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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

3

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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
16 industry and can cause abdominal discomfort if consumed by humans. There are only scattered
17 studies on the abundance or distribution of the sealworm in fish and seals in the Baltic Sea. To
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*)
21 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
25 ‰. Even though the northern Baltic proper and the southern Bothnian Sea have a high number of
26 grey seals, only one sealworm was found in a sculpin in that region, and none in cod. In grey seal
27 stomachs the sealworm was only found in samples from the central Baltic proper; further north
28 all anisakid nematodes identified in seals were *Contracaecum osculatum*. The results indicate
29 that seal presence drives the distribution in the southern parts of the Baltic and that low salinity,
30 or some other variable which correlates with salinity, limits the distribution in the northern part.
31 Keywords: Baltic Sea, cod, grey seal, *Pseudoterranova*, sealworm, sculpin.

32

33 **Introduction**

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

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

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

61 Along the Swedish west coast the sealworm is common in both harbour seals (Lunneryd 1991)
62 and in fish (Lunneryd et al. 2001) but in the Baltic Sea there have only been a few reports of
63 sealworms so far. The first major study of the occurrence of anisakid nematodes in Baltic cod,
64 conducted in 1976, showed no records of the sealworm. However, at that time there were very
65 few seals in the southern Baltic, so it was not surprising that there were no sealworms (Grabda
66 1976). Later studies (Myjak et al. 1994; Szostakowska et al. 2005; Buchmann and Kania 2012)
67 revealed that the sealworm was present in cod caught in ICES subdivision 25 and 26 southern
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
70 (1989) did a study of parasites and fish diseases in Swedish waters and found sealworms only in
71 cod caught off the Swedish west coast and not at all in the Baltic Sea, despite extensive
72 sampling. (Buchmann and Kania, 2012) compared the sealworm infection levels in cod collected
73 in 1982-83 from the Bornholm Basin with that in cod collected in 2011, and noted that no
74 nematodes were found during 1982-83 while at least 2% of the samples surveyed in 2011 were
75 infected with 1 to 4 sealworms. The presence of the nematodes in Baltic cod in recent times has
76 been well known among fishermen (several personal communications) but has not been public
77 knowledge in e.g. Sweden until very recently. It is therefore important to collect knowledge
78 about the biology and distribution of this parasite. Factors discussed as determinants of the
79 distribution of sealworms are seal distribution, intermediate host distribution and environmental
80 factors such as temperature and salinity (Measures 1996; Marcogliese 2001a; Hauksson 2011).
81 The spatial distribution in the Baltic Sea is of special interest because of its uniqueness in being a
82 brackish sea. Salinity has been shown to limit the survival of the sealworm larvae (Measures,
83 1996). As regards the survival and hatching rates of sealworm eggs in different salinity
84 environments, studies have shown no difference between sea water (35 ‰ salinity), brackish
85 water (17 ‰ salinity) and fresh water, but the larvae were much more sensitive: those hatched in
86 water with higher salinity survived about 10 times as long as those hatched in freshwater
87 (Measures 1996). In the Swedish coastal areas of the Baltic Sea there is a gradient of salinity
88 from around 9‰ in the southern Baltic proper to about 5‰ in the southern Bothnian Sea. In this
89 lower range of salinity in the Baltic Sea, it is not known how well the sealworms survive.
90 The aim of this study was to examine the relative presence and distribution of sealworms in the
91 middle Baltic Sea. The presence of the sealworm was investigated in cod, a commercially

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

98

99 **Materials and Methods**

100 Fish and parasite sampling

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

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

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

122

123 Seals and parasite sampling

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

131

132 Models for spatial distribution and infection presence of *Pseudoterranova sp.* in fish

133 To understand the influence of different variables on the spatial distribution and infection
134 patterns of the sealworm in cod and shorthorn sculpin, we used a generalized additive model
135 (GAM), with a backward stepwise approach. This method was chosen as it compensates for the
136 unbalanced sampling design between different seasons and collection areas (Hastie & Tibshirani
137 1990). The explanatory variables used were year, length of the fish, gear type (net, hook, pot),

