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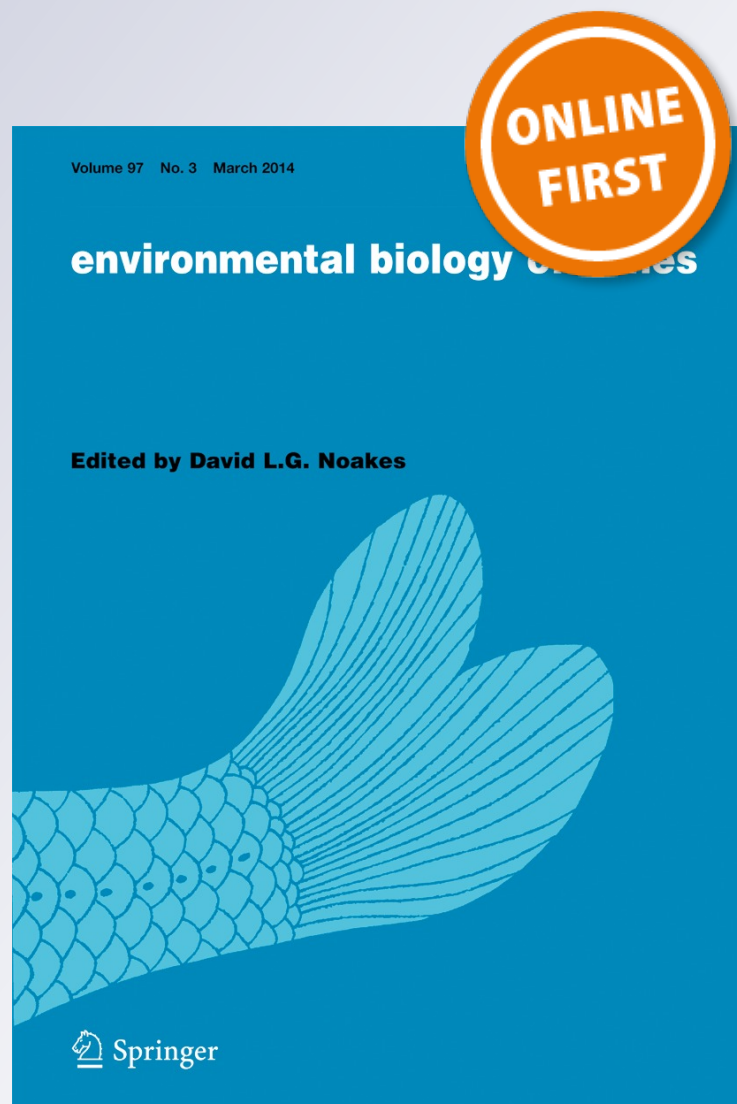
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# Effects of turbidity and habitat complexity on antipredator behavior of three-spined sticklebacks (*Gasterosteus aculeatus*)

## Antipredator behavior in sticklebacks

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**Abstract** Human-induced processes like eutrophication are increasing water turbidity and altering vegetated habitats in the Baltic Sea. Unfortunately, the influence of these environmental changes on predator–prey interactions remains poorly studied in mobile taxa of this region. We used three-spined stickleback (*Gasterosteus aculeatus*) as a model species to study the combined effects of turbidity and shoot density on habitat choice (shelter vs. open) in the presence and absence of piscivorous perch (*Perca fluviatilis*). Habitat choice of sticklebacks was video-monitored and compared between two paired observation periods: “control” (no predator) and “risk” (predator present). Though sticklebacks

exhibited a general preference for sheltered habitat across treatments, repeated measures ANOVA found that sticklebacks responded to predator presence by significantly increasing use of the sheltered habitat. However, shoot density of the shelter interacted with observation period, where risk-induced shifts of sticklebacks into sheltered habitat were most apparent and sustained at higher shoot densities. Stickleback activity level was generally reduced with predator presence at higher turbidity and shoot density levels, suggesting a possible adaptation to reduce predator encounter rates in visually deteriorated conditions. Overall, our study demonstrates that relatively minute increases (1’s – 10’s of NTU) in algal turbidity intensify three-spined stickleback sheltered habitat use, but vegetation density may still play a larger role in avoiding predators at these turbidity levels. When compared with a similar study on 0+ perch, our work suggests that eutrophication (i.e., increased turbidity, loss of habitat complexity) may have variable, taxon- and/or habitat-specific effects on predator–prey interactions of the Baltic Sea.

