Oligochaetes in variable or reduced salinity infralittoral muddy sediment

MarLIN – Marine Life Information Network
Marine Evidence–based Sensitivity Assessment (MarESA) Review

Dr Heidi Tillin

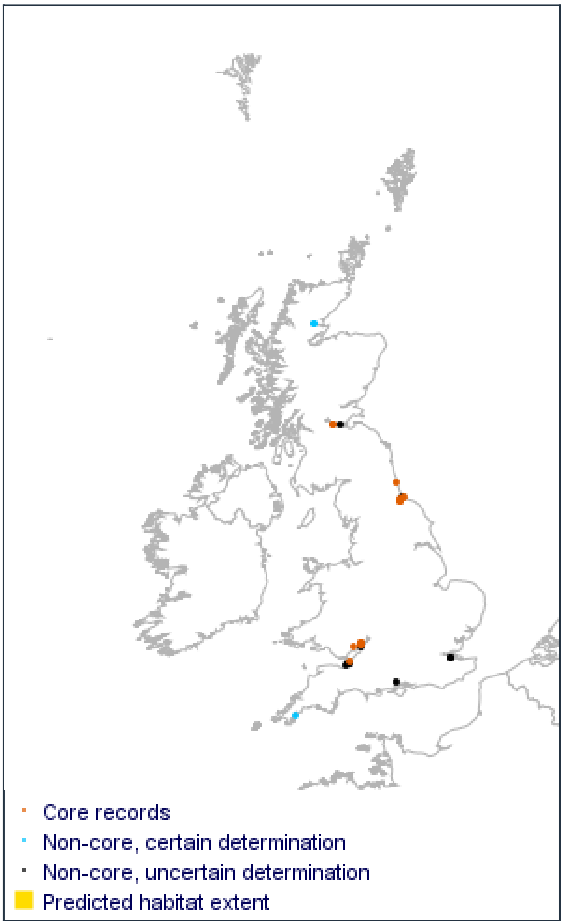
2016-06-01

A report from:
The Marine Life Information Network, Marine Biological Association of the United Kingdom.

Please note. This MarESA report is a dated version of the online review. Please refer to the website for the most up-to-date version [https://www.marlin.ac.uk/habitats/detail/115]. All terms and the MarESA methodology are outlined on the website (https://www.marlin.ac.uk)

This review can be cited as:
Tillin, H.M. 2016. Oligochaetes in variable or reduced salinity infralittoral muddy sediment. In Tyler-Walters H. and Hiscock K. (eds) *Marine Life Information Network: Biology and Sensitivity Key Information Reviews*, [on-line]. Plymouth: Marine Biological Association of the United Kingdom.
DOI https://dx.doi.org/10.17031/marlinhab.115.1

(page left blank)



17-09-2018
Biotope distribution data provided by EMODnet Seabed Habitats (www.emodnet-seabedhabitats.eu)

Researched by Dr Heidi Tillin **Refereed by** Admin

Summary

UK and Ireland classification

EUNIS 2008	A5.326	Oligochaetes in variable or reduced salinity infralittoral muddy sediment
JNCC 2015	SS.SMu.SMuVS.OIVS	Oligochaetes in variable or reduced salinity infralittoral muddy sediment
JNCC 2004	SS.SMu.SMuVS.OIVS	Oligochaetes in variable or reduced salinity infralittoral muddy sediment
1997 Biotope	SS.IMU.EstMu.Tub	*Tubificoides spp.* in reduced salinity infralittoral muddy sediment

Description

Reduced salinity muddy sediments characterized by oligochaetes, particularly of the genus *Tubificoides*. The abundance of the oligochaetes may vary by several orders of magnitude but very few other species will be present. This biotope is found towards the edges of tidal channels in estuaries where current velocities allow deposition of silt and the establishment of an infaunal community. Organic loading and poor water-exchange within the sediment lead to anoxic conditions which may explain the low species richness within this biotope. This biotope is found towards the edges of tidal channels in estuaries where current velocities allow deposition of silt and the establishment of an infaunal community. The biotope may occur downstream of SMU.LhofTtub, differentiated by the absence of the freshwater species, and adjacent to more mobile and sandier biotopes in the tidal channels (JNCC, 2015).

Depth range

0-5 m

Additional information

-

Listed By

- none -

Further information sources

Search on:

G G JNCC

Sensitivity review

Sensitivity characteristics of the habitat and relevant characteristic species

The biotope description and characterizing species is taken from (JNCC, 2015). This biotope comprises of a species-poor community of oligochaetes, particularly of the genus *Tubificoides* or from the group Enchytraeidae. The abundance of the oligochaetes may vary by several orders of magnitude but very few other species will be present. The sensitivity assessments are based on Tubificidae (largely based on *Tubificoides benedii* as this species is well studied) and Enchytraeid oligochaetes as most marine species belong to these families (Giere & Pfannkuche, 1982). Although the evidence base for assessing sensitivity of oligochaetes is limited compared to other taxa, it is clear that there are species-specific responses to environmental factors and perturbations (Rodriguez & Reynoldson, 2011) and the lack of species information is a key limitation in assessing sensitivity.

The sedimentary habitat and salinity conditions are key factors structuring this habitat and these factors are considered in the sensitivity assessments where the pressure may alter these.

Resilience and recovery rates of habitat

Usually for oligochaetes fertilization is internal and relatively few large eggs are shed directly into a cocoon that is secreted by the worm (Giere & Pfannkuche, 1982). Asexual reproduction is possible in some species by spontaneous fission (Giere & Pfannkuche, 1982). The naid oligochaete *Panais litoralis* can produce asexually producing clones, the rapid rate of increase (18 times population abundance in 3 months, Gillett *et al*., 2007) allows this species (which is sensitive to high temperatures, hypoxia and is exposed to predation due to shallow burial) to repopulate rapidly when conditions are favourable. However, few Tubificidae and Enchytraeidae produce asexually (Giere & Pfannkuche, 1982).

