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Burrowing behaviour of the European eel (*Anguilla anguilla*): effects of life stage

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ABSTRACT

The European eel (*Anguilla anguilla*) is a fascinating species, exhibiting a complex life cycle. The species is, however, listed as critically endangered on the IUCN Red List due to an amalgam of factors including habitat loss. This study investigated the burrowing behaviour and substrate preference of glass, elver and yellow stages of *A. anguilla* . Preference was determined by introducing eels in aquaria with different substrates and evaluating the chosen substrate for burrowing. Additionally, burrowing was recorded with a camera in all substrate types and analyzed for kinematics. The experiments showed that all of these life stages sought refuge in the sediments with particle sizes ranging from sand to coarse gravel. Starting from a resting position, they shook their head horizontally in combination with rapid body undulations until half of their body was within the substrate. High-speed X-ray videography revealed that once partly in the sediment, eels used only horizontal head sweeps to penetrate further, without the use of their tail. Of the substrates tested, burrowing performance was highest in fine gravel (diam. 1-2 mm; lower burrowing duration, less body movements and/or lower frequency of movements) and all eels readily selected this substrate for burrowing. However, glass eels and elvers were able to use coarse gravel (diam. > 8 mm) because their smaller size allowed manoeuvring through the spaces between the grains. Further, burrowing performance increased with body size: glass eels required more body undulations compared to yellow eels. Interestingly, the urge to hide within the sediment was highest for glass eels and elvers. Documentation of substrate preference and burrowing behaviour of *A. anguilla* provides new information about their potential habitat use. Considering that habitat alterations and deteriorations are partly responsible for the decline of the eel, this information can contribute to the development of more effective conservation measures.

Keywords: Behaviour, Burrow, European eel, Kinematics, sediment

INTRODUCTION

Despite many biomechanical (e.g. bio-energetic cost; Du Toit et al., 1985) and ecological challenges (e.g. risk of predation for sessile invertebrates (Smith and Merriner, 1985), burrowing is an important behaviour for many invertebrate and vertebrate organisms, both in terrestrial and aquatic environments. Some species constantly occupy the substrate and rarely, if ever, come to the surface, such as South-African mole-rats (*Bathyergus suillus* Shreber 1782; Thomas *et al.*, 2013) and earthworms (*Lubricidae;* Lavelle *et al.,* 2001). Other organisms are active on land or in the water column, but are also able to construct burrows for various purposes. American eels (*Anguilla rostrata* Lesueur 1817), for example, construct tunnels that may be used as a hiding place and/or winter refuge (Tomie *et al.*, 2017). The fivefinger wrasse (*Iniistius pentadactylus* L. 1758) burrows into sandy sediments to sleep subsurface at night (Clark, 1983). The common skink (*Scincus scincus* L. 1758), also known as ‘the sandfish’, uses the substrate for subsurface locomotion (Sharpe *et al.,* 2014; Maladen *et al.,* 2011) while many species bury to avoid predation (Griffiths & Richardson, 2006). In addition, burrows give access to other trophic opportunities and can be used as a base for feeding (Bozzano, 2003).

Some fishes (e.g. jawfishes, *Opistognathidae*) excavate permanent burrows (Colin, 1973), while others create tunnels that collapse behind them (e.g. sand lances, *Ammodytes* spp.) (Bizarro *et al.,* 2016). Many different burrowing mechanisms have evolved depending on the substrate characteristics, the speed of movement, the magnitude of forces exerted and the type of sediment (Herrel *et al.,* 2011). Examples are burrowing by crack propagation (Dorgan *et al.,* 2007), compaction (Wake, 1993), sand swimming (Sharpe *et al.,* 2014; Maladen *et al.,* 2011) and sand-diving (Tatom-Naecker & Westneat, 2018). Also among several Anguilliformes, burrowing behaviour has been observed. Anguilliform fish possess two mechanisms for burrowing: tail-first and/or head-first burrowing (De Schepper *et al.,* 2007a; De Schepper *et al.*, 2007b). *A. rostrata*, for example, construct burrows head-first (Tomie *et al.,* 2013). On the other hand, the spotted garden eel (*Heteroconger hassi* Klausewitz & Eibl-Eibsefeldt 1959) is an obligate tail-first burrower, while the Indian snake eel *(Pisodonophis boro* Hamilton 1822) can use both (De Schepper *et al.,* 2007a).

Surprisingly, although burrowing has already been reported for the European eel (*Anguilla anguilla* L. 1758) (Schafer, 1972; Christoffersen *et al.,* 2018), many questions about its behaviour remain unanswered. *A. anguilla* is a catadromous species, with mature silver eels spawning in the Sargasso Sea (Miller *et al*., 2019). From there, leptocephalus larvae move with the Gulf Stream towards the European and North-African coasts, subsequently metamorphosing into unpigmented glass eels (Arai *et al.,* 2000). Glass eels acquire pigment and turn into elvers. Upon reaching a length of 150 mm, eels are classified as yellow eels, the sedentary growing stage (Tesch, 2003; Lafaille et al., 2003). After 3 – 20 years or more, yellow eels undergo a metamorphosis to silver eels, which migrate downstream into the Atlantic Ocean to spawn and subsequently die (Van den Thillart et al., 2007). Worryingly, *A. anguilla* is currently labelled as critically endangered according to the IUCN Red List (Jacoby & Gollock, 2014), as glass eel recruitment declined to nearly 1.4% compared to the late 70's in the North Sea and to 6% elsewhere (ICES, 2019). A combination of different factors such as habitat loss (Kettle et al., 2011), migration barriers (Durif *et al.*, 2002), non-native parasites (Palstra *et al.,* 2007), overfishing (Dekker, 2003), pollution (Belpaire *et al.,* 2016), climate change and changes in oceanic currents (Castonguay *et al.,* 1994; Munk *et al*., 2010) are considered possible causes for this steep decline. To restore the *A. anguilla* population, EU Member States implemented eel management plans (European Eel Regulation no 1100/2007) to ensure 40% silver eel escapement, defined as the best estimate of the theoretical escapement rate if the stock were completely free of anthropogenic influences (European

Commission, 2007). With habitat loss being one of the threats for *A. anguilla,* insight into substrate preference and burrowing behaviour over different life stages may engender recommendations for both habitat restoration and selection of suitable habitats for restocking. Hence, this may play an important role in restoring the *A. anguilla* stock.