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

161 index ($k=4$). Gear code was treated as a factor. Gear type was excluded from the ZINB model for
162 shorthorn sculpin as the majority of the shorthorn sculpin were caught in nets and there was not
163 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
167 (Table 1). Two of the cod samples, X1 and X2 (in table 1 and figure 1), were collected offshore
168 and ended up as outliers and were therefore excluded from further analyses. 966 cod were thus
169 included in the analyses. All samples were collected along the Swedish coastline, from the
170 southern Baltic up to the southern part of the Bothnian Sea, between 2004 and 2011. A total of
171 2,029 nematodes were found in the musculature and all nematodes examined were sealworms.
172 A total of 2,169 anisakid nematodes were identified in subsamples from 82 grey seal stomachs,
173 however only six samples contained sealworms. Of the 20 sealworms found, two were in life
174 stage L3, two in L4 and 16 in the adult stage. The sealworms were found in seals collected in
175 ICES subdivision 25 and 27 (Table 2).

176

177 Spatial distribution and infection patterns

178 The final models for sealworm abundance in shorthorn sculpin and cod are presented in Tables 3
179 and 4. All the effects attained in the final models are highly significant. For the quasi-Poisson
180 models for both shorthorn sculpin and cod, the variables year, length, salinity and seal density
181 index all had a significant effect on the predicted abundance. For shorthorn sculpin the final
182 model explained 76.9 % and for cod 35.5 % of the abundance. In the zero inflated negative
183 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 %
185 and for cod 27.1 % of the abundance. Because shorthorn sculpin is a more sedentary species than
186 cod, it is more relevant to show the effects of the significant univariate predictors included in the
187 final version of the quasi Poisson model, where zero values were included (Figure 2). For cod we
188 present the predictors for the zero inflated negative binomial distribution, excluding the zero
189 values (Figure 3). For both species there is a trend of more nematodes with longer fish length,
190 with higher salinity and (for the southern part of the Baltic only) with higher seal density index.
191 An obvious trend further north with decreasing salinity is lower infection rate despite high seal
192 density index. In the south the pattern is more complex but the infection rates increase with
193 higher seal density. Analysis of the residuals did not reveal any major departure from the main
194 model assumptions of normality and homogeneity of variance.

195

196 **Discussion**

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

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

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

229 2002). Different macro invertebrates have different importance in transmitting the sealworm to
230 fish, e.g. mysids have been shown to be more important than amphipods (Margolise 2001).

231 No sealworms were found in cod north of latitude 59°, but one was found in a sculpin from the
232 Bothnian Sea, in subdivision 30. This means that the parasite occurs in the area but it is rare. This
233 observation was verified by local fishermen who on rare occasions have noticed the sealworm in
234 cod fillets. The higher latitudes showed the lowest abundance of sealworm despite the highest
235 seal density index (Table 2).

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

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

250 The seal population will probably continue to increase and as a consequence cause higher
251 sealworm infection rates in commercially valuable fish.

252

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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.

409

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.

415

416 Tables with their legends

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

Fish species	N	Subdivision	No. fish	Prevalence	Abundance	SD	Salinity	Seal density index
Shorthorn sculpin	1	30	18	0.06	0.06	4	5.2	6.8
	2	29	10	0	0	0	6.4	6.8
	3	29	56	0	0	0	5.7	7.1
	4	27	52	0.02	0.02	4	6.4	7.8
	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
	8	27	50	0.36	0.62	5	7.4	5.3
	9	27	129	0.22	0.43	2	7.3	5.5
	10	27	34	0.12	0.18	5	7.5	5.6
	11	25	48	0.79	6.4	7	7.5	6.4
	12	25	12	0.83	4.25	3	7.9	6
	13	24	52	1	14.17	6	8.6	6
Cod	3	29	106	0	0	0	6.3	7.1
	4	27	71	0.03	0.03	7	6.4	7.8
	7	27	50	0.34	0.5	6	7	5.6
	14	28	56	0.05	0.07	2	7.4	4.9
	9	27	52	0.08	0.08	7	7.2	5.5

15	27	60	0.12	0.15	0.4	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	
X					2.0			
1	24	47	0.17	0.21	4	10.2	5.7	
X					0.4			
2	24	30	0.2	0.43	6	9.6	2.8	

422

423 **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.