Tubificid populations tend to be large and to be constant throughout the year, although some studies have noticed seasonal variations (Giere & Pfannkuche, 1982). Many species, including *Tubificoides benedii* and *Baltidrilus costata* have a two-year reproductive cycle and only part of the population reproduces each season (Giere & Pfannkuche, 1982). Populations of *Tubificoides benedii* in the Fourth estuary have not demonstrated clear seasonality in recruitment (Bagheri & McLusky, 1982), although mature *Tubificoides benedii* (as *Peloscolex benedeni*) in the Thames Estuary were reported to occur in December with a maximum in late February (Hunter & Arthur, 1978), breeding worms increased from April and maximum cocoon deposition was observed in July (Hunter & Arthur, 1978). Tubificids exhibit many of the traits of opportunistic species. They often reach huge population densities in coastal areas that are enriched in organic matter and are often described as 'opportunist' species adapted to rapid environmental fluctuations and stress (Giere, 2006; Bagheri & McLusky, 1982). However, unlike other opportunist species they have a long lifespan (a few years, Giere, 2006), a prolonged reproductive period from reaching maturity to maximum cocoon deposition and exhibit internal fertilisation, with brooding rather than pelagic dispersal. These factors mean that recolonization is slower than for some opportunistic species such as *Capitella capitata* and nematodes which may be present in similar habitats.

Bolam and Whomersley (2003) observed faunal recolonization of fine sediments placed on saltmarsh as a beneficial use and disposal of fine grained dredged sediments. They found that tubificid oligochaetes began colonizing sediments from the first week following a beneficial use scheme involving the placement of fine-grained dredged material on a salt marsh in southeast

England. The abundance of *Tubificoides benedii* recovered slowly in the recharge stations and required 18 months to match reference sites and those in the recharge stations prior to placement of sediments. The results indicate that some post-juvenile immigration is possible and that an in-situ recovery of abundance is likely to require more than one year. Rapid recolonization has also been observed in the tubificid oligochaete *Baltidrilus costata (Tubifex costatus)* appeared in upper sediment layer s in experimentally defaunated patches ($4m^2$) after 3 weeks (Gamenick *et al*., 1996).

Resilience assessment. In general there was little information found for *Tubificoides benedii* and other oligochaetes, but, taking into consideration the information above (particularly Bolam & Whomersley, 2003) , this review considers that the recoverability of this species is generally 'High', so that recovery from defaunation is suggested to occur within two years and that therefore, recovery from any impact (resistance is 'None', 'Low' or 'Medium') is assessed as 'High'.

NB: The resilience and the ability to recover from human induced pressures is a combination of the environmental conditions of the site, the frequency (repeated disturbances versus a one-off event) and the intensity of the disturbance. Recovery of impacted populations will always be mediated by stochastic events and processes acting over different scales including, but not limited to, local habitat conditions, further impacts and processes such as larval-supply and recruitment between populations. Full recovery is defined as the return to the state of the habitat that existed prior to impact. This does not necessarily mean that every component species has returned to its prior condition, abundance or extent but that the relevant functional components are present and the habitat is structurally and functionally recognizable as the initial habitat of interest. It should be noted that the recovery rates are only indicative of the recovery potential.

Hydrological Pressures

	Resistance	Resilience	Sensitivity
Temperature increase (local)	High Q: High A: High C: High	High Q: High A: High C: High	Not sensitive Q: High A: High C: High

Deeper burrowing oligochaetes are protected from fluctuations in temperature by the overlaying sediments which dampen changes if poorly drained (Giere & Pfannkuche, 1982). Bamber & Spencer (1984) observed that *Tubificoides* were dominant species in an area affected by thermal discharge in the River Medway estuary. Sediments were exposed to the passage of a temperature front of approximately 10°C between heated effluent and estuarine waters during the tidal cycles.

Increased temperature was found to trigger the onset of reproduction in *Baltidrilus costata* (studied as *Tubifex costatus*) in the Thames (Birtwell & Arthur, 1980). This effect was non-lethal and may be beneficial to populations.

Sensitivity assessment. The dominance of *Tubificoides* spp. when exposed to a heated effluent suggests that this genus would be highly resistant to an increase in temperature at the pressure benchmark. Biotope resistance is therefore assessed as 'High' and resilience as 'High' (by default), so that the biotope is considered to be 'Not sensitive'.

Temperature decrease (local)	High Q: High A: High C: High	High Q: High A: High C: High	Not sensitive Q: High A: High C: High

Most littoral oligochaetes, including tubificids and enchytraeids, can survive freezing temperatures and can survive in frozen sediments (Giere & Pfannkuche, 1982). *Tubificoides benedii* (studied as *Peloscolex benedeni*) recovered after being frozen for several tides in a mudflat (Linke, 1939).

Sensitivity assessment. Based on freezing tolerances of *Tubificoides benedii* (Linke, 1939) biotope resistance is assessed as 'High' to decreases in temperature. Resilience is assessed as 'High' (by default) and the biotope is therefore considered to be 'Not sensitive'.

Salinity increase (local)	High	High	Not sensitive
	Q: High A: High C: High	Q: High A: High C: High	Q: High A: High C: High

This biotope is present in reduced (18-30 ppt) and variable (18-35 ppt) salinity habitats (JNCC, 2015), a change at the pressure benchmark therefore represents a change from reduced or variable to full salinity (30-35 ppt). Oligochaete dominated biotopes are recorded from a range of salinity regimes from full (LS.LSa.MoSa.Ol; LS.LSa.MoSa.Ol.FS), variable (SS.SMu.SMuVS.CapTubi) to low (SS.SMu.SMuVS.LhofTtub) habitats (JNCC,2015). The species characterizing these biotopes are likely to vary. Giere & Pfannkuche (1982) identified how species change over a hypothetical salinity gradient with marine stenohaline species present at full salinities replaced by more euryhaline oligochaete species including *Tubificoides benedii* and *Tubificoides pseudogaster*, *Paranais litoralis* and *Baltidrilus costata* (formerly *Heterochaeta costata*). Studies in the Rhine delta have found that *Tubificoides benedii*, is more tolerant of a range of salinities than *Baltidrilus costata* (as *Heterochaeta costata*) which preferred shallow water brackish stations (Verdonschot *et al.* 1982). However, numerous studies suggest that *Baltidrilus costata* tolerates a wide range of salinities from 1‰ to 28‰ (Giere & Pfannkuche, 1982 and references therein), suggesting that while tolerant of some changes, an increase to full salinity may lead to reductions in abundance of this species.

Sensitivity assessment. Based on the distribution of oligochaetes (including characterizing species) in full, variable and reduced salinity, the biological assemblage associated with the biotope is considered to have 'High' resistance and 'High' resilience and is therefore considered to be 'Not sensitive'.