Previous work on American yellow eels found a preference for burrowing into mud substrates (diam. 6-221 µm) during warmer periods and mud and cobble (average diam. 75 mm) in winter (Tomie *et al.,* 2017). For *A. anguilla*, however, it has only been found that elvers prefer coarse gravel for hiding (diam. 12-64 mm; Christoffersen *et al.,* 2018), but this is not necessarily the preferred substrate for burrowing, since elvers can easily enter the interstitial spaces of coarse gravel and therefore do not require active burrowing to enter this substrate (Lecomte-Finiger & Prodon, 1979). In addition, whether other *A. anguilla* life stages show the same sediment preference has not been tested yet. An organism's size relative to that of sediment particles could for example affect burrowing potential and/or sediment preference. Consequently, sediment preference and burrowing performance (*i.e.* burrowing duration, the number and frequency of body movements) might change as the eel becomes larger, with burrowing requiring a relatively higher effort for smaller glass eels compared to larger yellow eels. In addition, the skull of glass eels is not completely ossified yet and thus potentially lacks the robustness required to burrow into dense, hard substrates (De Meyer *et al.,* 2017b). By performing preference experiments on eels of different life stages, the hypothesis that substrate preference and burrowing performance changes as eels become larger can be tested. Specifically, we expect glass eels and elvers to prefer less compact substrates (coarse and fine gravel; larger interstitial spaces), whereas larger yellow eels, being able to exert higher forces, could prefer more compact substrates (sand; small interstitial spaces) to create stable burrows. Moreover, we evaluate whether the preferred substrate is linked to the most efficient burrowing performance (lower burrowing duration, less body movements and/or lower frequency of movements).

Determining substrate preference in different life stages allows the identification of habitats that can act as growing areas and are thus suitable for eels (e.g. for restocking measures), but also of degraded habitats that require restoration. As such, this study can play an important role in the conservation of *A. anguilla.*

METHODS

Sample collection and housing

In this study, we investigated the burrowing behaviour of the colonizing and sedentary life stages (*i.e.* a cross-sectional study of glass, elver and yellow eel stage), since these life stages are directly associated with substrates and thus potentially show burrowing behaviour. Glass eels are unpigmented eels of ca. 70 mm in length, while elvers are defined as fully pigmented eels < 150 mm and yellow eels as eels > 150 mm. Yellow eels were classed as small [151-300 mm], medium [301-450 mm] and large [>451 mm] (Laffaille *et al.,* 2003). In total, 28 individuals were used (the small sample size was due to limited catch numbers). Age was not determined as this requires lethal sampling. Sex was not determined as the gonadal system only starts developing during the yellow eel stage (Tesch, 2003) and requires dissection. The care and use of experimental animals were in accordance with Belgian legislation (EC approved by ethical committee EC2018-063).

Glass eels (N=10), elvers (N=3) and small yellow eels (N=4) were obtained from the tidal sluice 'Maertenssas' (Bredene, Belgium) using fyke nets (mesh size = 1 mm) attached to the sluice in March 2019. In addition, medium (N=3) and large yellow eels (N=8) were captured downstream from the tidal weir in the freshwater part of the Zeeschelde (Merelbeke, Belgium, N=9) using double fyke nets (mesh size = 8 mm) and at the Veurne-Ambacht pumping station (Nieuwpoort, Belgium; N=2) using fyke nets (mesh size = 8 mm) attached to the gravitational discharge openings in August 2018. Eels were transferred to the laboratory and acclimatized to the new water conditions by gradually adding water from the experimental tank. Eels were temporally anesthetized with clove oil (0.1 ml/L) in the laboratory (Walsh and Pease, 2002) and the total length (TL, to the nearest mm), measured from the tip of the snout to the tip of the caudal fin, and body weight (W, to the nearest mg) of each eel was measured. Afterwards, the eels were kept in an aerated water tank until fully recovered.

Elvers and yellow eels were marked individually by injection with green or yellow Visible Implant Elastomer (VIE) staining (Northwest Marine Technology, Inc., USA) in different parts of the lip. VIE staining was used because this method has no significant effect on survival and does not affect the eel's locomotor behaviour (Imbert *et al.,* 2007). Elvers and yellow eels similar in size (maximal difference of 10 cm) were housed together, with a maximum of four individuals per aquarium (120x55x50 cm, water depth = ca. 40 cm). A maximum of three easily identifiable glass eels (based on degree of pigmentation) were housed together (40x20x25 cm, water depth = ca. 20 cm). All aquaria were filled with fresh water (salinity < 0.5 ‰) and fitted with rocks and tubes to hide in. Water temperature of the housing and testing aquaria was on average 14 ± 1.95 °C. Glass eels were fed *Daphnia* spp., while elvers and yellow eels were fed *Tubifex* spp., chironomid larvae and earthworms (*Dendrobena veneta* Michaelsen 1890), ad libidum. In the laboratory, no artificial light was used and natural light-dark cycles were retained. Before the experiment, we evaluated whether the eels were in good physical condition (i.e., were active, showed cryptic behaviour in their housing aquaria and had no visible abrasions). Experimental trials were conducted during the day in random order. When eels were transferred to the experimental tanks by means of a small tank, they were acclimatized by gradually adding water from the experimental tank to avoid any abrupt physiological changes. After data collection, all individuals were returned into the wild (Zeeschelde, Merelbeke, Belgium). Yellow eels were captive for seven months. Glass eels and elvers were captive for three weeks.