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## Introduction

The role of habitat selection in structuring marine communities has become an increasingly important theme in ecology (Kiflawi et al. 2003; Morris 2003). However, man-made processes such as eutrophication are

changing habitat structure and visual conditions in various aquatic systems. These phenomena are now considered worldwide threats to fish fauna due to the associated declines in food resources (Diehl 1998), but the impacts on community interactions (e.g., predator–prey interactions) are relatively unknown. Due to heightened anthropogenic pressure, nutrient runoff from agriculture and industry have enhanced the eutrophication process and thus increased algal turbidity in several lakes and seas (Utne-Palm 2002; Engström-Öst and Mattila 2008). Bonsdorff et al. (2002) reviewed the influence of eutrophication on the Baltic Sea and recognized it as a primary environmental issue of this water body, with some parts becoming increasingly turbid during last few decades (Sandén and Håkansson 1996).

Turbidity is a common natural feature of many aquatic environments. In coastal areas, algae and clay are considered the two main components of turbidity (Mobley 1994). Algal turbidity is desirable within certain levels since algae add oxygen and remove nutrients from water (Boyd 1982). However, turbidity reduces visibility in aquatic systems by light attenuation (Krik 1985), which can considerably influence community structure. For example, increased light penetration can have “top-down” effects by expanding the visual feeding habitat of predatory fishes and allowing consumption of lower trophic levels (Aksnes et al. 2004). Contrastingly, more turbid environments are considered somewhat optimal for larval fishes and planktivores that rely on detecting small particles at close range and are negatively affected by high amounts of light scattering (Utne-Palm 2002). Thus, while most fishes have well developed eyes and rely on vision for foraging, predation and antipredator behavior (Guthrie and Muntz 1993), the influence of turbidity on marine fish communities is likely to vary among various feeding guilds and environmental conditions (Bowmaker 1995). Given this variation in visual capabilities among fishes, the impact of increased turbidity is likely to be taxon- and/or life-stage dependent.

Multiple studies demonstrate that behavioral strategies of fish may change with turbidity. For example, the northern Pike *Esox lucius* exhibits a higher degree of behavioral diversity in a turbid lake than in a clear water lake (Andersen et al. 2008). Engström-Öst and Mattila (2008) showed that algae-induced water turbidity affects both direct (i.e., feeding and habitat choice) and indirect qualities (i.e., weight) of pike larvae and may therefore influence larval survival. Ferrari et al. (2010) indicated

that turbidity alters the quality and quantity of visual information received by the minnow (*Pimephales promelas*), with prey recognition significantly impaired under turbid conditions. In addition, increased algal turbidity may induce some other behaviors of fish such as migration, reduced use of shelter and increased use of open water (reviewed by Utne-Palm 2002). Algal-based turbidity may also influence prey reaction distances to predators (Quesenberry et al. 2007), or general predator avoidance behavior (Abrahams and Kattenfeld 1997).

When exposed to predators, fish often seek shelter in structurally complex submerged vegetation and avoid open habitats (Werner et al. 1983; Gotceitas and Colgan 1989). However, many studies suggest turbidity alone provides shelter against predators, sometimes in place of complex benthic habitat (Gregory 1993; Gregory and Northcote 1993; Abrahams and Kattenfeld 1997; Maes et al. 1998; Snickars et al. 2004; Engström-Öst et al. 2006). As such, several authors have suggested the idea of “turbidity as cover,” where reduced visual fields lessen predation risk (Gregory 1993; Aksnes and Utne 1997). Like turbidity, dense vegetation cover also reduces visibility and can decrease encounter rates and foraging efficiency of predators (Nelson and Bonsdorff 1990; Mattila 1992; Candolin and Voigt 2001). Furthermore, differences in density and morphology of vegetation may influence foraging rates of predators and the degree of predator avoidance by prey (Dionne and Folt 1991; Wychera et al. 1993; Newbrey et al. 2005; Sass et al. 2006; Shoji et al. 2007; Burfeind et al. 2009). However, prey refuge and survival in vegetated habitat may depend on factors other than vegetation density alone, such as the density of prey, predators and the ratio of these two densities to one another and vegetation density (Mattila et al. 2008; Canion and Heck 2009; Scheinin et al. 2011).

Unfortunately, the interaction between turbidity and vegetation density on predator–prey relationships remains sparsely studied, though these factors may play a combined role in community interactions of shallow benthic environments. The few studies that assess the relative roles of turbidity and vegetation density suggest that their combined effect may have substantial implications on the behavior of prey and predatory fishes (Snickars et al. 2004; Skov et al. 2007). The aim of the present study was to examine the fine-scale (i.e. minute-by-minute) habitat choice of the three-spined stickleback when confronted with multiple environmental factors, such as turbidity, habitat complexity and a predator.