Salinity decrease (local)	Low	High	Low
	Q: High A: High C: High	Q: High A: Low C: High	Q: High A: Low C: High

This biotope is present in reduced (18-30 ppt) and variable (18-35 ppt) salinity habitats (JNCC, 2015); a change at the pressure benchmark therefore represents a change from reduced to low (<18 ppt) salinity. Oligochaete dominated biotopes are recorded from a range of salinity regimes from full (LS.LSa.MoSa.Ol; LS.LSa.MoSa.Ol.FS), variable (SS.SMu.SMuVS.CapTubi) reduced (SS.SMu.SMuVS.CapTubi; LS.LMu.UEst.Tben) and low (SS.SMu.SMuVS.LhofTtub) habitats (JNCC,2015). In very low salinities from <15 to 0 ‰ species such as *Limnodrilus* spp. and *Tubifex tubifex* are found (Giere & Pfannkuche, 1982). It is therefore considered that a decrease in salinity at the pressure benchmark would result in replacement by oligochaete species more tolerant of lower salinities such as *Limnodrilus hoffmeisteri* and *Tubifex tubifex* that characterize the low salinity biotope SS.SMu.SMuVS.LhofTtub. This would result in the loss of the characterizing biotope. Numerous studies suggest that *Baltidrilus costata* tolerates a wide range of salinities from 1‰ to 28‰ (Giere & Pfannkuche, 1982 and references therein), suggesting that this species is likely to still be present in the biotope.

Sensitivity assessment. A reduction in salinity at the pressure benchmark may lead to species replacement and biotope reclassification to SS.SMu.SMuVS.LhofTtub. Biotope resistance is therefore assessed as 'Low' and resilience as 'High' (following a return to usual habitat conditions), so that biotope sensitivity is assessed as 'Low'.

Water flow (tidal current) changes (local)	Medium Q: High A: Medium C: Medium	High Q: High A: Low C: High	Low Q: High A: Low C: Medium

This biotope is found in areas where tidal streams are estimated to range from moderately strong (0.5-1.5 m/s) to weak (<0.5 m/s), (JNCC, 2015). Increases and decreases in water velocity may lead to increased erosion or deposition. The associated pressures alteration to sediment type and siltation are assessed separately. Experimental increases in near-bed current velocity were achieved over intertidal sandflats by placing flumes on the sediment to accelerate water flows (Zuhlke & Reise, 1994). The increased flow led to the erosion of up to 4cm depth of surface sediments. No significant effect was observed on the abundance of *Capitella capitata* and numbers of *Tubificoides benedii* and *Tubificoides pseudogaster* were unaffected, as they probably avoided suspension by burrowing deeper into sediments. This was demonstrated by the decreased abundance of oligochaetes in the 0-1cm depth layer and increased abundance of oligochaetes deeper in sediments (Zuhlke & Reise, 1994). A single storm event had a similar result with decreased abundance of oligochaetes in surficial layers, coupled with an increase in deeper sediments (Zuhlke & Reise, 1994). Although *Tubificoides* spp. can resist short-term disturbances their absence from sediments exposed to higher levels of disturbance indicate that they would be sensitive to longer-term changes in sediment mobility (Zuhlke & Reise, 1994). Birtwell and Arthur (1980) reported seasonal changes in abundance in *Baltidrilus costata* (as *Tubifex costatus*) which they attributed to erosion of the upper sediment layers caused by high river flows and wave action.

In the turbid waters of estuaries, where many mud habitats develop, a reduction in water flow is likely to result in a significant increase in siltation increasing the silt and clay content of the substratum. Decreases in water flow with increased siltation of fine particles are considered unlikely to alter the physical character of this habitat type as it is already found in sheltered areas where siltation occurs and where particles are predominantly fine. Reductions in waterflow occurring through the presence of trestles (for off-bottom oyster cultivation) arranged in parallel rows in the intertidal area (Goulletquer & Héral, 1997) reducing the strength of tidal currents (Nugues *et al*., 1996) has been observed to limit the dispersal of pseudofaeces and faeces in the water column and thus increase the natural sedimentation process by several orders of magnitude (Ottman & Sornin, 1985, summarised in Bouchet & Sauriau, 2008). As the characterizing oligochaetes can live relatively deeply buried and in depositional environments with low water flows (based on habitat preferences) and low oxygenation they are considered to be not sensitive to decreases in water flow.

Sensitivity assessment. Where increased or decreased water flows alter the sediment type this could lead to sediment reclassification, this change is assessed in the sedimentary change assessment. As muds tend to be cohesive and the surface tends to be smooth reducing turbulent flow, an increase at the pressure benchmark may not lead to increased erosion. Biotope resistance is assessed as 'Medium' based on the tidal stream range (JNCC, 2015). Resilience is assessed as 'High' (following restoration of usual conditions) and sensitivity is assessed as 'Low'. The biotope is not considered to be sensitive to decreased flows due to its presence in sheltered habitats and the tolerance of *Tubificoides benedii* for low oxygen and sediment deposition.

Emergence regime changes	Not relevant (NR) Q: NR A: NR C: NR	Not relevant (NR) Q: NR A: NR C: NR	Not relevant (NR) Q: NR A: NR C: NR

Not relevant to subtidal habitats.

Wave exposure changes (local)	High Q: High A: Medium C: High	High Q: High A: High C: High	Not sensitive Q: High A: Medium C: High

This biotope occurs in habitats that are sheltered from strong wave action. Disturbance of sediment by waves may reduce oligochaete abundance (Giere, 1977) and oligochaetes may be absent from very wave exposed shores (Giere & Pfannkuche, 1982 and references therein). As this biotope occurs across two wave exposure categories; extremely sheltered and very sheltered, JNCC (2015), this is considered to indicate that mid-range biotopes would tolerate both an increase or decrease in wave exposure at the pressure benchmark. Resistance is therefore assessed as 'High' and resilience as 'High' by default and the biotope is considered to be 'Not sensitive'. An increase in wave exposure at the pressure benchmark would be likely to re-suspend sediments and increase erosion altering sediment type. Some oligochaete dominated biotopes occur in areas with mobile sediments and it is possible the biotope would revert to one of these.

Chemical Pressures

	Resistance	Resilience	Sensitivity
Transition elements & organo-metal contamination	Not Assessed (NA) Q: NR A: NR C: NR	Not assessed (NA) Q: NR A: NR C: NR	Not assessed (NA) Q: NR A: NR C: NR

This pressure is **Not assessed** but evidence is presented where available.

Contamination at levels exceeding the pressure benchmark may have negative effects. A 2-year microcosm experiment was undertaken to investigate the impact of copper on the benthic fauna of the lower Tyne Estuary (UK) by Hall & Frid (1995). During a 1-year simulated contamination period, 1 mg l1copper was supplied at 2-weekly 30% water changes, at the end of which the sediment concentrations of copper in contaminated microcosms reached 411 g g1. Toxicity effects reduced populations of the four dominant taxa including *Tubificoides* spp.). When copper dosage was ceased and clean water supplied, sediment copper concentrations fell by 50% in less than 4 days, but faunal recovery took up to 1 year, with the pattern varying between taxa. Since the copper leach rate was so rapid it is concluded that after remediation, contaminated sediments show rapid improvements in chemical concentrations, but faunal recovery may be delayed taking up to a year.