Sediment Preference

To test whether eels prefer a certain sediment type, they were individually introduced in aquaria containing three substrate types: sand (diam. < 1 mm), fine gravel (diam. 1-2 mm) and coarse gravel (diam. 8-12 mm) (Table 1). Sediment depth ranged from 5 cm for glass eels to 20 cm for yellow eels. This depth was chosen based on the maximum burrow depth of American yellow eels (Tomie *et al.,* 2013). All specimens were randomly introduced at a side of the aquarium. A minimum of two and a maximum of six replicates were obtained per individual. Both the random introductions and the low numbers of replicates were used to minimize eel learning behaviour. Maximum three trials were done per individual a day to minimize fatigue effects. Upon introduction, the researchers distanced themselves behind a corner to prevent the eel from seeing them. When eels immediately dug into the substrate upon introduction (11% of the cases), this was not taken into account for determining substrate preference, since these individuals may not have chosen a sediment type. Instead, they may have buried themselves into the sediment type closest to their introduction location in the aquarium. As soon as the eel burrowed into a substrate, the trial was considered complete. Trials took a maximum of 60 minutes. If no burrowing behaviour was observed within this time limit, the observation was considered as “non-burrowing”. As we observed that glass eels and elvers showed a strong preference to move between the interstitial spaces of the coarse gravel (see Results), a separate set-up with only sand and fine gravel was used to determine which of these two substrates glass eels and elvers preferred to effectively bury themselves (three replicates per individual).

Videography and High-speed X-ray Videography

To evaluate the burrowing behaviour of eels, all eels were introduced into aquaria containing either sand or fine gravel (Table 1) and burrowing was recorded with three JVC-HD Everio GZ-GX cameras (50 fps). The cameras were positioned such that burrowing could be recorded in dorsal, lateral and frontal views. Coarse gravel was left out of this experiment, as glass eels and elvers swum inbetween the interstitial spaces, rather than actually burrowing, and because yellow eels did not select this substrate (see Results). A minimum of two and a maximum of six replicates were obtained per specimen for each substrate. If no burrowing behaviour was observed within 60 minutes of introduction, the recordings were considered as "non-burrowing". Several factors were considered in determining which recordings were included in the analysis. Only recordings on which the eels were completely within the substrate and were completely in the field of the camera view were used. In addition, recordings where water turbidity was too high to track the movement of the eel accurately were left out of the analysis. After this selection, 217 recordings were obtained.

In order to track the eel’s movements underneath the substrate, which is impossible with visible-light cameras, high speed X-ray video recordings were applied (**Ethical approval ECD2019-01**) using two elvers and two small yellow eels. Since it’s impossible to detect the eel in sand or gravel due to the high radio-opacity of these silicates, couscous (diam. 1-3 mm) was used as an experimental substrate. When compacted and moistened, this granular medium approximates natural sediment reasonably well, as was demonstrated in previous research on digging moles (Lin *et al.,* 2019). The couscous was compressed prior to each digging event. Because the X-ray movies did not clearly distinguish between water and couscous, a piece of metal was placed on the couscous surface to visibly mark this interface in X-ray movies. After compression, couscous depth was 15 cm, both for elvers and for yellow eels. X-ray videos were recorded at 500 fps, with 70kV, and 50mA using the 3D2YMOX system (Sanctorum *et al.,* 2019). In addition, the above-substrate behaviour was recorded with a visible-light Redlake MotionPro 2000 camera (125 fps), synchronized with the X-ray movies.

Burrowing Technique and Performance

To evaluate whether substrate preference was related to burrowing performance, the video-recordings were used to evaluate the burrowing technique. These recordings were analyzed frame by frame in VirtualDub. Snout-touch was used as the initiation point, and the point at which the tail was completely covered by substrate as the end point. Three different parameters were analyzed: (1) total burrowing duration, from snout-touch till tail-covered, (2) duration of the eel making accelerated body undulations and (3) total number of body undulations needed to construct a burrow. The first three variables were obtained from 94 recordings in fine gravel and 97 in sand. Counting of body undulations was possible for 85 and 74 recordings in fine gravel and sand, respectively. Subsequently, body undulation frequency was calculated as total number of body undulations (parameter 4) divided by the time that the eel made accelerated swimming motions (parameter 2). In addition, the angle between the head and sediment was measured using ImageJ (Abràmoff, Magalhães & Ram, 2004).

Kinematic analyses were performed to study burrowing behaviour in more detail, using visible-light videography recordings. For this, only the high-quality recordings where the eel moved without stopping or changing direction were retained, resulting in a total of 22 recordings: six burrowing sequences of glass eels (N = 3 for both sand and fine gravel) and elvers (N = 3 for both sand and fine gravel) and ten burrowing sequences of small yellow eels (N = 6 for sand, N = 4 for fine gravel). Each AVI file was converted as a JPEG sequence using VirtualDub. Subsequently, the X and Y coordinates of the tip of the tail on each frame were obtained using ImageJ (Abràmoff, Magalhães & Ram, 2004). This body point was chosen as it was easily detectable on all video recordings during the whole burrowing sequence. X and Y coordinates were plotted against time to visualize the number and the amplitude of tail movements. No high-quality recordings were obtained for medium and large yellow eels, since the tip of the tail could not easily be distinguished in the video recordings.