Previous studies have overlooked the potential impact of environmental changes on fine temporal scales and have not assessed changes over relatively small incremental changes in turbidity. These aspects are most relevant since predator–prey interactions are often sudden in nature and both predators and prey may live in environments where turbidity changes gradually over time (e.g., in river mouth areas or along archipelago gradients). We utilized the three-spined stickleback (*Gasterosteus aculeatus*) as our representative prey species as it is widely distributed freshwater-anadromous species (Bell and Foster 1994) and a visual predator of seagrass environments affected by periodic turbidity (Hart and Gill 1994). As such, the three-spined stickleback served as a practical model to study the relative effects of turbidity and habitat loss (i.e., seagrass density) on fish antipredator behavior. We used the perch (*Perca fluviatilis*) as a predator since three-spined sticklebacks are naturally consumed by this co-occurring piscivore (Wootton 1984; Remichen 1994).

## Materials and methods

The main purpose of our experiments was to study effects of sudden encounters of prey with predators at different turbidity and habitat complexity levels. All experiments were carried out at the Husö Biological Station (60°17' N, 19°128 50' E), Åland, Finland, from mid-August to the first week of September 2008. Three-spined sticklebacks were collected from a nearby breeding area using a seine net. Only female sticklebacks were used in the experiments. Male sticklebacks were not suitable for such a mesocosm experiment as they can exhibit high levels of aggression and social dominance hierarchies when grouped together (Bakker and Sevenster 1983; Rowland 1984; Bakker 1986; McLennan and McPhail 1989). Perch were collected from Husö Bay using gillnets and quickly transported to tanks. Approximately 800 sticklebacks were housed inside the laboratory in six aquariums (100×100×100 cm) for 4 weeks and ten perch were held in one large aquarium (300×300×50 cm) outside the laboratory for 1 week prior to the experiment for acclimatization. All aquaria were connected with a continuous flow-through of unfiltered sea water (Temperature: 12–18 °C, Salinity: 5.20–5.45 psu). Inside the laboratory, the photoperiod regime was maintained 16 L: 8D, which was approximately the same as the natural photoperiod

during the late boreal summer. The sticklebacks were maintained on a daily diet of frozen blood worms while perch were fed juvenile sticklebacks from the holding tank.

Variations in algal and clay turbidity across a range of ca. 1.5–15 NTU are known to cause significant changes in refuge use and behavior of sticklebacks (Engström-Öst et al. 2009) and other small-sized fish (e.g. juvenile perch and roach; Snickars et al. 2004; Sundblad et al. 2009; Nurminen et al. 2010) which thrive in seagrass areas of the northern Baltic Sea (Boström et al. 2003). Three different levels of turbidity were used during trials: low (2–3 NTU), medium (7–9 NTU) and high (13–15 NTU). These turbidity levels simulate the natural turbid conditions in some shallow coastal areas as well as the breeding grounds of three-spined sticklebacks of the Baltic Sea during summer. We found turbidity varied between 5 and 15 NTU during field collections for sticklebacks (S. Sohel, pers. obs.). Granqvist and Mattila (2004) reported that the turbidity level of sticklebacks breeding area may vary from 0.5 to 45 NTU, though in nature turbidity levels exceeding 30 NTU are rare. Lundberg et al. (2009) reported a range of turbidity levels between 0.2 and 22 NTU in the inner coastal areas of Bothnian Sea, northern part of the Baltic Sea. Abrahams and Kattenfeld (1997) used a turbidity level of 13 NTU in their study. In our study area, a turbidity of 2–3 NTU corresponds to Secchi depths about 4–5 m and 13–15 NTU to ca. 2.5 m (Granqvist and Mattila 2004), which are typical for seagrass beds and other vegetated areas of the northern Baltic Sea. Thus, our experimental levels of turbidity levels were representative of ranges observed locally.

Cultures of a 10–15 µm unicellular planktonic algae (*Brachiomonas submarina*) were used to create different turbidity levels. The initial culture was collected from Tvärminne Zoological Station (59°50' N, 23°145 15' E), Finland. Algae were grown from sea water (filtered through a 20 µm sieve to eliminate other possible grazers) in white 60 L buckets with continuous aeration under natural light. Following Järvenpää and Lindström (2004), we added fertilizer containing nitrogen and phosphorus to the culture to ensure sufficient nutrients. The levels of turbidity were set manually before running each experiment by adding the initial culture to clear sea water (<1 NTU). A HACH Turbid meter 2100P was used to measure the turbidity prior to and after the experiments. The turbidity level remained within the desired level (low (2–3±0.5 NTU), medium

( $7-9 \pm 0.5$  NTU) and high ( $13-15 \pm 0.5$  NTU)) during the experiment.

