Effect of sediment, salinity, and velocity on the behavior of juvenile winter flounder (*Pseudopleuronectes americanus*)

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Abstract

Winter flounder (*Pseudopleuronectes americanus)* is a benthic flatfish that is economically important for recreational and commercial fishing in North America. In the last twenty years, the species has undergone a drastic decline, mainly due to anthropic influence. The goal of this study was to gain knowledge on habitat preferences and behavior of juvenile winter flounder to improve the management of natural stocks and optimize release sites of juveniles produced for stock enhancement. Three abiotic factors (sediment, current, and salinity) potentially influencing the distribution of flatfish species were tested in a recircurlating flume with juvenile winter flounder. Time budgets of observed behaviors including swimming, orientation, and burying capacity were analyzed. Sediment texture was the only factor that significantly influenced the burying behavior of winter flounder juveniles; shear velocity, salinity, and sediment had no effect on the orientation of juveniles.

Keywords: Winter flounder, sediment, salinity, current, swimming behavior

Introduction

Winter flounder (*Pseudopleuronectes americanus*) is a eurythermal and euryhaline flatfish found in the shallow coastal waters of North America from Georgia (U.S.) to the Labrador coast (Canada) (Scott and Scott 1988). This species supports a commercial fishery in Canada and the United States that is mainly driven by market price (Atlantic States Marine Fisheries Commission, 2012; Fisheries and Oceans Canada, 2012). Because the American and Canadian stocks have declined since the 1980s, the conservation of winter flounder populations is a major concern on the east coast of the United States (Atlantic States Marine Fisheries Commission 2012; Fisheries and Oceans Canada 2012). To support sport fishing, the release of hatchery-reared juveniles to stimulate the renewal and size of natural populations is an option to be considered (Fairchild 2010). However, the survival rate of released juveniles remains low (Fairchild and Howell 2004; Fairchild 2013). The lack of natural stimuli in the hatchery environment could suppress anti-predatory behaviors or decrease cryptic abilities when juveniles are released in the wild (Kellison et al. 2000; Fairchild and Howell 2004).

Young-of-the-year (0+) flounder are found over a wide range of depths and sediment types (Able and Fahay 1998), but habitat preference seems to be size dependent, as seen in both laboratory and field experiments (Phelan et al. 2001). While juveniles from 50 to 95 mm in size prefer sandy substrate (Phelan et al. 2001), 0+ may prefer cobble of an intermediate complexity (Pappal et al 2009). High densities of 0+ have also been observed in eelgrass habitats (Lazzari 2015) and the presence of prey has been shown to modify habitat selectivity (Gibson 1994; Phelan et al. 2001; Fairchild and Howell 2004). The presence of complex three-dimensional structures such as macroalgae or pebbles has also been shown to influence burying behavior (Stoner et al. 2001; Fairchild et al. 2005; Pappal et al. 2009). However, 1- to 3-year-old fish have been far less studied than 0+ winter flounder juveniles.

The effects of both salinity and the benthic boundary layer flows on the behavior of juvenile winter flounder have been poorly documented. Wirjoatmodjo and Pitcher (1984) suggested that salinity likely has a limited impact on the distribution of estuarine fish based on their adaptive osmoregulatory capacity, but the abundance of *Pleuronectes platessa* juveniles seems to be salinity dependent (Poxton and Nasir 1985). Greer Walker et al. (1978) suggested that *Limanda yokohamae* juveniles use tidal currents to preserve their energetic reserves, but the effect of currents on the energy budget is not available for *P. americanus*. Juveniles and adults have been observed to undergo foraging tidal migrations (Tyler 1971), but the main mechanisms driving these migrations remain largely unknown.