Statistical Analysis

The number of individuals choosing each substratum was compared with the predicted number if the choice was random using a Chi-square test. The null hypothesis states that all sediment types will be chosen equally. If one sediment type was chosen more often than predicted by the null hypothesis ($0.01 < p < 0.05$), it was defined as a moderate preference for that particular substrate. If differences were significant at the $p < 0.01$ level, their preference was considered strong. In addition, Chi-square tests were used to evaluate whether substrate preferences differed significantly between subsequent life stages. To determine whether burrowing performance (specifically: burrowing speed, total number of body undulations and body undulation frequency) differed significantly between sand and fine gravel, Welch Two Sample t-tests were performed for each size class (assumption of homogeneity not met). To detect differences in burrowing speed, total number of body undulations and body undulation frequency between size classes, ANOVAs were performed for both sand and fine gravel. Subsequently, Post-Hoc Tukey HSD Tests were used to evaluate whether subsequent life stages differ in performance measures. Statistical analyses were conducted in R version 3.5.0 (R Core Team, 2018).

RESULTS

Sediment Preference

The sediment preference did not differ significantly between glass eels and elvers (Table 2), both having a significant preference for coarse gravel (Chi-square test: $\chi^2_2 = 48.6$, $P < 0.01$ and $\chi^2_2 = 12.67$, $P < 0.01$ respectively). The subsequent small yellow eel stage differed significantly in preference from glass eels and elvers (Table 2), strongly preferring fine gravel over the other substrates (Chi-square test: $\chi^2_2 = 12.83$, $P < 0.01$). Medium and large yellow eels did not construct burrows in 20% and 29% of the trials respectively (Table 2). No significant difference in sediment preference was observed between small and medium yellow eels and between medium and large yellow eels (Table 2). Medium and large yellow eels showed respectively a moderate and strong preference for fine gravel (Chi-square test: Medium: $\chi^2_2 = 7$, $P = 0.03$; Large: $\chi^2_2 = 17.82$, $P < 0.01$).

Glass eels, elvers and small yellow eels did not show burrowing behaviour when selecting coarse gravel; they simply swam through the interstitial spaces. To determine the preferred substrate for burrowing, these eels were also introduced in aquaria with only sand and fine gravel substrates. Fine gravel was selected over sand in the majority of the replicates, independent of life stage (Table 2). However, this preference was only significant for glass eels (Chi-square test: $\chi^2_2 = 34.91$, $P < 0.01$).

Burrowing Technique

Kinematics and X-ray videography

Identical burrowing behaviour was observed in sand and fine gravel, independent of life stage. The burrowing sequence generally consisted of four phases. Each burrowing sequence started with the eel lying with its ventral side on the substrate. During phase one (P1), the eel lifted its body until its head made an angle of on average 36° ± 6° (mean ± S.D.; based on 54 measurements of 18 specimens) with the substrate. It then moved its snout vertically into the substrate, while also sweeping its head laterally in the horizontal plane. This way, the eel started penetrating the substrate. Subsequently, in phase two (P2), the eel used full body undulations with a high amplitude and frequency. As the eel advanced further into the substrate, body undulations continued at a lower intensity (lower amplitude and frequency), which corresponds to phase 3 (P3). Finally, phase four (P4) initiated when about half of the body was within the substrate and corresponded to the ceasing of body undulation movements above the water-substrate interface (Figure 1). X-ray video analysis further revealed that at the onset of this phase the eel made a curvature within the sediment and then used within-substrate movements to burrow further in a more horizontal direction. For this, the eel mainly relied on horizontal head sweeps, with the rest of the body acting as an anchor. After being completely burrowed, the snout of the eel often appeared at the substrate surface. In some cases, the end of the tail remained above the substrate surface as well. When initial attempts of burrowing failed, the eel restarted this process at another location. While the burrowing sequence was identical for all life stages, glass eels showed alternative behaviour during P3 (fine gavel: 23%; sand: 56% of the cases). Specifically, the amplitude of the body undulations decreased substantially while the body undulation frequency first increased and then remained constant. Phase 4 then did not take place as glass eels kept using body undulation movements during the entire burrowing sequence.

The above description held for sand and fine gravel. Coarse gravel, however, was not selected by medium and large yellow eels, while glass eels, elvers and small yellow eels did not actually burrow into this substrate, but rather swam through the interstitial spaces between these larger particles (see above).

Burrowing Performance

Burrowing duration

Elvers and small yellow eels burrowed significantly quicker in fine gravel than in sand (Welch Two Sample t-test: $t_{24} = 2.81$, $P < 0.01$ and $t_{41} = 3.76$, $P < 0.01$ respectively; Figure 2), whereas no significant difference between the substrates was observed in glass and large yellow eels (Welch Two Sample t-test: $t_{113} = 1.10$, $P = 0.27$ and $t_7 = 2.34$, $P = 0.05$ respectively). Medium yellow eels were left out of this analysis, since too few replicates were obtained.

Among life stages, significant differences in burrowing duration (from snout-touch till tail-covered) were observed in both sand (ANOVA: $F_4 = 8.74$, $P < 0.01$) and fine gravel (ANOVA: $F_4 = 7.38$, $P < 0.01$). Post-hoc Tuckey tests revealed that glass eels burrow significantly slower than elvers in fine gravel (Tuckey Test: $P_{adj} = 0.02$), but not in sand (Tuckey Test: $P_{adj} = 0.98$). In turn, burrowing duration did not differ between elver and small yellow eels (Tuckey Test: $P_{adj} = 0.97$ for sand and $P_{adj} = 0.95$ for fine gravel). As medium yellow eels were left out of the analysis, we compared the burrowing duration between small and large yellow eels. The latter were significantly slower in constructing burrows in both substrates (Tuckey Test: $P_{adj} < 0.01$ for sand and $P_{adj} = 0.02$ for fine gravel). In general, elvers, small and medium yellow eels burrowed faster than glass eels and large yellow eels (Figure 2).