Experimental trials were carried out in a square shape ( $100 \times 100 \times 100$  cm) plastic aquarium during day time (10:00–14:00) under natural light conditions. The bottom of the aquarium was filled with 5 cm of gravel sand and divided into two halves; one half treated as vegetated habitat (artificial seagrass grid) and the other half as open habitat. Before each trial the aquarium was filled with turbid water (depth=20 cm) from one of the three turbidity levels and then fixed with an artificial seagrass grid with one of the three shoot densities. We used artificial eelgrass in the form of polypropylene deep green ribbons (Width  $\times$  Height: 0.5 cm  $\times$  15 cm) tied to a plastic grid. The grid covered half of the tank during the trial and contained three different shoot densities of green ribbons (100, 400 and 800 shoot/m<sup>2</sup>). Polypropylene ribbon is a good mimic of eelgrass *Zostera marina* and has been previously used in several predation and habitat complexity experiments (James and Heck 1994; Boström and Mattila 1999; Boström and Bonsdorff 2000). Similar shoot densities also have been used in several habitat complexity related studies (Boström and Bonsdorff 2000). We chose only one type of artificial eel grass leaf morphology to minimize problems with interpretation (Edger and Robertson 1992). *Zostera marina* forms distinct patches of eelgrass in coastal areas of the Baltic Sea. Eelgrass sites in the archipelago region of Finland vary in terms of patch size (1–75 m<sup>2</sup>), shoot density (50–500 shoots/m<sup>2</sup>), shoot length (20–100 cm) and biomass (10–32.1 g ash-free dry weight/m<sup>2</sup>) (Lappalainen and Hällfors 1977).

In each trial, 20 female three-spined sticklebacks (mean  $\pm$  S.E. =  $6.20 \pm 0.03$  cm FL) were released randomly in either open or in vegetated area and allowed to roam around the aquarium for 10 min. One perch ( $14.5 \pm 0.5$  cm FL) was introduced after 10 min and allowed to move around the aquarium with sticklebacks for another 10 min. The perch was released in the middle of the two habitats by slowly lowering the animal into the experimental area from a small container of mesocosm water. This technique ensured the experimenters did not disturb sticklebacks during the predator introduction. A video camera was suspended above the aquaria and recorded the whole trial (20 min), monitoring movements and habitat selection of the sticklebacks and solitary perch. The experimental zone was covered with black opaque plastic to minimize the outside disturbance and glare during experiments. All treatments (three

turbidity levels  $\times$  three vegetation densities over predator presence/absence) were completed over the course of a single day and replicated 7 times (7 days of filming). Each individual prey and predator fish was introduced to a randomly assigned experimental treatment at most once per experiment day to minimize potential learning or acclimation effects. At the end of each trial, a divider was lowered between the two habitats to recapture sticklebacks and confirm habitat choice at the final minutes of video. All experimental fishes were released to their natural habitat after completion of the experiments.

### Video analyses

Over each 20-min trial (10 min in absence/presence of predator) we counted the number of sticklebacks in vegetated (sheltered) and open habitat, as well as the location of the predatory fish, at 1-min intervals. We then averaged the number of sticklebacks in sheltered and open habitats in the absence/presence of a predator. To assess the potential impacts of predatory perch on stickleback activity levels, we monitored the number of times sticklebacks shifted between sheltered and open habitats before and after the predator introduction. To account for the disturbance associated with introducing the perch to the experimental mesocosm, we did not include shifts associated with risk period until 1 min after the predator addition. No sticklebacks were actively consumed by perch predators during experimental trials.

Two-way repeated measures analyses of variance (ANOVA) were run to examine the influence of turbidity, vegetation density and their interaction effects on stickleback antipredator response (i.e., change in distribution after predator addition). In this design, we used our two observation periods (before/after predator introduction) as the repeated measure since we monitored the distribution of the same individuals over time. Environmental effects on activity levels (number of movements between habitats) during the two observation periods were similarly tested using separate paired t-tests for each habitat complexity and turbidity treatment. Because habitat use data were all proportional, all values were arc-sine square-root transformed to better meet the assumptions of ANOVAs (Underwood 1997; Zar 2010). Tukey's post-hoc tests were used to determine the source of variation within each significant

effect ( $\alpha \leq 0.05$ ). Statistical analyses were conducted using SYSTAT 11.0 (SPSS, Inc.).