The overall aim of this study was to define the most suitable habitats for juveniles. This type of information could be useful for managing releases of hatchery-produced juveniles through a restoration program, for fishing management, and for the development of marine protected areas. The objectives of this study were to test how the burying, orientation, and swimming behaviors of 2+ hatchery-reared juvenile winter flounder are influenced by sediment texture, salinity, and shear-stress intensity. Our first hypothesis was that the time spent in positive rheotaxis would increase proportionally when shear stress increases. We also hypothesized that the rate of burial would be higher in finer sediment, and that no effect of salinity would be found on any of the three behaviors studied.

Methods

Juvenile hatchery conditions

Two-year-old winter flounder juveniles (mean length 10.35 ± 0.82 cm, mean weight 17.1 ± 3.4 g) produced at the Pointe-au-Père Aquaculture Research Station (ISMER, UQAR, Quebec, Canada) were used for this study. Scott and Scott (1988) reported a mean length of 11.4 cm in Passamaquody Bay (NB, Canada) and 17.8 cm in St. Marys Bay (NS, Canada) for two-year-old wild winter flounder. Considering that these areas are warmer than the St. Lawrence Estuary, the size of juveniles likely was close to the wild juveniles from this area. In the Gulf of St. Lawrence, length at 50% maturity has been estimated to be 21 cm in males and 24 cm in females (DeCelles and Cadrin 2011). Egg fertilization was done according to Ben Khemis et al. (2000), and larval and post-settlement juvenile rearing followed Vagner et al. (2013). During the experimental period, juveniles were reared in rectangular open-flow tanks supplied with filtered sea water (50 μm, 5 L min^{-1}) pumped from the St. Lawrence Estuary; the inlet was 1 km off shore from the station. Tanks were exposed to artificial light

(6.5 μEinstens m^{-2} s^{-1}, natural photoperiod). Commercial filtration sand was used as the substratum and juveniles were fed with a commercial food (Lansy microdiet, INVE Aquaculture Inc.) at a daily rate of 3% of their body weight.

Flume experiments

Experiments were conducted using the Aquatron racetrack flume at the Aquaculture Research Station. This recirculating flume was designed to generate steady, turbulent, benthic boundary flows induced by the friction of 12 rolling plastic disks (0.5 cm thick). A description of the benthic boundary layer (BBL) conditions and the flume's technical details can be found in Redjah et al. (2010). Briefly, the experimental zone (91.5 × 45 cm) was filled with a layer of at least 5 cm of sediment. For each trial, water depth was set at 15 cm to maximize development of the BBL (Olivier et al. 1996), resulting in a water volume of 800 L.

Two weeks before the beginning of the experimental period, which lasted four weeks (29 May to 24 June 2015), we decreased the salinity in two of the four rearing tanks to 15‰ by mixing saltwater with dechlorinated tap water. The other two tanks were subjected to natural salinity (26.44 ± 0.76‰) and temperature (6.53 ± 0.62°C) variations.

For each experiment, five juvenile winter flounder were randomly chosen from the two saltwater or brackish water tanks for the salinity treatment. Different juveniles were used for each experiment, for a total of 100 individuals for the whole experimental period. Four treatments with five replicates each (total of 20 trials) were evaluated. Each treatment included one salinity (15 or 25) and one sediment (gravel [-1 phi, 2.23 mm] or sand [+1 phi, 0.75 mm]) type, for a total of four possible combinations. The mean shear velocity (u*) was increased from 0.26 to 1.78 cm s^{-1} for the sandy sediment and from 0.35 to 2.14 cm s^{-1} for the gravel. This corresponds to an increase in current speed interval (Uz) from 5 cm s^{-1} to 30 cm s^{-1} for the two sediment types. Five Uz values were considered for the analysis: 10 min plateaus of 5, 5 to 20, 20, 20 to 30, and 30 cm s^{-1} (Fig. 1). An increase of Uz = 5 cm of 1 cm s^{-1} per minute was used to avoid substrate erosion.