Rygg (1985) classified *Tubificoides* spp as highly tolerant species, common at the most copper polluted stations (>200 mg Kg-1) in Norwegian fjords.

Hydrocarbon & PAH contamination	Not Assessed (NA) Q: NR A: NR C: NR	Not assessed (NA) Q: NR A: NR C: NR	Not assessed (NA) Q: NR A: NR C: NR

This pressure is **Not assessed** but evidence is presented where available.

In Finland in oligohaline inland waters near an oil refinery, *Baltidrilus costata* (as *Tubifex costatus*) appeared to be sensitive to oil pollution and had completely disappeared from sediments exposed to pollution and did not recolonize during a four year post pollution period (Leppäkoski & Lindström, 1978). *Tubificoides benedii* appears to be more tolerant and was found in UK waters near oil refineries as the sole surviving member of the macrofauna. Populations were however apparently reduced and the worms were absent from areas of oil discharge and other studies indicate sensitivity to oiling (Giere & Pfannkuche, 1982, references therein).

Synthetic compound contamination	Not Assessed (NA) Q: NR A: NR C: NR	Not assessed (NA) Q: NR A: NR C: NR	Not assessed (NA) Q: NR A: NR C: NR

This pressure is **Not assessed** but evidence is presented where available.

Radionuclide contamination	No evidence (NEv) Q: NR A: NR C: NR	No evidence (NEv) Q: NR A: NR C: NR	No evidence (NEv) Q: NR A: NR C: NR

No evidence was found for radionuclide uptake by marine oligochaetes.

Introduction of other substances	Not Assessed (NA) Q: NR A: NR C: NR	Not assessed (NA) Q: NR A: NR C: NR	Not assessed (NA) Q: NR A: NR C: NR

This pressure is **Not assessed**.

De-oxygenation	High Q: High A: High C: High	High Q: High A: High C: High	Not sensitive Q: High A: High C: High

Oligochaete species vary in their tolerance of hypoxia and associated high sulphide levels. Most enchytraaids and naidids are sensitive to hydrogen sulphide and hypoxia while tubificids are often more resistant (Giere, 2006).

Tubificoides benedii has a high capacity to tolerate anoxic conditions, its extreme oxygen tolerance is based on an unusually low respiration rate (Giere *et al*., 1999). Respiration rates of *Tubificoides benedii* measured at various oxygen concentrations showed that aerobic respiration is maintained even at very low oxygen concentrations (Giere *et al*., 1999). Birtwell & Arthur (1980) showed that *Tubificoides benedii* could tolerate anoxia in the Thames Estuary (LT_{50} = 58.8 hours at 20°C, 26.6 hours at 25°C and 17.8 hours at 30°C in experiments with worms acclimated to 20°C.)

Tolerance experiments by Gamenick *et al*. (1996) found that *Baltidrilus costata* (as *Heterochaeta costata*) was not affected by hypoxic conditions for at least 3 days but the addition of sulphide 91.96 mmol/litre) caused mortality after 1 day (Gamenick *et al*., 1996)

Sensitivity assessments. As this biotope is found in the tidal oxygen levels will be recharged during the tidal cycle lowering exposure to this pressure for worms that migrate to surface layers. Based on the reported tolerances for anoxia, biotope resistance is assessed as 'High' based on *Tubifioides benedii*, resilience is assessed as 'High' (by default) and the biotope is considered to be 'Not sensitive'.

Nutrient enrichment	High Q: High A: High C: High	High Q: High A: High C: High	Not sensitive Q: High A: High C: High

In nutrient enriched tidal sediments oligochaetes can dominate assemblages (Gray, 1971; Leppäkoski, 1975; Birtwell & Arthur, 1980). Green algae such as *Enteromorpha* spp. may form mats on the surface of the mud during the summer months, particularly if nutrient enrichment occurs.

Sensitivity assessment. As the benchmark is relatively protective and would not lead to blooms of *Ulva* spp. (although green algae may be present on the surface layers of sediments in the summer), biotope resistance is assessed as 'High', resilience is assessed as 'High' and the biotopeis considered to be 'Not sensitive'.

Organic enrichment	High Q: High A: High C: High	High Q: High A: High C: High	Not sensitive Q: High A: High C: High

Tubificoides benedii and *Baltidrilus costatus* are both very tolerant of high levels of organic enrichment and often dominate sediments where sewage has been discharged or other forms of organic enrichment have occurred (Pearson & Rosenberg, 1978; Gray, 1971; McLusky *et al*., 1980). Their tolerance for organic enrichment is attributed to their adaptation to live in and feed on enriched organic deposits (Pearson & Rosenberg, 1978) and their high population densities in such areas is enhanced by the lack of predation and competition. *Tubificoides benedii* are abundant in mussel beds (mussel relaying may be the source of smothering) which has been attributed to their tolerance of organically rich deoxygenated sediment (Commito & Boncavage, 1989). *Tubificoides benedii* has also been found in elevated abundances in areas of organic enrichment around fish farms (Haskoning, 2006).

Sensitivity assessment. Based on the high tolerance of the characterizing species *Tubificoides benedii* for organic enrichment, biotope resistance is assessed as 'High' and resilience as 'High', so that the biotope is considered to be Not sensitive'.

Physical Pressures

	Resistance	Resilience	Sensitivity
Physical loss (to land or freshwater habitat)	None Q: High A: High C: High	Very Low Q: High A: High C: High	High Q: High A: High C: High

All marine habitats and benthic species are considered to have a resistance of 'None' to this pressure and to be unable to recover from a permanent loss of habitat (resilience is 'Very Low'). Sensitivity within the direct spatial footprint of this pressure is therefore 'High'. Although no specific evidence is described confidence in this assessment is 'High', due to the incontrovertible nature of this pressure.

Physical change (to another seabed type)	None Q: High A: High C: High	Very Low Q: High A: High C: High	High Q: High A: High C: High

The biotope is characterized by the sedimentary habitat (JNCC, 2015), a change to an artificial or rock substratum would alter the character of the biotope leading to reclassification and the loss of the sedimentary community including the characterizing oligochaetes that live buried within the sediment.

Sensitivity assessment. Based on the loss of the biotope, resistance is assessed as 'None', recovery is assessed as 'Very low' (as the change at the pressure benchmark is permanent and sensitivity is assessed as 'High'.