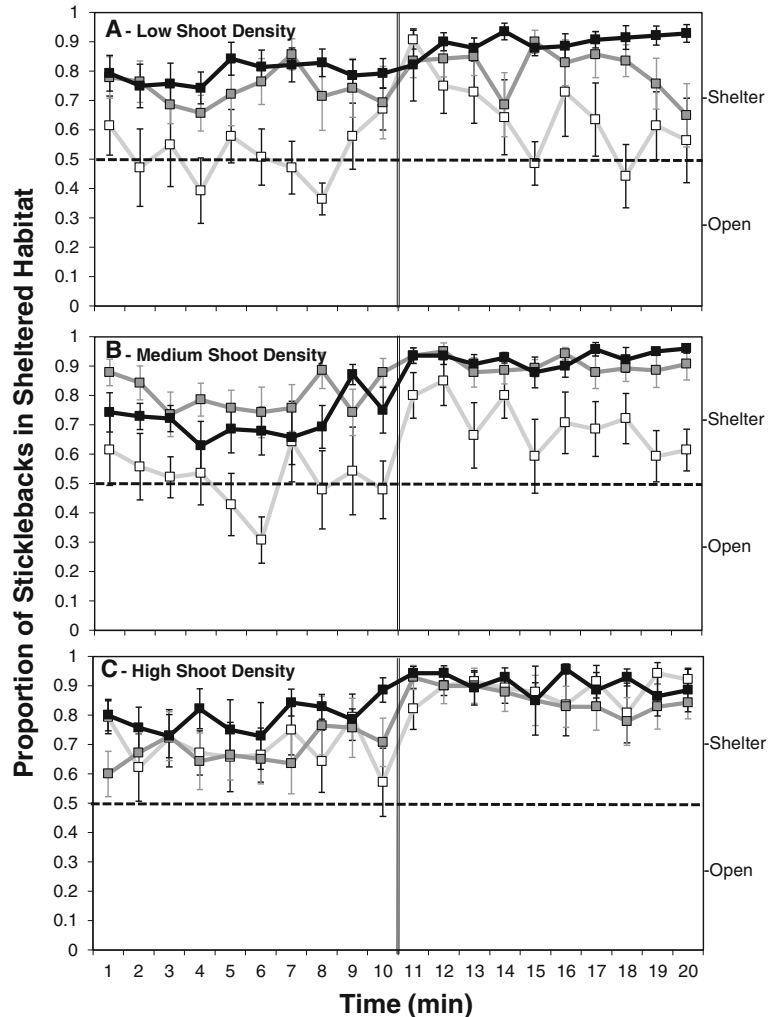
## Results

Prior the introduction of the predator, stickleback habitat preference varied greatly among turbidity levels (Fig. 1). After the predator was added to the mesocosm (i.e., minute 11), a strong pulse of sticklebacks was observed into the sheltered habitat, particularly at low turbidity. Stickleback proportions in sheltered habitat then decreased with time (11–20 min) at low and medium turbidity levels (Fig. 1a, b), but appeared to remain constant at the high turbidity level (Fig. 1c). On average,

preference for sheltered habitat was lowest at low turbidity levels, whether the predator was present or absent.

Without accounting for predator introductions, turbidity significantly influenced stickleback distribution behavior (Two-way ANOVA:  $F_{2,54}=8.929$ ;  $P<0.0001$ ; Table 1a) while vegetation density did not (Two-way ANOVA:  $F_{2,54}=1.293$ ;  $P=0.283$ , Table 1a). With all vegetation densities pooled, significantly greater proportions of sticklebacks were observed in vegetated habitat at high ( $m=0.839\pm 0.019$  S.E.) and medium ( $m=0.798\pm 0.026$  S.E.) turbidity than low turbidity ( $m=0.659\pm 0.035$  S.E.) (Tukey's test,  $P<0.05$ ), although high and medium turbidity levels were not significantly different from one another (Tukey's test,  $P=0.545$ ). The predator introduction significantly influenced stickleback sheltered habitat use (Repeated

**Fig. 1** Proportion of sticklebacks in the sheltered habitat (mean  $\pm$  1SE) under different shoot densities: **a** low, **b** medium, and **c** high. Turbidity levels are represented by colour gradation: low (open square, light grey line), medium (dark grey square/line) and high (black square/line)



Measures ANOVA:  $F_{1,54}=132.395$ ;  $P<0.0001$ , Table 1b); predator presence caused a significantly higher proportion of sticklebacks to move into sheltered habitat ( $m=0.838\pm 0.016$  S.E.) than predator absence ( $m=0.693\pm 0.016$  S.E.). However, this predator effect interacted with vegetation density at a marginally significant level ( $F_{2,54}=2.852$ ,  $P=0.066$ ; Table 1b). Subsequent visual inspection of interaction plots showed a trend of increased sheltered habitat use with higher densities of vegetation. To better identify the source of variation in the interaction, separate paired  $t$ -tests were conducted comparing mean distribution between control and risk periods for the three vegetation densities. We found the greatest difference occurred at high densities ( $t=-7.957$ ;  $P<0.001$ ), followed by medium ( $t=-7.101$ ;  $P<0.001$ ) and low ( $t=-4.888$ ;  $P<0.001$ ) densities (Fig. 2). The interaction between observation period (before and after predator introduction) and turbidity was not significant (Repeated Measures ANOVA:  $F_{2,54}=0.926$ ,  $P=0.402$ ; Table 1b).