Body undulations

All eels required more body undulations to construct a burrow in sand than in fine gravel (Table 3; Figure 3). The number of body undulations differed significantly between the two substrates for glass, elver and small yellow eels (Welch Two Sample t-test: $t_{61} = 2.91$, $P < 0.01$ for glass eels; $t_{16} = 2.68$, $P = 0.02$ for elvers and $t_{28} = 3.36$, $P < 0.01$ for small yellow eels). No clear trends were observed in body undulation frequency between the two substrates, independent of life stage. Accordingly, none of the t-tests found significant differences in undulation frequency between the two substrates (Welch Two Sample t-tests: $t_{60} = 1.70$, $P = 0.09$ for glass eels, $t_{23} = -1.28$, $P = 0.21$ for elvers, $t_{35} = -2.00$, $P = 0.05$ for small yellow eels and $t_7 = -0.03$, $P = 0.98$ for large yellow eels).

When comparing the required number of body undulations for burrowing across life stages, significant differences were found in both sand (ANOVA: $F_4 = 5.18$, $P < 0.01$) and fine gravel (ANOVA: $F_4 = 15.67$, $P < 0.01$). Post-hoc Tuckey tests revealed that glass eels required significantly more body undulations than elvers both in sand and fine gravel (Tuckey Test: $Padj = 0.02$ for sand and $Padj < 0.01$). Also, the body undulation frequency differed significantly between life stages in the two sediment types (ANOVA: $F_4 = 19$, $P < 0.01$ for sand, $F_4 = 16$, $P < 0.01$ for fine gravel). Body undulation frequency tended to decrease with body size (Figure 3). Elvers had a higher body undulation frequency than small yellow eels (Tuckey Test: $Padj = 0.02$ for sand, $Padj = 0.03$ for fine gravel). In turn, small yellow eels had a higher frequency than large yellow eels, but this was only significant in fine gravel (Tuckey Test: $Padj < 0.01$).

DISCUSSION

Our study shows that *A. anguilla* is an efficient head-first burrower, contrary to the views of De Schepper (2007c) and Herrel *et al.* (2011). The burrowing mechanism is similar to that described for *A. rostrata* and the speckled worm eel (*Myrophis punctatus* Lütken 1852) (Tomie *et al.,* 2013; Able *et al.,* 2011). Eels form burrows by forcing their head, followed by their body, into the substrate with the aid of rapid body undulations and horizontal head sweeps. Schafer (1972) reported that yellow-stage *A. anguilla* make burrows by swimming rapidly through open water followed by ramming their heads into the substrate, while continuing body and tail undulations. However, no such behaviour was observed during this study, even though the aquaria were large enough to allow it. Instead, burrowing always started from a resting position, from where eels tilted their head and pushed their snout into the substrate (P1). Next, body undulations occured at high frequencies and high amplitudes (P2), subsequently followed by a substantial decrease in undulation frequency and amplitude (P3; Figure 1). This change in frequency and amplitude could be related to substrate fluidization. By using rapid body movements, species can decrease the weight of the overlying sediment, increase the distance between substrate particles and reduce the friction between the grains, thus facilitating burrowing into the substrate (Dorgan *et al.,* 2006). Such behaviour has indeed been observed in several burrowing species (Gidmark *et al.,* 2011. Baumgartner *et al.,* 2008. Tatom-Naecker & Westneat, 2018) and might thus also be applicable for *A. anguilla*. The initial high frequency might be necessary to initiate substrate fluidization, with the subsequent lower frequency being necessary to retain this. Alternatively, the changes in frequency and amplitude might be due to a change between locomotion in water and in a granular substrate. The eel, initially completely surrounded by water, used high frequency and high amplitude body undulations to penetrate the sediment and experience very little resistance from the water. When burrowing proceeded, the resistance imposed by the substrate increased, causing a dampening of the undulation frequency and amplitude. When the body undulations ceased (P4), eels could then use the body underneath the substrate as an anchor to propel themselves forward. Finally, it is possible that both substrate fluidization and changes in resistance have a mutual effect on the frequency and amplitude.

While Aoyama *et al.* (2005) stated that anguillid eels only burrow into muddy sediments, our study shows that this is not the case for *A. anguilla*. Similarly, Tomie *et al.* (2017) showed that yellow stage *A. rostrata* preferred mud over cobble in summer, but chose mud and cobble at similar frequencies during winter. Moreover, Aoyama *et al.* (2005) suggested that Japanese eels (*Anguilla japonica* Temminck & Schlegel 1846) construct mud burrows only when other, more spatially complex habitats are unavailable. During the experiments of this study, supra-substrate hiding material was not offered and the hypothesis whether *A. anguilla* prefer hiding in supra-substrate materials (e.g. vegetation, rocks,…) over active burrowing was not tested. Recent research indicated, however, that in 62% of the trials, *A. anguilla* (small yellow eel stage) preferred making burrows in sand, fine and coarse gravel, even when supra-substrate hiding materials were present (Steendam *et al.,* 2017). Whether this is also the case for the other life stages requires further research. Furthermore, our study was limited to three sediment types. We acknowledge that eels possibly show burrowing behaviour and different preferences when other substrate types (e.g. mud and cobble – which were not included in this study) are available (Tomie *et al.,* 2017). Given their phenotypic plasticity and occurrence in a wide range of freshwater, transitional and marine habitats (e.g. Daverat *et al.,* 2006), they can encounter a variety of substrates during their life cycle, from fine silt to large cobbles. Nonetheless, the results of this study on a selection of three sediment types illustrate that European glass eels, elvers and yellow eels readily hide in bottoms and hence indicate the importance of substrates for the various life stages.