Physical change (to another sediment type)	None Q: High A: High C: High	Very Low Q: High A: High C: High	High Q: High A: High C: High

Tubificoides benedii (studied as *Peloscolex benedeni*) are found in a range of substratum types from sandy mixed habitats, fine sands and coarse sands (Giere & Pfannkuche, 1982 and references therein). Similarly, *Baltidrilus costata* (as*Tubifex costatus*) is found in mud/silts (Giere & Pfannkuche,1982 and references therein). Giere & Pfannkuche (1982) suggest that factors that correlate to substratum types such as organic matter availability, size and shape of the intertstitial space between grains, the level of sediment disturbance and water content, rather than the sediment type alone are the key factors influencing distribution.

Sensitivity assessment. A change in sediment type to mixed or coarser particles could lead to changes in the oligochaete community depending on species specific responses. However, if other factors, such as the low salinity, that structure this biotope are not altered, oligochaetes are likely to colonise and a similar biological assemblage could be present (based on the range of sediments *Tubificoides benedii* inhabits. However, the loss of the mud that characterizes this habitat would change the character of the biotope and is likely to lead to reclassification. Based on a change in character, the biotope is considered to have 'No' resistance to this pressure, resilience is assessed as Very 'Low' as a change at the pressure benchmark is permanent and biotope sensitivity is assessed as 'High'.

Habitat structure changes - removal of substratum (extraction)	None Q: High A: High C: High	High Q: High A: Low C: Low	Medium Q: High A: Low C: Low

Sedimentary communities are likely to be highly intolerant of substratum removal, which will lead to partial or complete defaunation, expose underlying sediment which may be anoxic and/or of a different character or bedrock and lead to changes in the topography of the area (Dernie *et al*., 2003). Any remaining species, given their new position at the sediment / water interface, may be exposed to conditions to which they are not suited, Removal of 30 cm of surface sediment will remove the oligochaete community and other species present in the biotope. Recovery of the biological assemblage may take place before the original topography is restored, if the exposed, underlying sediments are similar to those that were removed. Hydrodynamics and sedimentology (mobility and supply) influence the recovery of soft sediment habitats (Van Hoey *et al*. 2008).

Sensitivity assessment. Extraction of 30 cm of sediment will remove the characterizing biological component of the biotope. Resistance is assessed as 'None' and biotope resilience is assessed as 'High'. Biotope sensitivity is therefore 'Medium'.

Abrasion/disturbance of the surface of the substratum or seabed	Medium	High	Low
	Q: High A: High C: High	Q: High A: High C: High	Q: High A: High C: High

Tubificoides benedii can be relatively deeply buried and could avoid direct exposure to abrasion although sediment disturbance and compaction could damage these soft-bodied species and oligochaetes in general are not found in high abundances in sediments with high levels of disturbance from wave action.

Experimental studies on crab-tiling impacts have found that densities of *Tubificoides benedii* and *Tubificoides pseudogaster* were higher in non-trampled plots (Sheehan *et al*. 2010), indicating that these oligochaetes have some sensitivity to trampling. Whomersley *et al*., (2010) conducted experimental raking on intertidal mudflats at two sites (Creeksea- Crouch estuary England and Blackness- lower Forth estuary, Scotland), where *Tubificoides benedii* were dominant species. For each treatment 1 m^2 plots were raked twice to a depth of 4cm (using a garden rake). Plots were subject to either low intensity treatments (raking every four weeks) or high (raking every two weeks). The experiment was carried out for 10 months at Creeksea and a year at Blackness. The high and low raking treatments appeared to have little effect on *Tubificoides benedii* (Whomersley *et al*., 2010)

Sensitivity assessment. The experiments by Whomersley *et al*. (2010), suggest that disturbance of the surficial layers has little effect on *Tubificoides benedii*. Many individuals are likely to be buried more deeply and can migrate to the surface following disturbance, so that little impact is observed through sampling. Abrasion with associated compaction (as in trampling) may have a greater impact. Resistance is therefore assessed as 'medium' and resilience as 'High' (by default) so that sensitivity is assessed as 'Low'.

Penetration or disturbance of the substratum subsurface	Medium	High	Low
	Q: High A: High C: High	Q: High A: High C: High	Q: High A: High C: High

Tubificoides benedii can be relatively deeply buried and could avoid direct exposure to penetration and disturbance of upper sediment layers although sediment disturbance and compaction could damage these soft-bodied species and oligochaetes in general are not found in high abundances in sediments with high levels of disturbance from wave action.

Whomersley *et al*., (2010) conducted experimental raking on intertidal mudflats at two sites (Creeksea- Crouch estuary England and Blackness- lower Forth estuary, Scotland), where *Tubificoides benedii* were dominant species. For each treatment 1 m^2 plots were raked twice to a depth of 4cm (using a garden rake). Plots were subject to either low intensity treatments (raking every four weeks) or high (raking every two weeks). The experiment was carried out for 10 months at Creeksea and a year at Blackness. The high and low raking treatments appeared to have little effect on *Tubificoides benedii* (Whomersley *et al*., 2010). These results are supported by observations that two experimental passes of an oyster dredge that removed the sediment to a depth of between 15-20 cm did not significantly affect *Tubifcoides benedii* (EMU, 1992).

Sensitivity assessment. The experiments by Whomersley *et al*., (2010) and EMU (1992), suggest that penetration and disturbance of the upper surface has little effect on *Tubificoides benedii*. Many individuals are likely to be buried more deeply and can migrate to the surface following disturbance, so that little impact is observed through sampling. Resistance is therefore assessed as 'Medium' and resilience as 'High' so that sensitivity is assessed as 'Low'.

Changes in suspended solids (water clarity)	Medium Q: Low A: NR C: NR	High Q: High A: Low C: High	Low Q: Low A: Low C: Low

Estuaries where this biotope is found form can be naturally turbid systems due to sediment resuspension by wave and tide action and inputs of high levels of suspended solids, transported by rivers. The level of suspended solids depends on a variety of factors including: substrate type, river flow, tidal height, water velocity, wind reach/speed and depth of water mixing (Parr *et al.* 1998). Transported sediment including silt and organic detritus can become trapped in the system where the river water meets seawater. Dissolved material in the river water flocculates when it comes into contact with the salt wedge pushing its way upriver. These processes result in elevated levels of suspended particulate material with peak levels confined to a discrete region (the turbidity maximum), usually in the upper-middle reaches, which moves up and down the estuary with the tidal ebb and flow. Intertidal mudflats depend on the supply of particulate matter to maintain mudflats and the associated biological community is exposed naturally to relatively high levels of turbidity/particulate matter.