Stickleback activity levels were generally lower after predator introductions when compared to control periods for all combinations of turbidity and shoot density levels (Fig. 3). However, repeated measures ANOVAs found that stickleback activity levels were dependent on both turbidity and shoot density levels (Table 2). Stickleback activity was significantly reduced at high shoot density for low turbidity ( $F_{1,13}=7.231$ ;  $P<0.05$ ), medium shoot density for medium turbidity ( $F_{1,13}=9.775$ ;  $P<0.05$ ), and medium ( $F_{1,13}=15.346$ ;  $P<0.01$ ) and high ( $F_{1,13}=8.361$ ;  $P<0.05$ ) shoot densities for high turbidity. Despite a trend towards lowered activity levels after predator introductions, the low shoot density did

not significantly reduce stickleback activity at any turbidity level.

## Discussion

Our mesocosm experiments confirmed stickleback preference of vegetated over open space habitats as sticklebacks aggregated in sheltered, vegetated habitat even in the absence of a predator (Fig. 1). The adherence to this type of habitat reflects this species' affinity to structurally complex environments in the wild; in natural conditions, sticklebacks are generally encountered over seagrass (e.g. *Zostera marina*) or rock weed (*Fucus*, *Ascophyllum* sp.) where they feed on crustaceans, insect larvae, small fish fry, fish eggs, and other small prey (Wootton 1976). As such, preference for sheltered areas by this naturally seagrass-associated species was anticipated in these experiments regardless of predator presence.

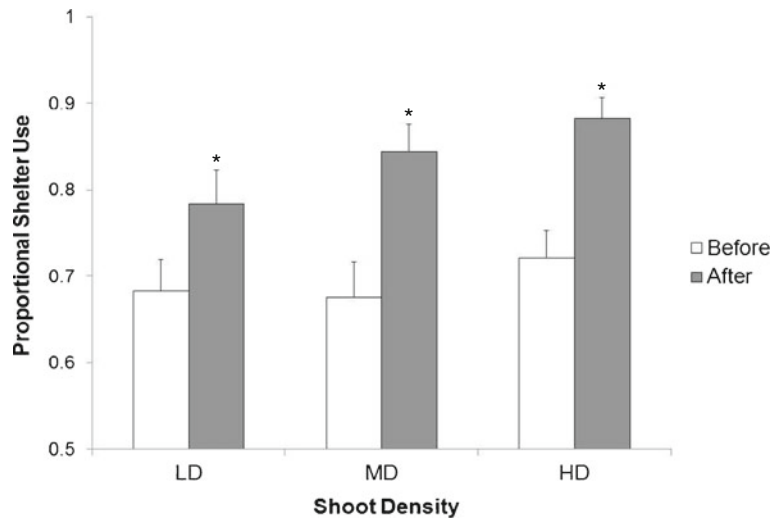
Several studies emphasize the importance of habitat complexity and its role in governing predator-prey interactions (Heck and Thomson 1981; Robertson 1984; Nelson and Bonsdorff 1990). Although we observed general use of sheltered habitat across all treatments in this experiment, sticklebacks responded to predator introductions by significantly increasing their use of the vegetated area and generally reducing overall activity levels. We attribute the increased use of sheltered habitat as an antipredator strategy by the sticklebacks, since a multitude of studies indicate the foraging efficiency of predators is significantly reduced in these seagrass habitats (Orth et al. 1984; Heck and Crowder 1991; Heck

**Table 1** Results from repeated measures ANOVA on the impact of Vegetation Density and Turbidity on stickleback distribution based on 10 min mean distributions before and after predator introduction (observation period = obs. period). Results are shown for between- (a) and within-subjects (b) effects. Items in bold represent significant values ( $P<0.1$ )

Source	SS	df	MS	F	P
<b>a - Between subjects</b>					
Vegetation density	0.155	2	0.077	1.293	0.283
Turbidity	1.069	2	0.534	8.929	<b>&lt;0.0001</b>
Vegetation density $\times$ Turbidity	0.466	4	0.116	1.945	0.116
Error	3.232	54	0.06		
<b>b - Within subjects</b>					
Obs. Period	1.178	1	1.178	132.395	<b>&lt;0.0001</b>
Obs. Period $\times$ Vegetation density	0.051	2	0.025	2.852	<b>0.066</b>
Obs. Period $\times$ Turbidity	0.016	2	0.008	0.926	0.402
Obs. Period $\times$ Vegetation density $\times$ Turbidity	0.034	4	0.008	0.954	0.44
Error	0.48	54	0.009		



**Fig. 2** Proportional shelter use of sticklebacks (mean  $\pm$  1SE) at three different shoot densities (low (LD), medium (MD) and high (HD)) before (*open bars*) and after (*dark bars*) predator introduction to the mesocosm. Asterisks (\*) indicate significant differences in shelter use between before and after predator introduction (Paired *t*-test,  $P < 0.001$ )