Impact of life stage and size on burrowing performance and substrate preference

Anguilla anguilla inhabit a variety of habitats, including lakes, rivers, marshes and estuaries (Tesch, 2003), but can also be found in coastal marine habitats without ever entering fresh water (Tsukamoto *et al.,* 1998) or move between different salinity zones (Daverat *et al.,* 2006). There appears to be a link between the habitat where an eel occurs and its body size (Laffaille *et al.,* 2003): small eels are mainly found in shallow habitats with a high abundance of aquatic vegetation, whereas larger eels tend to be found in deeper habitats with small to intermediate abundances of aquatic vegetation. Here, we show that all tested life-stages of *A. anguilla* (glass eels, elvers and yellow eels) are able to burrow into a variety of substrates and that also substrate preference depends on life stage and body size; young glass eels and elvers preferred less compact substrates, especially coarse gravel, confirming earlier observations of Christoffersen *et al.* (2018). However, glass eels and elvers did not actively burrow in this substrate, but rather swam through the interstitial spaces, indicating that this material can act as a hiding space. Contradicting to the observations of Christoffersen *et al*. (2018) who reported that elvers spent 30 min searching for favourable susbstrata, we observed that glass eels and elvers quickly selected a substrate to burrow in (ca. 3 minutes per trial, Pers. Obs.). From the yellow eel stage onwards, fine gravel (diam. 1-2 mm) was preferred, with the preference for this substrate becoming more pronounced with increasing eel size. As such, our expectation that larger eels would prefer the more compact sandy substrates for burrowing is rejected.

In general, eels chose substrates that require the least body undulations and lowest body undulation frequency and are thus energetically the least costly. As glass eels and elvers were able to simply swim through the gaps between the coarse gravel particles, they preferred this substrate. However, when there was no coarse gravel to hide in, they preferred fine gravel over sandy substrates, as this allows the fastest and easiest burrowing. Sandy substrates are more compact in comparison to fine gravels and might be more difficult to fluidize and will provide more resistance during burrowing. Yellow eels, too large to hide between the interstitial spaces of the coarse gravel, actively preferred the fine gravel over the other substrate types. Moreover, burrowing performance increased with body size as well, with the large yellow eels requiring the least body undulations and lowest undulation frequencies and glass eels requiring the most body undulations and highest frequencies to burrow into the substrate. This thus supports the hypothesis that burrowing performance differs between life stages.

Based on our results, we hypothesize that burrowing performance might increase with body size (Figure 4). A first potential explanation is that glass eels are still in full musculoskeletal development (De Meyer *et al.,* 2017b). Secondly, biomechanical scaling theory also predicts an increase in burrowing performance with increasing size: for an eel growing isometrically, the increase in propulsive power (i.e. propulsive force multiplied by velocity) will be faster (proportional to muscle mass, so increasing with length to the third power) than the increase in snout surface and body cross-sectional area (increasing with length to the second power).This implies that throughout growth larger eels should be capable of exerting higher pressures by its snout on the substrate to penetrate a substrate at a given absolute velocity (assuming that eel's head tissues are structurally capable of withstanding such higher pressures). The observed decrease in body undulation frequency for larger eels concurs with the general observation that tail beat frequency tends to decrease with body size in fish (Bainbridge, 1958), as swimming kinematics are fine-tuned to hydrodynamic efficiency (Gazolla *et al.,* 2014), and presumably also because of some metabolic constraints on the scaling of muscle power (Hill 1950).

It could be expected that higher burrowing performance will be associated with more frequent burrowing behaviour. However, no burrowing behaviour was observed in 20% of the medium and 29% of the large yellow eel trials. In contrast, glass, elver and small yellow eels always looked for shelter in the sediment. This contrasting result could be related to the energetic costs of burrowing *versus* predation risk. For anguillid eels, hiding from predators could be especially important because their burst swimming speed to flee predators is slower than that of fusiform fish (Wolter and Arlinghaus, 2003). Accordingly, eels react to disturbances either by burrowing or by pulling back into denser vegetation, rather than by fleeing (Westerberg *et al.,* 1993). The use of burrows as a hiding spot during the day concurs with the observation that *A. anguilla* often left their burrow as soon as darkness fell (pers. obs. in lab). Large yellow eels, however, likely have a lower predation risk through their larger body size and might prioritize saving energy over making burrows, potentially explaining the presence of non-burrowing events during our experiments (which took place during the day). In contrast, for smaller eels, the predation risk is higher and predator avoidance through burrowing might be prioritized over energy loss. To test this hypothesis, it would be interesting for future studies to evaluate burrowing behaviour in absence/presence of predators.

While our study provides important insights in the burrowing behaviour of *A. anguilla*, several biological factors, including presence of food and predators, were excluded during the experiments. Hence, only the physical characteristics of the offered substrates determined selection of the preferred substrate. Further research is necessary to determine whether other environmental variables influence burrowing behaviour. Nyman (1972) found, for example, that burrowing behaviour depends on water temperature; eels are more likely to burrow in cold water, whereas little to no burrowing behaviour is observed in warm water (>16° C). However, whether temperature influences substrate preference as well and whether this is linked to body size has not been examined yet. Similarly, recent reseach showed that the preference of *A. anguilla* elvers for small pebbles (16 – 32 mm) as a hiding place is not altered by piscivore chemical cues (Nilsson *et al.,* 2020). However, future research is necessary to determine whether predator presence has an effect on the preferred substrate for burrowing.