The same protocol was used for each trial. One day before an experiment, the flume was emptied and rinsed with freshwater. Sediments to be tested were disinfected with a Vircon solution (10 g per 1 L of water; Vircon, Vétoquinol, Lavaltrie, Quebec, Canada) for 12 h and then rinsed with freshwater for another 12 h. Sediment was placed in the experimental section and the flume was filled with either brackish or salt water. Batches of juveniles, which were transported in a jar with a solution of 0.13 L of stresscoat per 1 L of brackish or salt water (Stresscoat+, Mars Fishcare Inc., Hamilton, PA, USA) then were introduced into the experimental zone. Because preliminary tests showed that the juvenile winter flounder dispersed throughout the flume, we installed plastic grid barriers to restrict them to the experimental section and applied a low current (shear velocity less than u* = 0.20 cm s^{-1}) and aeration for the night. This acclimation period lasted for 16 hours, during which the photoperiod and light intensity conditions were similar to those of the rearing tanks.

Juveniles were starved for 24 h before the beginning of the experiments to avoid digestion during the experiments, which could potentially reduce their activity. The following morning, the barriers were removed and the experiment started with the increase in u* as detailed in Figure 1. Flounder juveniles were weighed and measured after each trial. Because the flume was located in a room with no air-temperature control, seawater temperature increased from 7 ± 1°C at the beginning of each trial to 14.25 ± 1°C at the end. This means that the acclimation period in the flume started at the juvenile rearing temperature but that observations were made at a higher temperature range. Experimental conditions were the same for all trials.

A GoPro HERO3 Silver Edition camera (GoPro Inc., San Mateo, California, USA) was used in dorsal view to record fish behavior in the experimental zone during all experiments. Based on all video recordings, we identified three types of behavioral responses of juvenile winter flounder to salinity and sedimentary treatments: swimming activity, orientation relative to the main current, and burying ability. To establish time budgets (% of active behavior per observation period), we only considered the activity of flounder in the experimental zone. Moreover, individuals staying more than 75% of the observation period outside of the experimental zone were excluded from the time-budget analyses of behaviors for that observation period. We adopted this approach to avoid attributing a very high behavior score to a fish staying most of its time outside the experimental zone.

Behavioral variables

Several variables were defined according to the particular type of behavioral response. Burying-dependent variables included six states: “not buried,” “body covered less than 25% by sediment,” “body covered from 25% to 50%,” “body covered from 50% to 75%,” “body covered from 75% to 100%,” and “totally buried.” Orientation-dependent variables included “positive rheotaxis,” “negative rheotaxis,” and “transverse position.” The first two situations were scored if juveniles were at least at a 70 degree’ angle from the transverse position (for scoring of orientation, see Champalbert et al. 1994). Orientation variables were only scored when juveniles were in contact with the sediment. Variables related to swimming activity included: “swimming close to the sediment with periods of rest,” “passive drifting,” “swimming far from the sediment,” and “carried away by the current.” These four swimming variables were combined to form the variable “total swimming activity.” Time spent outside the experimental zone also was recorded. The Observer XT 9 software (Noldus Information Technology B.V., Wageningen, Netherlands) was used to analyze the videos and create the time-budget database.

Statistical analyses

The effect of salinity and sediment treatments as well as the shear velocity range on behavioral variables (burying, orientation, and swimming) were analyzed by three-way ANOVA with repeated measures for each hydrodynamic level using STATISTICA v6.0

(Dell Inc., Tulsa, Oklahoma, USA). Normality was verified with the Kolmogorov-Smirnov test and heteroscedasticity with Levene's test. We performed a square-root transformation on "total swimming activity" and "not buried" data to attain normality. We also combined the "75% buried," "50% buried," and "25% buried" variables into a single "25% to 75% buried" variable to allow data normality. We were not able to analyze all the data relative to the "less than 25% buried" variable because this condition did not occur for sand treatments. We thus only analyzed data associated with the gravel treatment for that variable. It should be noted that treatment effects of the whole set of variables also were analyzed using a three-way PERMANOVA. Data were transformed prior to the analysis (square root of arc cosinus). The software Primer 6.1.1.12 and the PERMANOVA+ add-on (PRIMER-E Ltd, Ivybridge, United Kingdom) were used. The PERMANOVA analyses (9999 permutations) were based on an Euclidian distance matrix. Homoscedasticity was tested with the PERMDISP procedure ($p < 0.05$). Because the results were similar to those obtained with ANOVA, they are not presented here. Length and weight of juveniles were compared with a two-way ANOVA (sediment and salinity factors) to validate the absence of differences between treatments. We did not use these data as covariates because no differences between treatments were found ($p > 0.05$).