ICES	No		Number				
	seals	No seals with	nematodes				
		Sealworm	C.		Unidenti		
			Sealworm	Sealworm <i>osculatum</i>	<i>A. simplex</i>		
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	

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

425

426 **Table 3.** Alternative quasi Poisson models fitted for estimating the influence of predictive factors
427 on the abundance of sealworm in shorthorn sculpin and cod. The initial model included year,
428 length of the fish, gear type (net/ hook/ pot), salinity and a seal population density index. DEV is
429 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

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

Species	Model	Covariates	DE	
			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

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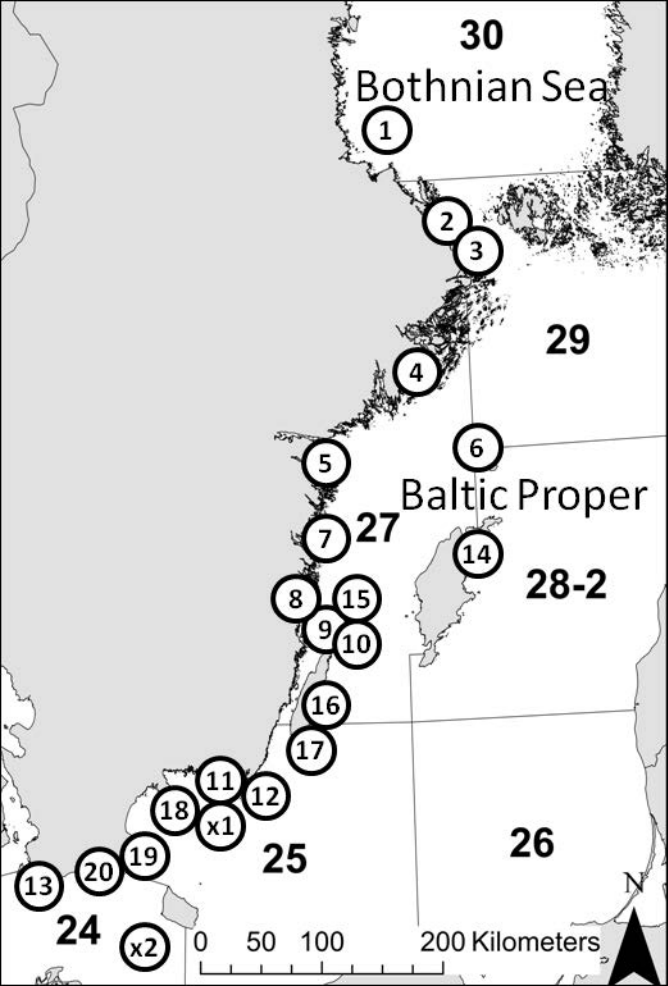
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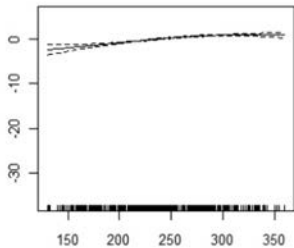
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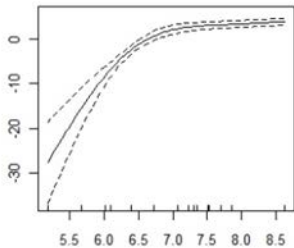


s(Length, 2.44)



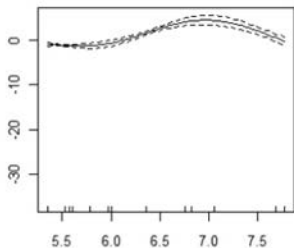
Length

s(Salt, 2.93)



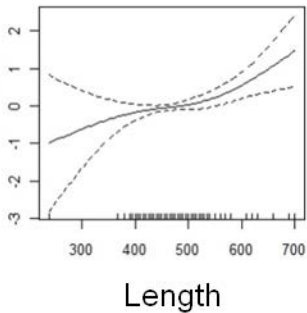
Salinity

s(Seal Index, 2.97)

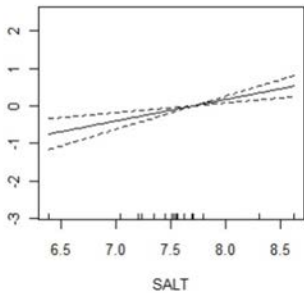


Seal Density Index

s(Length, 2.22)



s(Salt, 1)



s(Seal Index, 1)