Conservation implications of burrowing

Anthropogenic changes to substrates could negatively impact burrowing species, for example, by increasing exposure to sediment-bound pollution. Benedetti *et al.* (2008) indeed found that eels readily absorb contaminants (especially heavy metals) from sediments and accumulate them in their liver tissues. During migration, these contaminants are released by lipolysis inside the body, where they can subsequently affect the eel’s metabolism, disrupt gonadogenesis or impair the production of high quality gametes. Increased exposure to sea- and riverfloor pollutants can thus have detrimental effects on the eel’s spawning success (Robinet & Feunteun, 2002). In addition, burrowing fishes suffer from the increase in anoxic and hypoxic waters caused by increased anthropogenic eutrophication (Diaz & Rosenberg, 2008). Because eels in burrows respire water from the water column (Tomie *et al.,* 2013), an increase in anoxic or hypoxic bottom waters could mean a loss of habitat.

Finally, bottoms of many coastal areas, estuaries, and inland waters have been profoundly altered by anthropogenic activities including dredging (Gage *et al.,* 2005), run-off and erosion (Colodey & Wells, 1992), and the extraction of sand and gravel (de Groot, 1986). These activities, together with potential effects of pollution (Sühring *et al.,* 2016) and/or severe occurrences of hypoxia (Schmidtko *et al.,* 2017) can alter the spatial distribution of the vegetation community and even lead to changes in sediment type (ICES, 2015). Because not all substrate types, such as mud, have been evaluated in this study, we cannot recommend which substrate types should be preserved for eel conservation. Still, our results are important for eel conservation, as we clearly demonstrate that substrate preference differs depending on life stage and body size. Consequently, measures in terms of habitat restoration or preservation should take into account that not all eels might be affected equally by specific measures. Moreover, the clear preference of small eels for coarse gravel (Christoffersen *et al.,* 2018, Nillson *et al.,* 2020) suggests that installing coarse gravel beds, both at areas with absence of supra-substrate hiding material, such as canals with limited riparian and submerged vegetation, and at migration barriers with eel ladders where glass eels accumulate, could be beneficial for eel survival by lowering the predation risk, decreasing intra-specific competition for space (Westerberg *et al.,* 1993) and/or by providing a safe base for feeding (Bozzano, 2003; Ménard *et al.,* 2008). The eel's diet includes prey items such as amphipods and chironomids (Thurow, 1985; Proman & Reynolds, 2000), which can easily and safely be obtained from burrows. As such, providing and retaining proper growing areas is crucial for eel conservation. This study thus illustrates the importance of fully understanding the eel's ecology for implementing proper management measures.

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Contribution

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- C. S. performed the experiments, analyzed the data, prepared figures and tables, reviewed and revised drafts of the paper.
- P. V. contributed materials, reviewed and revised drafts of the paper.
- S. V. W. designed the experiments, contributed materials, reviewed and revised drafts of the paper.
- J. D. M. designed the experiments, contributed materials, reviewed and revised drafts of the paper, approved the final draft.

Significance Statement

European management plans are currently failing to reach the goals set to restore the critically endangered European eel population. A lack of knowledge about the eel's ecology and behaviour can partially explain this. Here, we show that the urge to burrow and the preferred substrate changes during the eel's ontogeny, with eels generally preferring the substrate that allows the quickest burrowing. These insights provide useful information for implementing more appropriate and concrete management plans.

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Figure legend

Figure 1: Schematic representation of the burrowing sequence of *Anguilla anguilla* (L. 1758). This sequence is composed of four distinct kinematic phases. P1: The eel tilts its head after which the snout is pushed into the substrate. P2: Full body undulations occur with a high amplitude and frequency. P3: The amplitude and frequency of body undulations minimize. P4: Body undulations cease and only horizontal head sweeps within the substrate occur to completely drag the tail into the sediment.

Figure 2: Boxplots of burrowing duration in sand and fine gravel for different size classes of *Anguilla anguilla* (L. 1758). (a) total time needed to construct a burrow. GE: glass eels, ELV: elvers (<150 mm), SYE: small yellow eels (151-300 mm), MYE: medium yellow eels (301-450 mm), LYE: large yellow eels (>451 mm). For the medium yellow eels, only 1 individual was included: 2 measurements were made in sand and 3 in fine gravel.

Figure 3: Burrowing performance of *Anguilla anguilla* (L. 1758). (left) Mean number of body undulations per individual in sand (S) and fine gravel (FG). (right) Mean body undulation frequency per individual in sand (S) and fine gravel (FG). GE: glass eels, ELV: elvers (<150 mm), SYE: small yellow eels (151-300 mm), MYE: medium yellow eels (301-450 mm), LYE: large yellow eels (>451 mm).

Figure 4: Schematic representation of how burrowing urge and performance may change during the eel's life cycle. BLUE: relation between burrowing performance and life stage. Glass eels are the least efficient life stage for burrowing. Burrowing performance is hypothesized to follow the displayed curve. RED: relation between burrowing urge and life stage. For glass eels and elvers, burrowing is of high importance, but becomes less important as eels increase in size.