Results

Sediment treatment was the only factor that significantly influenced the burying behavior of winter flounder juveniles (Table 1A, 1B &1C), yet sediment had no effect on rheotaxis behaviors (Table 1D, 1E & 1F). The time budgets of the three burying variables (100% buried, 25–75% buried, and not buried) were significantly different between sand and gravel treatments. Burying behavior was hindered in the gravel treatment: juveniles spent significantly more time not buried or 25% to 75% covered in the gravel treatment experiments (Fig. 2). Close to 80% of juveniles tested on sand were 100% covered in sediment compared to less than 10% in those tested on gravel (Fig. 2).

Neither shear velocity, salinity, nor sediment affected juvenile orientation (Table 1D, 1E & 1F); our first hypothesis was then rejected. The only significant interaction was found between sediment and u* (Table 1G), and this was explained by a significant increase in flounder found outside the experimental zone when submitted to high shear velocities with gravel in the experimental zone (Fig. 3). At intermediate u*, i.e., from 0.29 to 1.18 cm s^{-1} for sand and 0.35 to 1.42 cm s^{-1} for gravel (Uz corresponding to 5 cm s^{-1} to 20 cm s^{-1}), the average percentage of time spent in the experimental zone in positive rheotaxis, negative rheotaxis, or in a transverse orientation were 30.4 ± 6.1, 30.1 ± 6.1, and 37.5 ± 4.8%, respectively. Orientation was not affected by any of the three variables (Table 1D, 1E, 1F). Although shear velocity did not influence the total swimming activity (Table 1H), there was no occurrence of “passive drifting” or “carried away by the current” during the first two observation periods for shear velocity. We could only determine time budgets for these variables starting at

$u^*=1.18$ cm s^{-1} for sand and 1.42 cm s^{-1} for gravel (Uz of 20 cm s^{-1}). For the "passive drifting" swimming type, fish propelled themselves in the water column close to the sediment in the same direction as the flow and glided by being pushed by the current. Individuals were seen in various conditions floating through the experimental zone without touching the bottom. A few individuals attempted to glide, but pushed themselves too far from the bottom ($z>5$ cm) and were carried away by the flow while tumbling backwards. Thus, the swimming type "carried away by the flow" only happened when fish were swimming against the flow. At high hydrodynamic conditions, swimming far from the bottom was the least efficient swimming response. Without quantifying it, we observed that the heads of fish were higher than the rest of the body when they propelled themselves in the water column. No fish was carried away when buried or resting on top of sediment.

Discussion

Swimming behavior

The first contribution of this work is the detailed characterization of the swimming modes used by winter flounder juveniles. While the experimental conditions did not modify the time spent in total swimming activity, we observed that more individuals used the flow and were passively transported at high shear velocities ($u^* > 1$ up to 2.14 cm s^{-1}). Below this threshold value, we did not observe any passive drifting. Indeed, mathematical calculations previously showed that passive drifting can reduce the cost (energy consumption) of swimming by 90% per unit distance in juvenile flatfishes (Weihs 1978), but such transport is restricted to the direction of the flow. When studying tidal migrations of juvenile and adult winter flounder with underwater cameras at Passamaquody Bay (NB, Canada), Tyler (1971) observed that fish followed the direction of the tidal current. Although Tyler (1971) did not describe the type of swimming, it is reasonable to hypothesize that passive drifting was used during these tidal migrations. More recently, He (2003) reported an average swimming speed of 0.96 body lengths per second at 4.4 °C in adult winter flounder (27 to 48 cm), which corresponds to adult size.