Sensitivity assessment. The biological assemblage characterizing this biotope is infaunal and consists of sub-surface deposit feeders. Increased suspended solids are unlikely to have an impact and resistance is assessed as 'High' and resilience as 'High', so that the biotope is considered to be 'not sensitive'. A reduction in suspended solids may reduce deposition and supply of organic matter, resistance to a decrease is therefore assessed as 'Medium' as a shift between deposition and erosion could result in the net loss of surficial sediments. A reduction in organic matter as suspended solids could also reduce production within this biotope. Resistance is assessed as 'Medium' as over a year the impact may be relatively small and resistance is assessed as 'High', following restoration of usual conditions. Biotope sensitivity is therefore assessed as 'Low'.

Smothering and siltation rate changes (light)	High Q: High A: High C: High	High Q: High A: High C: High	Not sensitive Q: High A: High C: High

Intertidal mudflats occur in sheltered environments and, in general, are accreting environments meaning that deposition rather than erosion is the dominant process, this means that the assemblages present (primarily deposit feeders) are adapted to natural levels of siltation through life history traits and can withstand burial (by repositioning in sediment or similarly extending tubes or feeding and respiration structures above the sediment surface). At low levels of siltation the high bioturbatory nature of mudflat organisms decreases sensitivity to effects (Elliott *et al.* 1998) as sediment turnover rates are relatively rapid. *Tubificoides* live relatively deeply buried and can tolerate periods of low oxygen that may occur following the deposition of a fine layer of sediment. In addition the presence of this species in areas experiencing deposition, such as estuaries, indicate that this species is likely to have a high tolerance to siltation events. *Tubificoides* spp. showed some recovery through vertical migration following the placement of a sediment overburden 6cm thick on top of sediments (Bolam, 2011).

Whomersley *et al.*, (2010) experimentally buried plots on intertidal mudflats at two sites (Creeksea- Crouch Estuary, England and Blackness- lower Forth Estuary, Scotland), where *Tubificoides benedii* were dominant species. For each treatment anoxic mud was spread evenly to a depth of 4cm on top of each treatment plot. The mud was taken from areas adjacent to the plots, and was obtained by scraping off the surface oxic layer and digging up the underlying mud from approximately 20cm depth. Plots were subject to either low intensity treatments (burial every four weeks) or high (burial every two weeks). The experiment was carried out for 10 months at Creeksea and a year at Blackness. At Creeksea numbers of *Tubificoides benedii* increased in both burial treatments until the third month (high burial) and sixth month (low burial). At Blackness increased numbers of *Tubificoides benedii* were found in both burial treatments after one month (Whomersley *et al*., 2010)..

Sensitivity assessment. The characterizing species *Tubificoides benedii* is considered to be able to survive under a deposit of fine grained sediment up to 5cm thick and to burrow and reposition within this. The assessment is supported by the burial experiments conducted by Whomersley *et al*. (2010).

Smothering and siltation rate changes (heavy)	Low Q: High A: Low C: NR	High Q: High A: Low C: High	Low Q: High A: Low C: Low

The pressure benchmark (30 cm deposit) represents a significant burial event and the deposit may remain for some time in a sheltered mudflat. Some impacts on *Tubificoides benedii* and other characterizing oligochaetes may occur and it is considered unlikely that signficiant numbers of the population could reposition, based on (Bolam, 2011). Placement of the deposit will therefore result in a defaunated habitat until the deposit is recolonized. Biotope resistance is therefore assessed as 'Low' as some removal of deposit and vertical migration through the deposit may occur. Resilience is assessed as 'High' as migration and recolonization of oligochaetes is likely to occur within two years, biotope sensitivity is therefore assessed as 'Low'.

Litter	Not Assessed (NA) Q: NR A: NR C: NR	Not assessed (NA) Q: NR A: NR C: NR	Not assessed (NA) Q: NR A: NR C: NR

Not assessed.

Electromagnetic changes	No evidence (NEv) Q: NR A: NR C: NR	No evidence (NEv) Q: NR A: NR C: NR	No evidence (NEv) Q: NR A: NR C: NR

A number of studies have investigated the effects of electromagnetic fields on terrestrial oligochaetes, notable earthworms. Some negative effects have been observed e.g. Tkalec *et al*., 2013. However no evidence was found to support an assessment at the pressure benchmark for the marine oligochaetes that characterize this biotope.

Underwater noise changes	Not relevant (NR) Q: NR A: NR C: NR	Not relevant (NR) Q: NR A: NR C: NR	Not relevant (NR) Q: NR A: NR C: NR

Not relevant.

Introduction of light or shading	Not relevant (NR) Q: NR A: NR C: NR	Not relevant (NR) Q: NR A: NR C: NR	Not relevant (NR) Q: NR A: NR C: NR

No evidence was found to assess this pressure. Studentowicz (1936) found that the enchytraeid oligochaete *Enchytraeus albidus*, retracted from light, although the worms accumulated at the surface even when illuminated to avoid low oxygen and hydrogen sulpfide. Giere and Pfannkuche (1982) considered that other enchytraeids and tubificids are likely to react in the same way. As the biological assemblage occurs within the sediment and can be deeply buried (to 10cm or more) this pressure is considered 'Not relevant'.

Barrier to species movement	High Q: Low A: NR C: NR	High Q: High A: High C: High	Not sensitive Q: Low A: Low C: Low

As the tubificid oligochaetes that characterize this biotope have benthic dispersal strategies (via egg cocoons laid on the surface, Giere & Pfannkuche, 1982), water transport is not a key method of dispersal over wide distances, as it is for some marine invertebrates that produce pelagic larvae. The biotope (based on the biological assemblage) is therefore considered to have 'High' resistance to the presence of barriers that lead to a reduction in tidal excursion, resilience is assessed as 'High' (by default) and the biotope is considered to be 'not sensitive'.

Death or injury by collision	Not relevant (NR) Q: NR A: NR C: NR	Not relevant (NR) Q: NR A: NR C: NR	Not relevant (NR) Q: NR A: NR C: NR

Not relevant' to seabed habitats. NB. Collision by grounding vessels is addressed under 'surface abrasion.

Visual disturbance	Not relevant (NR) Q: NR A: NR C: NR	Not relevant (NR) Q: NR A: NR C: NR	Not relevant (NR) Q: NR A: NR C: NR

Most aquatic oligochaetes have no eyes although a few have simple ocelli (eyspots) which are light receptors. Visual disturbance is not considered relevant to this biotope.