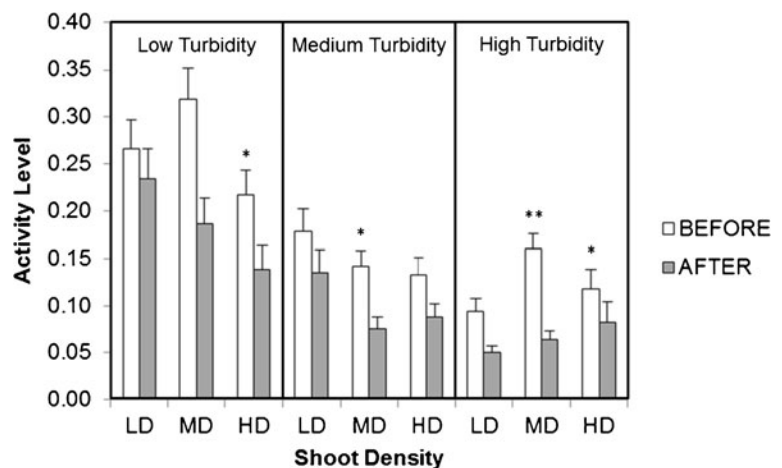


et al. 2003; Heck and Orth 2006). Although sticklebacks significantly increased shelter use under predation risk at all vegetation densities, the noticeably greater use at higher shoot densities (Fig. 2) suggests these prey were sensitive to habitat availability. At lower vegetation densities (i.e., habitat limiting conditions), protection may be compromised as individuals crowd the less abundant shoots of seagrass. As such, sticklebacks remained active around the mesocosm during low density treatments in the presence of the perch, regardless of turbidity level. These findings indicate that higher shoot densities ( $>400$  shoots/m<sup>2</sup>) may play an important role in reducing predation risk of three-spine sticklebacks, yet comparable densities have become increasingly rare in the Baltic Sea.

Interestingly, Snickars et al. (2004) observed sheltered habitat use of 0+ perch decreased with increasing density of vegetation in clear conditions (1–4 NTU). They suspected that, in more complex environments, space was too reduced and may have negatively impacted prey maneuverability (sensu Bartholomew et al. 2000). The disparity in complex habitat use between sticklebacks and perch may be related to structural differences between rigid wooden dowels and more flexible polypropylene ribbon, or due to behavioral differences between the two species.

As a schooling and social species, three-spined sticklebacks may more effectively communicate predation threats to conspecifics than more solitary perch (Frommen et al. 2007). Schooling behavior is known

**Fig. 3** Activity levels of sticklebacks (proportion of individuals shifting habitats during a 10-min period, mean  $\pm$  1SE) before (*open bars*) and after (*shaded bars*) the predator introduction to the mesocosm. Graphs in different panels refer to different turbidity levels: low (*left panel*) to high (*right panel*) turbidity. Asterisks indicate significant differences in activity before and after predator introduction (Paired *t*-test, \* $P < 0.05$ , \*\* $P < 0.01$ )



**Table 2** Results from separate paired t-tests on the impact of vegetation density and turbidity on stickleback activity levels before and after predator introduction

Turbidity	Density	SS	MS	F	P
LT	LD	0.006	0.006	0.457	0.524
LT	MD	0.108	0.108	3.631	0.105
LT	HD	0.059	0.059	7.231	<b>0.036</b>
MT	LD	0.023	0.023	1.223	0.311
MT	MD	0.057	0.057	9.775	<b>0.020</b>
MT	HD	0.031	0.031	4.753	0.072
HT	LD	0.019	0.019	2.755	0.158
HT	MD	0.091	0.091	15.346	<b>0.008</b>
HT	HD	0.042	0.042	8.361	<b>0.028</b>

to increase foraging efficiency in a wide variety of taxa, but may also reduce mortality of prey when confronted with predators. Traveling around the experimental mesocosm as a school may have thus reduced stickleback sensitivity to predator introductions.

Past studies show that increased turbidity could be detrimental to prey due to a reduced reaction distance relative to the predator (Vinyard and O'Brien 1976; Hecht and van der Linger 1992; Miner and Stein 1996). While turbidity significantly influenced stickleback distribution behavior in the absence of a predator, we found no impact of this factor on shelter use with a predator present. Contrastingly, Snickars et al. (2004) work on 0+ perch found that, under predation risk, these fish used significantly less vegetated habitat at higher turbidity levels. This finding suggested that perch

**Table 3** Summary table of select predator-prey studies that examined the effect of turbidity and habitat complexity on a variety of parameters (1 – Survival: Gregory and Levings, 1996; 2 – Survival and growth: Skov et al. 2002; 3 – Antipredator behaviour: Snickars et al. 2004; 4 – Foraging and prey selection:

Stuart-Smith et al. 2004, 5 – Antipredator behaviour: Present Study). Under findings, we show the conclusions based on the turbidity effect (T), vegetation (V) and, in some cases, the effect of turbidity and vegetation interaction (TxV)