In the present study, a few cases of juveniles unable to withstand the flow were observed (“carried away by the flow” behavior), but only when fish were swimming away from the sediment. We speculate that by exposing more body surface to the flow, juveniles increase the drag force, causing them to tumble backwards. When this happens, they are no longer able to swim, and some individuals tumbled backwards over the entire flume. This could be problematic in natural habitats because the fish could be carried away to unsuitable habitats.

The behavior “swimming close to the sediment with periods of rest” was expressed by juveniles actively swimming close to the sediment with clear fin movement, often for a short distance followed by a rest period. This type of swimming was observed in all directions at all the tested shear velocities, and visual observations suggest that the juveniles glided because of fin movements and not because of a flow effect. This type of swimming has also been observed in juvenile plaice in natural habitats (Gibson 1980). When actively swimming close to the bottom, juvenile winter flounder were not using BBL flows for transport. Although our experimental design was such that fish observations were made only from the flume's water surface, we hypothesized that transport was driven by fin movements and body propulsion. The resting periods observed between these short swimming activities could indicate that this type of swimming was energetically costly. The experimental flume work of Joaquim et al. (2004) used a cardiac function test to demonstrate this energy demand for a similar swimming type in adult winter flounder. However, the major advantage of this type of swimming compared to passive drifting is that fish can swim in any direction relative to the flow while remaining close to the sediment.

In contrast to sandy treatments, juveniles that had settled on gravel left the experimental zone significantly more often and remained longer at rest on the flume's bottom under high shear velocity conditions. This fish movement was clearly active: no sediment erosion occurred and fish were not passively carried away. Juvenile plaice and turbot tested for sediment selection in the laboratory were observed to favor a bare surface over a coarse substrate (Nasir and Poxton, 2001); this may suggest that coarse sediments are unsuitable substrates for juveniles. Indeed, areas of high hydrodynamics associated with coarse sediments have been shown to reduce growth in juvenile winter flounder in a New Hampshire estuary, and it was suggested that the energy spent in unsuccessful burying could explain the low growth rates (Fairchild et al. 2005).

If these movements were active, why would fish select a bare surface over gravel? When submitted to high current speeds, winter flounder juveniles could use postures to prevent being carried away by the current, as described for plaice in Arnold and Weihs (1978) and Gerstner and Webb (1998). Behavioral responses include evacuating water under the body by fin burying, body undulations, and fin beating. Similarily, it was observed that juvenile sturgeons (*Acipenser brevirostrum*) increase station-holding with high current velocity, probably to reduce energetic costs from swimming (Kieffer et al. 2009). We can hypothesize that juveniles were not able to burrow deep enough into coarse sediments (lack of strength) or correctly perform the postures to prevent themselves from being carried away by the current. We suggest that it was easier to maintain contact with the bottom on resin-covered wood than on gravel. Unfortunately, the walls of the flume were opaque, preventing lateral observations of the fish.

Effects of sediment and salinity on juvenile flounder behavior

The selectivity of winter flounder juveniles for sandy over gravelly sediments was evident in the present work, strengthening previous results of experiments conducted on both cultured and wild juveniles (age-0 winter flounder: gravel [18 mm], coarse sand [1.55 mm], fine sand [0.137 mm], Manderson et al. 2000; age-0 winter flounder, muddy sand [0.27 mm], fine sand [0.21 mm], coarse sand [0.54 mm], fine gravel [1.58 mm], gravel [3.21 mm], Phelan et al. 2001; 20–32 mm juveniles, small grain size [250–1000 mm], large grain size [1000–2000 mm], Fairchild and Howell 2004) as well as from caging studies and beam trawl field surveys (Goldberg et al. 2002). However, none of these cited studies integrated time-budget assessments or characterization of swimming behaviors and orientation in BBL flows, especially when considering shear velocity – sediment texture interactions.