Biological Pressures

	Resistance	Resilience	Sensitivity
Genetic modification & translocation of indigenous species	Not relevant (NR) Q: NR A: NR C: NR	Not relevant (NR) Q: NR A: NR C: NR	Not relevant (NR) Q: NR A: NR C: NR

Key characterizing species within this biotope are not cultivated or translocated. This pressure is therefore considered 'Not relevant' to this biotope group.

Introduction or spread of invasive non-indigenous species	None Q: High A: High C: Low	Very Low Q: Low A: NR C: NR	High Q: Low A: Low C: Low

The biotope may be exposed to a number of invasive species that can cause impacts. The largest effect may be from species that significantly alter the character of the biotope, such as reef-forming species and invasive vegetation. Commito (1987) found that the population density of *Tubificoides benedii* was the same or higher in mussel beds than in open areas, suggesting that colonization of sediments by the Pacific oyster *Magallana gigas* would not necessarily impact the population (although it would alter the character of the biotope). Tang & Kristensen (2010) found that abundance of macrofauna, including *Tubificoides* was lower in marsh invaded by the hybrid cordgrass *Spartina anglica* than in mudflats. Colonization of upper mudflats by this species would alter the character of the biotope resulting in loss and reclassification.

Infaunal non-natives may impact the biotope through sediment disturbance, predation or competition for resources. No examples were found. The polychaete *Marenzellaria viridis* has become established in estuaries in Europe but a recent paper on its impacts where *Tubificoides* were abundant did not report on oligochaete impacts (Delefosse *et al*., 2012).

Sensitivity assessment. The biotope may be sensitive to invasion by *Spartina anglica* which would alter the character of the mudflat and the biological assemblage. Resistance is assessed as 'None' and resilience as 'very Low' as the biotope will not recover unless the INIS is removed. Sensitivity is therefore assessed as 'High'.

Introduction of microbial pathogens	High Q: High A: High C: Low	High Q: High A: High C: High	Not sensitive Q: High A: High C: Low

Marine oligochaetes host numerous protozoan parasites without apparent pathogenic effects even at high infestation levels (Giere & Pfannkuche, 1982 and references therein)

Sensitivity assessment. Based on the lack of evidence for mass mortalities in oligochaetes from microbial pathogens resistance is assessed as 'High' and resilience as 'High', by default, so that the biotope is assessed as 'Not sensitive'.

Removal of target species	Not relevant (NR) Q: NR A: NR C: NR	Not relevant (NR) Q: NR A: NR C: NR	Not relevant (NR) Q: NR A: NR C: NR

No species within the biotope are targeted by commercial or recreational fishers or harvesters. This pressure is therefore considered 'Not relevant'.

Removal of non-target species	Low Q: Low A: NR C: NR	High Q: High A: Low C: Medium	Low Q: Low A: Low C: Low

Incidental removal of the characterizing species would alter the character of the biotope and the delivery of ecosystem services such as secondary production and bioturbation. Populations of oligochaetes provide food for macroinvertebrates fish and birds. For example up to 67% of flounder and plaice stomachs examined from the Medway estuary (UK) (Van den Broek, 1978) contained the remains of *Tubificoides benedii (*studied as *Peloscolex benedeni)* and shrimps which in turn support higher trophic levels (predatory birds and fish). For some migratory birds the characterizing species *Tubificoides benedii* can form an important part of the diet during winter (Bagheri & McLusky, 1984). Polychaetes and crustaceans are also predators of oligochaetes and may significantly reduce numbers (Giere & Pfannkuche, 1982 and references therein). The loss of the oligochaete population could, therefore, impact other trophic levels.

Sensitivity assessment. Removal of the characterizing species would alter the character of the biotope. Resistance is therefore assessed as 'Low' and resilience as 'High' so that sensitivity is categorised as 'Low'.

Bibliography

Bagheri, E. & McLusky, D., 1982. Population dynamics of oligochaetes and small polychaetes in the polluted forth estury ecosystem. *Netherlands Journal of Sea Research*, **16**, 55-66.

Bagheri, E.A. & McLusky, D.S., 1984. The oxygen consumption of *Tubificoides benedeni* (Udekem) in relation to temperature and its application to production biology. *Journal of Experimental Marine Biology and Ecology*, **78**, 187-197.

Bamber, R.N. & Spencer, J.F. 1984. The benthos of a coastal power station thermal discharge canal. *Journal of the Marine Biological Association of the United Kingdom*, **64**, 603-623.

Birtwell, I.K. & Arthur, D.R., 1980. The ecology of tubificids in the Thames Estuary with particular reference to *Tubifex costatus* (Claparède). In *Proceedings of the first international symposium on aquatic oligochaete biology, Sydney, British Colombia, Canada, May 1-4, 1979. Aquatic oligochaete biology* (ed. R.O. Brinkhurst & D.G. Cook), pp. 331-382. New York: Plenum Press

Bolam, S. & Whomersley, P., 2003. Invertebrate recolonization of fine-grained beneficial use schemes: An example from the southeast coast of England. *Journal of Coastal Conservation*, **9** (2), 159-169.

Bolam, S.G., 2011. Burial survival of benthic macrofauna following deposition of simulated dredged material. *Environmental Monitoring and Assessment*, **181** (1-4), 13-27.

Bouchet, V.M. & Sauriau, P.-G., 2008. Influence of oyster culture practices and environmental conditions on the ecological status of intertidal mudflats in the Pertuis Charentais (SW France): A multi-index approach. *Marine Pollution Bulletin*, **56** (11), 1898-1912.

Brinkhurst, R. & Kennedy, C., 1962. Some aquatic Oligochaeta from the Isle of Man with special reference to the Silver Burn Estuary. *Archive fur Hydrobiologie*, **58** (3), 367-766.

Commito, J.A. & Boncavage, E.M., 1989. Suspension-feeders and coexisting infauna: an enhancement counterexample. *Journal of Experimental Marine Biology and Ecology*, **125** (1), 33-42.

Commito, J.A., 1987. Adult-larval interactions: predictions, mussels and cocoons. *Estuarine, Coastal and Shelf Science*, **25**, 599-606.

Delefosse, M., Banta, G.T., Canal-Vergés, P., Penha-Lopes, G., Quintana, C.O., Valdemarsen, T. & Kristensen, E., 2012. Macrobenthic community response to the *Marenzelleria viridis* (Polychaeta) invasion of a Danish estuary. M*arine Ecology Progress Series*, **461**, 83-94.

Dernie, K.M., Kaiser, M.J., Richardson, E.A. & Warwick, R.M., 2003. Recovery of soft sediment communities and habitats following physical disturbance. *Journal of Experimental Marine Biology and Ecology*, **285-286**, 415-434.

