	6.1
11	25	157	0.5	1.44	2.5	7.5	6.4
					1.4		
18	25	85	0.31	0.65	5	7.7	5.3
					0.4		
19	25	50	0.18	0.2	5	7.8	3.7
					3.5		
13	24	50	0.74	3.08	2	8.6	6
					2.3		
20	24	49	0.57	1.49	6	8.3	5.4
Х					2.0		
1	24	47	0.17	0.21	4	10.2	5.7
Х					0.4		
2	24	30	0.2	0.43	6	9.6	2.8

Table 2. The number of grey seal stomachs examined in each ICES subdivision. Number of seals

424 with the sealworm present and the number of nematodes for each species identified.

	No			Number			
ICES	seals	N	o seals with	nematodes			
					С.		Unidentifi
			Sealworm	Sealworm	osculatum	A. simple	exed
23		1	0	0	0	51	0
24		1	0	0	8	0	0
25		1	1	8	6	0	0
27		37	5	12	1 078	0	15
28		2	0	0	38	0	0
29		8	0	0	132	0	1

⁴²²

30	28	0	0	844	0	10
Total	82	6	20	2 098	51	26

Table 3. Alternative quasi Poisson models fitted for estimating the influence of predictive factors
on the abundance of sealworm in shorthorn sculpin and cod. The initial model included year,
length of the fish, gear type (net/ hook/ pot), salinity and a seal population density index. DEV is
the total deviance explained by the models. GCV is the generalized cross validation value (i.e.

430 lower GCV indicated more parsimonious models).

Species	Model	Covariates	DEV	GCV
Sculpin	QP1	year, length, gear type, salinity, seal density index	76.9	1.6444
Sculpin	QP2	year, length, salinity, seal density index	76.9	1.6431
Cod	QP1	year, length, gear type, salinity, seal density index	38.9	1.6213
Cod	QP2	year, length, salinity, seal density index	35.5	1.6993

431

Table 4. Alternative zero inflated negative binomial distribution models fitted for estimating the influence of predictive factors on the abundance of sealworm in shorthorn sculpin and cod. The initial model included year, length of the fish, gear type (net/ hook/ pot (excluded for sculpin)), salinity and a seal density index (the potential fish density pressure of seals, see explanation in text). DEV is the total deviance explained by the models. UBRE is the generalized cross validation value (i.e. lower UBRE indicated more parsimonious models).

			DE	
Species	Model	Covariates	V	UBRE
Sculpin	ZINB1	year, length, salinity, seal density index	71.5	-0.62071
Sculpin	ZINB2	length, salinity, seal density index	71.4	-0.63003
Cod	ZINB1	year, length, gear type, salinity, seal density index	29.8	-0.59062
Cod	ZINB2	length, gear type, salinity, seal density index	29.8	-0.59782
Cod	ZINB3	length, salinity, seal density index	27.1	-0.60044





Seal Density Index





Seal Density Index