In sand, fish were able to cover themselves entirely just after their introduction to the flume. Ellis et al. (1997) demonstrated that the cryptic ability in plaice is a learned behavior and observed that flatfish reared without sediment in tanks need time to learn how to bury themselves properly. Our juveniles were reared in tanks filled with filtration sand from hatch until metamorphosis, which could explain the high burying speed observed in our study.

In our work, salinity had no influence on the behavior or selectivity of sediment by the flounder. We chose to acclimate juveniles to the salinity conditions of the two treatments (salinity of 15 and 25 for at least one week of acclimatization) before transfer to the flume to avoid osmotic shock that could have generated subsequent erratic or unnatural behavioral responses.

Orientation

Unlike what has been shown in other flatfish species, our results did not indicate that juvenile winter flounder adopt a rheotaxic position with increasing current, which should be the most hydrodynamic position (Arnold and Weihs 1978). Rheotaxis has been observed at current speeds lower than the maximum value used in our study in many flatfish species such as adult plaice on a bare surface (Arnold and Weihs 1978) and juvenile sole on sediment (Champalbert and Marchand 1994). In sole, only buried juveniles could withstand a current of 20 cm s^{-1} when placed in a hydrodynamic tunnel with a bottom covered with sediments (Champalbert and Marchand, 1994). This study was carried out on smaller fish (2–3 cm) than the ones we used and they positioned themselves in positive rheotaxic positions as soon as the current increased. It is possible that rheotaxis and burial are mechanisms used by the juvenile sole to resist current. The absence of such behaviors in winter flounder juveniles could be explained by a strong adhesion to the substrate: no juvenile flounder on the ground (covered or not with sediments) was carried away by the force of the current, even at a speed of 30 cm s^{-1}. The only observations of animals carried by the current were made on individuals swimming in the water column.

Conclusion

Our results clearly indicate that winter flounder juveniles use different types of swimming depending on current speed, that they more readily abandon a gravel bottom for bare smooth surfaces at high shear stress, and that they have a higher burial rate in sand. In both sediment types, the effect of salinity on swimming, orientation, and burying behaviors was negligible. This information is not only of interest in terms of the ecological understanding of habitat preference, but also can be used to facilitate habitat selection for the purpose of population enhancement. Our results indicate that coastal areas characterized by fine sediment and low current speeds have a high potential for the successful establishment of juvenile winter flounder nurseries.

A better understanding of the effects of abiotic factors on the behavior of juvenile winter flounder will improve our understanding of their distribution and habitat preferences. The release of juveniles in unsuitable environments, i.e., with regard to hydrodynamics and sediment texture, may induce greater dispersion rates. Unnatural behaviors of juvenile cultured flatfishes, such as spending more time off-bottom, have been shown to increase the risk of predation (Kellison et al. 2000; Fairchild and Howell 2004). Our finding that the presence of high current speeds and gravel sediment increases swimming activity in fish can lead to an informed choice of release environments for cultured juvenile winter flounder that could prevent dispersion and reduce their visibility to predators.

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Table 1: ANOVA table for the different types of measured behaviours (Table sections A through H)

Variable tested	A - Time budget of "100% buried"				B - Time budget of "25% to 75% buried"			
	SS	DF	F	P	SS	DF	F	P
Slope intercept	14.657	1	120.99	<0.001	15.442	1	104.054	<0.001
Sediment	11.229	1	92.696	<0.001	2.525	1	17.015	<0.001
Salinity	0.057	1	0.471	0.502	0.021	1	0.144	0.709
Sediment x Salinity	0.056	1	0.469	0.503	0.017	1	0.116	0.737
Error	1.817	15			2.226	15		
Shear stress	0.023	4	0.423	0.791	0.161	4	1.59	0.188
Shear stress × Sediment	0.058	4	1.061	0.383	0.023	4	0.233	0.918
Shear stress × Salinity	0.013	4	0.24	0.914	0.109	4	1.076	0.375
Shear Stress × Sediment × Salinity	0.04	4	0.725	0.577	0.114	4	1.12	0.355
Error	0.828	60			1.527	60		
	C - Time budget of "not buried"				D - Time budget of "positive rheotaxis"			
	SS	DF	F	P	SS	DF	F	P