Elliot, M., Nedwell, S., Jones, N.V., Read, S.J., Cutts, N.D. & Hemingway, K.L., 1998. Intertidal sand and mudflats & subtidal mobile sandbanks (Vol. II). An overview of dynamic and sensitivity for conservation management of marine SACs. *Prepared by the Scottish Association for Marine Science for the UK Marine SACs Project*.

EMU, 1992. An experimental study on the impact of clam dredging on soft sediment macro invertebrates. English Nature Research Reports. No 13.

Gamenick, I., Jahn, A., Vopel, K. & Giere, O., 1996. Hypoxia and sulphide as structuring factors in a macrozoobenthic community on the Baltic Sea shore: Colonization studies and tolerance experiments. *Marine Ecology Progress Series*, **144**, 73-85.

Giere, O., 1977. An ecophysiological approach to the microdistribution of meiobenthic Oligochaeta. I. *Phallodrilus monospermathecus* (Knöllner)(Tubificidae) from a subtropical beach at Bermuda. *Biology of benthic organisms*. Pergamon Press New York, 285-296.

Giere, O., 2006. Ecology and biology of marine oligochaeta–an inventory rather than another review. *Hydrobiologia*, **564** (1), 103-116.

Giere, O. & Pfannkuche, O., 1982. Biology and ecology of marine Oligochaeta, a review. *Oceanography and Marine Biology*, **20**, 173-309.

Giere, O., Preusse, J. & Dubilier, N. 1999. *Tubificoides benedii* (Tubificidae, Oligochaeta) - a pioneer in hypoxic and sulfide environments. An overview of adaptive pathways. *Hydrobiologia*, **406**, 235-241.

Gillett, D.J., Holland, A.F. & Sanger, D.M., 2007. On the ecology of oligochaetes: monthly variation of community composition and environmental characteristics in two South Carolina tidal creeks. *Estuaries and Coasts*, **30** (2), 238-252.

Goulletquer, P. & Heral, M., 1997. Marine molluscan production trends in France: from fisheries to aquaculture. *NOAA Tech. Rep. NMFS*, **129**.

Gray, J.S., 1971. The effects of pollution on sand meiofauna communities. *Thalassia Jugoslovica*, **7**, 76-86.

Hall, J.A. & Frid, C.L.J., 1995. Response of estuarine benthic macrofauna in copper-contaminated sediments to remediation of sediment quality. *Marine Pollution Bulletin*, **30**, 694-700.

Haskoning UK Ltd. 2006. Investigation into the impact of marine fish farm deposition on maerl beds. *Scottish Natural Heritage Commissioned Report No.* 213 (ROAME No. AHLA10020348).

Hunter, J., & Arthur, D.R., 1978. Some aspects of the ecology of *Peloscolex benedeni* Udekem (Oligochaeta: Tubificidae) in the Thames estuary. *Estuarine and Coastal Marine Science*, **6**, 197-208.

JNCC, 2015. The Marine Habitat Classification for Britain and Ireland Version 15.03. (20/05/2015). Available from https://mhc.jncc.gov.uk/

JNCC, 2015. The Marine Habitat Classification for Britain and Ireland Version 15.03. (20/05/2015). Available from https://mhc.jncc.gov.uk/

Leppäkoski, E. & Lindström, L., 1978. Recovery of benthic macrofauna from chronic pollution in the sea area off a refinery plant, southwest Finland. *Journal of the Fisheries Board of Canada*, **35** (5), 766-775.

Leppäkoski, E., 1975. Assessment of degree of pollution on the basis of macrozoobenthos in marine and brackish water environments. *Acta Academiae Åboensis*, Series B, **35**, 1-90.

Linke, O., 1939. Die Biota des Jadebusenwatts. *Helgolander Wissenschaftliche Meeresuntersuchungen*, **1**, 201-348.

McLusky, D.S., 1982. The impact of petrochemical effluent on the fauna of an intertidal estuarine mudflat. *Estuarine, Coastal and Shelf Science*, **14**, 489-499.

McLusky, D.S., Teare, M. & Phizachlea, P., 1980. Effects of domestic and industrial pollution on distribution and abundance of aquatic oligochaetes in the Forth estuary. *Helgolander Wissenschaftliche Meeresuntersuchungen*, **33**, 384-392.

Nugues, M., Kaiser, M., Spencer, B. & Edwards, D., 1996. Benthic community changes associated with intertidal oyster cultivation. *Aquaculture Research*, **27** (12), 913-924.

Parr, W., Clarke, S.J., Van Dijk, P., Morgan, N., 1998. Turbidity in English and Welsh tidal waters. Report No. CO 4301/1 to English Nature.

Pearson, T.H. & Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanography and Marine Biology: an Annual Review*, **16**, 229-311.

Rygg, B., 1985. Effect of sediment copper on benthic fauna. *Marine Ecology Progress Series*, **25**, 83-89.

Sheehan, E., Coleman, R., Thompson, R. & Attrill, M., 2010. Crab-tiling reduces the diversity of estuarine infauna. *Marine Ecology Progress Series*, **411**, 137-148.

Studentowicz, J., 1936. Der Einfluss des Lichtes auf das Verhalten des Oligochaeten *Enchytraeus albidus* Henle: *Bulletin International Academy of Polish Science Letters, Series B*. .

Tang, M. & Kristensen, E., 2010. Associations between macrobenthos and invasive cordgrass, *Spartina anglica*, in the Danish Wadden Sea. *Helgoland Marine Research*, **64** (4), 321-329.

Van den Broek, W., 1978. Dietary habits of fish populations in the Lower Medway Estuary. *Journal of Fish Biology*, **13** (5), 645-654.

Van Hoey, G., Guilini, K., Rabaut, M., Vincx, M. & Degraer, S., 2008. Ecological implications of the presence of the tube-building polychaete *Lanice conchilega* on soft-bottom benthic ecosystems. *Marine Biology*, **154** (6), 1009-1019.

Verdonschot, P., Smies, M. & Sepers, A., 1982. The distribution of aquatic oligochaetes in brackish inland waters in the SW Netherlands. *Hydrobiologia*, **89** (1), 29-38.

Whomersley, P., Huxham, M., Bolam, S., Schratzberger, M., Augley, J. & Ridland, D., 2010. Response of intertidal macrofauna to multiple disturbance types and intensities – an experimental approach. *Marine Environmental Research*, **69** (5), 297-308.

Zühlke, R. & Reise, K., 1994. Response of macrofauna to drifting tidal sediments. *Helgoländer Meeresuntersuchungen*, **48** (2-3), 277-289.