Study	Prey	Predator	Turbidity type Levels (NTU)	Vegetation type Densities (m <sup>-2</sup> )	Findings
1	<i>Oncorhynchus tshawytscha</i> <i>Oncorhynchus keta</i> <i>Oncorhynchus nerka</i>	<i>Oncorhynchus clarkii</i>	Resuspended sediment 0.5–2.4 NTU 12–87 NTU	Polypropylene rope 1000	T: Generally not significant V: Higher survival
2	<i>Esox lucius</i>	<i>Esox lucius</i>	Copper Chlorophyllin 0, 20 cm secchi depth	<i>Phragmites</i> mimics Artificial ivy n/a	T: No effect on survival/growth V: No effect on growth, slight effect on survival
3	<i>Perca fluviatilis</i>	<i>Esox lucius</i>	Clay 1–4 NTU 12–16 NTU 25–36 NTU	<i>Phragmites</i> mimics 97 383 960	T: Greater shelter use at low levels V: Density effect in clear water, not turbid TxV: Reduced shelter use with increased shoot density at low turbidity
4	<i>Austrochilonia australis</i> <i>Daphnia carinata</i>	<i>Galaxias auratus</i>	Various Material 0 NTU 50 NTU 100 NTU	Macrophytes n/a	T: No effect on foraging, prey selection V: Influences prey selection
5	<i>Gasterosteus aculeatus</i>	<i>Perca fluviatilis</i>	Unicellular Algae 2–3 NTU 7–9 NTU 13–15 NTU	<i>Zostera</i> mimics 100 400 800	T: Greater shelter use at high levels, no effect on antipredator behaviour V: Greater shelter use at high levels under predation risk TxV: No effect

antipredator behavior may be reduced at higher turbidity levels (Snickars et al. 2004). The lack of predator influence with turbidity on sticklebacks may be explained by the prey having already been situated in this habitat prior to predator introduction, and therefore sticklebacks were pre-distributed in a protective environment. Thus, unlike perch in Snickars et al. (2004), sticklebacks may not have perceived the turbid environment as shelter from predator presence and instead utilized available habitat as a mechanism to further reduce predation risk. At higher turbidity levels (>5 NTU), sticklebacks may recognize greater predation risk due to the inability to register the location of a predator until within close proximity. Thus, in a deteriorated visual environment, sticklebacks may quickly adhere to available sheltered habitat and reduce activity levels to decrease their risk of being encountered. Despite being at the highest turbidity level (i.e., highest hypothesized cover), sticklebacks in this environment more significantly reduced their activity levels when compared to other turbidities. In relatively clear conditions (0–3 NTU), prey have increased visual field and may more adeptly perceive predation risk. As a result of this recognition of heightened “security,” prey may be less threatened to move around freely in low turbidity conditions. Thus, while turbidity may not always directly affect the antipredator behavior of prey, the overall response of prey to this property (i.e., increased use of complex habitats and reduced overall activity) may represent an adaptation to ensure survival when predators arrive in that habitat.

Some studies of fish habitat preference have counted the number of individuals in a particular habitat after a pre-determined time (Snickars et al. 2004). In essence, this technique represents a single “snapshot” of prey distribution, without an appreciation of the various movements that may occur throughout the trial run. In this experiment, we utilized video footage to grab multiple minute-by-minute observations of stickleback habitat choice behavior over a variety of environmental conditions. Indeed, such a method for understanding habitat selection is limited by the conditions as very turbid water can cause issues with video analysis. It should also be noted that we used a relatively low range of turbidity levels (2 NTU – 17 NTU) and a reduced time scale compared with other studies (Table 3). Despite this reduced range, we still observed significant changes in prey distribution and activity levels with a small change in algal turbidity. Our study therefore demonstrates that relatively minute changes in turbidity

may have significant impacts on antipredator capacity of fishes, which should be strongly considered in future studies of these taxa.

## Conclusion

Behavioral decisions by animals are made based on both energy intake and predation risk. In this study, we only focused on antipredator behavior and found an effect of vegetation density and not turbidity, though we recognize turbidity influences overall prey distribution and activity levels and thus may indirectly reduce risk. For a more comprehensive understanding of the influence of various anthropogenically-induced environmental changes on three-spined stickleback, future work should simultaneously examine how foraging behavior and habitat choice are influenced by turbidity, habitat complexity and predator presence. For example, similar experiments to the one herein could include food resources for prey animals. This would examine tradeoffs between predation risk and energy acquisition, and build a more comprehensive understanding of the influence of various environmental conditions on prey survival. Integrative and experimental approaches like these would likely enhance our ability to estimate the impacts of anthropogenic inputs on complex ecological interactions of the Baltic Sea.

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