Slope intercept	4.832	1	47.905	<0.001	8.124	1	38.657	<0.001
Sediment	1.748	1	17.336	<0.001	0.147	1	0.699	0.416
Salinity	0.05	1	0.503	0.488	0.086	1	0.411	0.53
Sediment × Salinity	0.006	1	0.068	0.796	0.031	1	0.147	0.705
Error	1.513	15			3.152	15		
Shear stress	0.198	4	1.333	0.267	0.03	4	0.213	0.929
Shear stress × Sediment	0.3	4	2.018	0.103	0.336	4	2.34	0.065
Shear stress × Salinity	0.078	4	0.53	0.713	0.12	4	0.834	0.508
Shear Stress × Sediment × Salinity	0.119	4	0.805	0.526	0.122	4	0.852	0.497
Error	2.233	60			2.157	60		

	E - Time budget of "negative rheotaxis"				F- Time budget of "transverse orientation"			
	SS	DF	F	P	SS	DF	F	P
Slope intercept	6.185	1	30.599	<0.001	17.978	1	93.421	<0.001
Sediment	0.001	1	0.009	0.923	0.12	1	0.625	0.441
Salinity	0.032	1	0.154	0.699	0.007	1	0.036	0.85
Sediment × Salinity	0.018	1	0.093	0.763	0	1	0.001	0.97
Error	3.031	15			2.886	15		
Shear stress	0.134	4	1.324	0.271	0.246	4	2.353	0.063
Shear stress × Sediment	0.246	4	2.426	0.057	0.156	4	1.499	0.213
Shear stress × Salinity	0.101	4	1.001	0.414	0.073	4	0.699	0.595

Shear Stress × Sediment × Salinity	0.082	4	0.812	0.522	0.182	4	1.741	0.152
Error	1.524	60			1.569	60		
	G - Time budget of "outside the experimental zone"				H - Time budget of "total swimming activity"			
	SS	DF	F	P	SS	DF	F	P
Slope intercept	10.112	1	99.659	<0.001	0.389	1	39.47	<0.001
Sediment	0.949	1	9.357	0.007	0.015	1	1.557	0.231
Salinity	0.226	1	2.229	0.154	0.001	1	0.118	0.735
Sediment × Salinity	0.032	1	0.323	0.577	0.003	1	0.363	0.555
Error	1.623	16			0.148	15		
Shear stress	0.557	4	9.877	<0.001	0.019	4	0.892	0.474
Shear stress × Sediment	0.376	4	6.67	<0.001	0.011	4	0.507	0.73
Shear stress × Salinity	0.126	4	2.244	0.073	0.047	4	2.137	0.087
Shear Stress × Sediment × Salinity	0.061	4	1.098	0.365	0.003	4	0.172	0.951
Error	0.902	64			0.33	60		

Figure 1: Experimental design for the increase of shear stress according to time for the treatments “sand” and “gravel”. Time = 0 minutes is the beginning of the experiment, after the acclimation period.

Figure 2: Time budget for the behaviors “not buried”, “25%-75% buried” and “100% buried”, observed at current speeds of 20–30 cm s^{-1}. Mean ± SE. All results on gravel are significantly different ($p \leq 0.05$) than the one on sand. N = 10 for each bar.

Figure 3: Current speed and sediment effects (current speed × sediment interaction, $p \leq 0.05$) on time budget for juveniles that spent time outside of the experimental zone. Mean ± SE. Means with different letters are significantly different ($p \leq 0.05$). N = 5 for each bar.