Figure 1

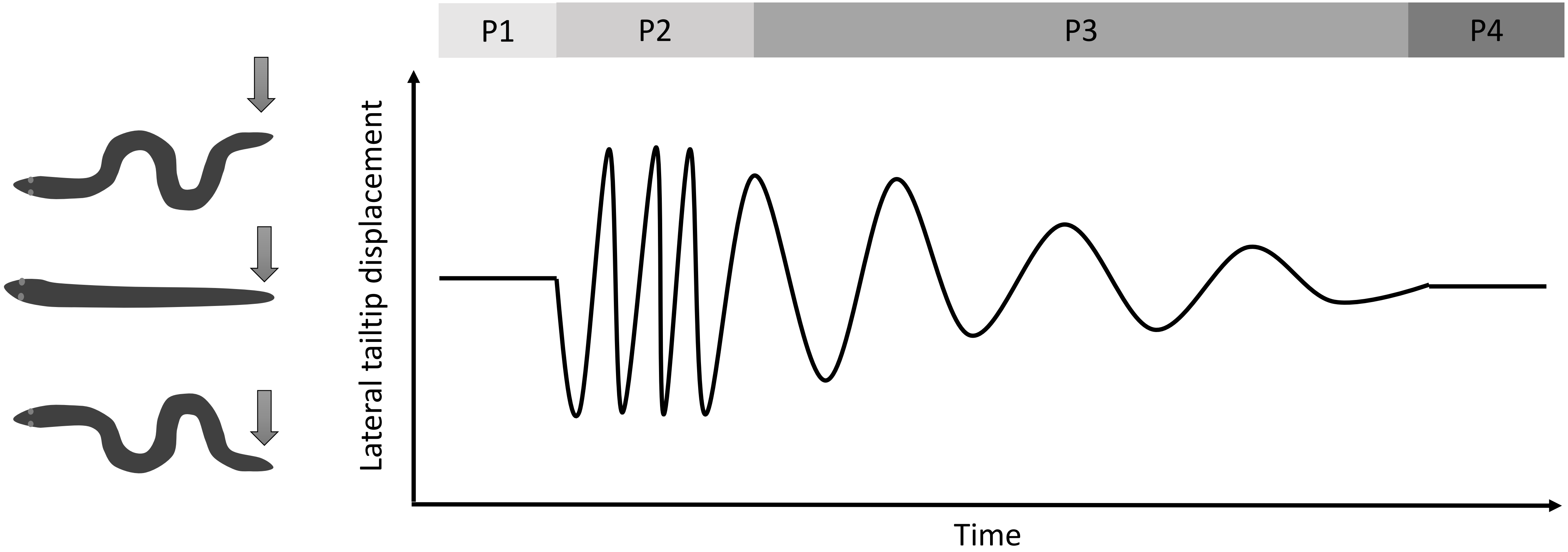


Figure 2

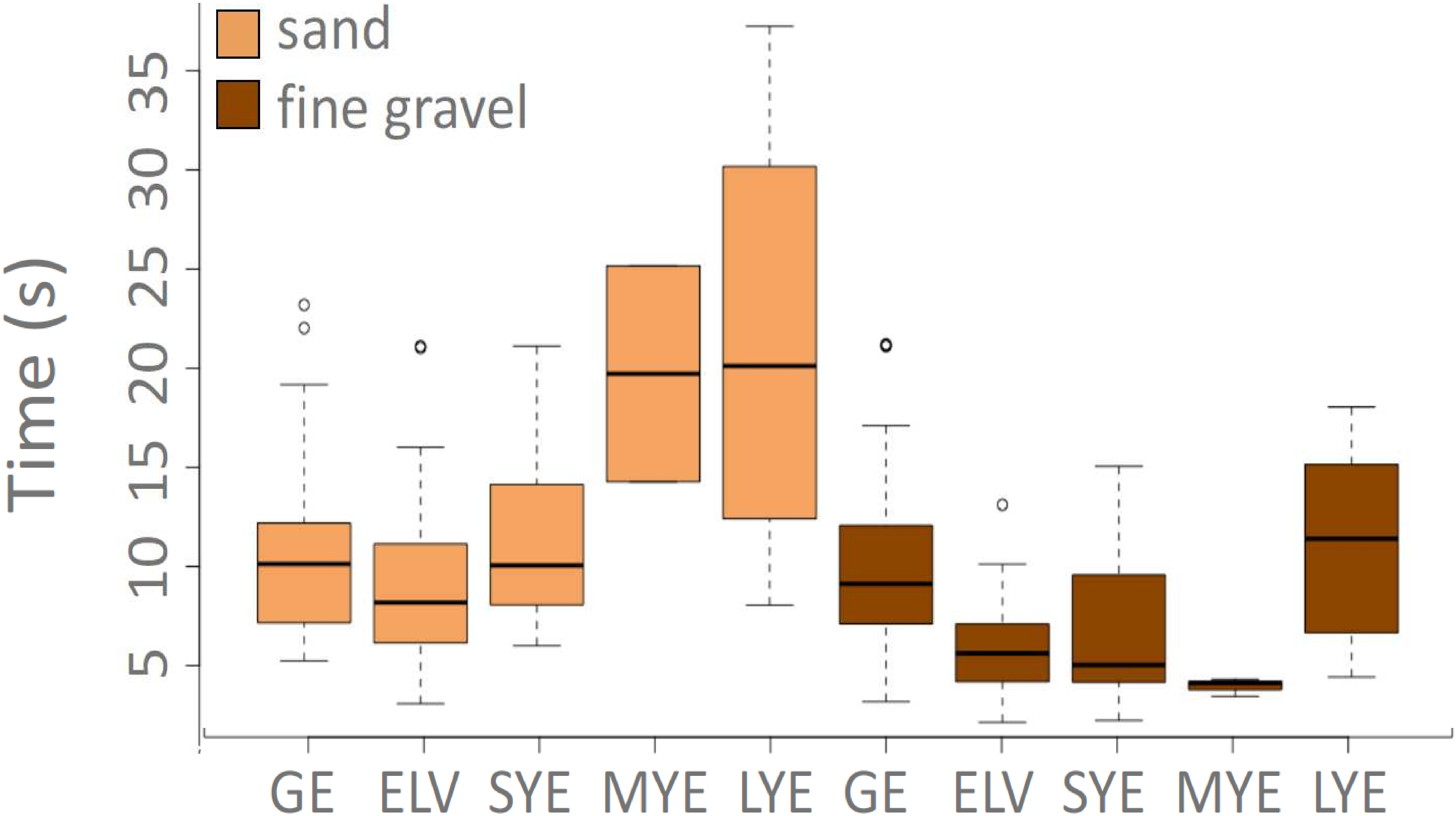
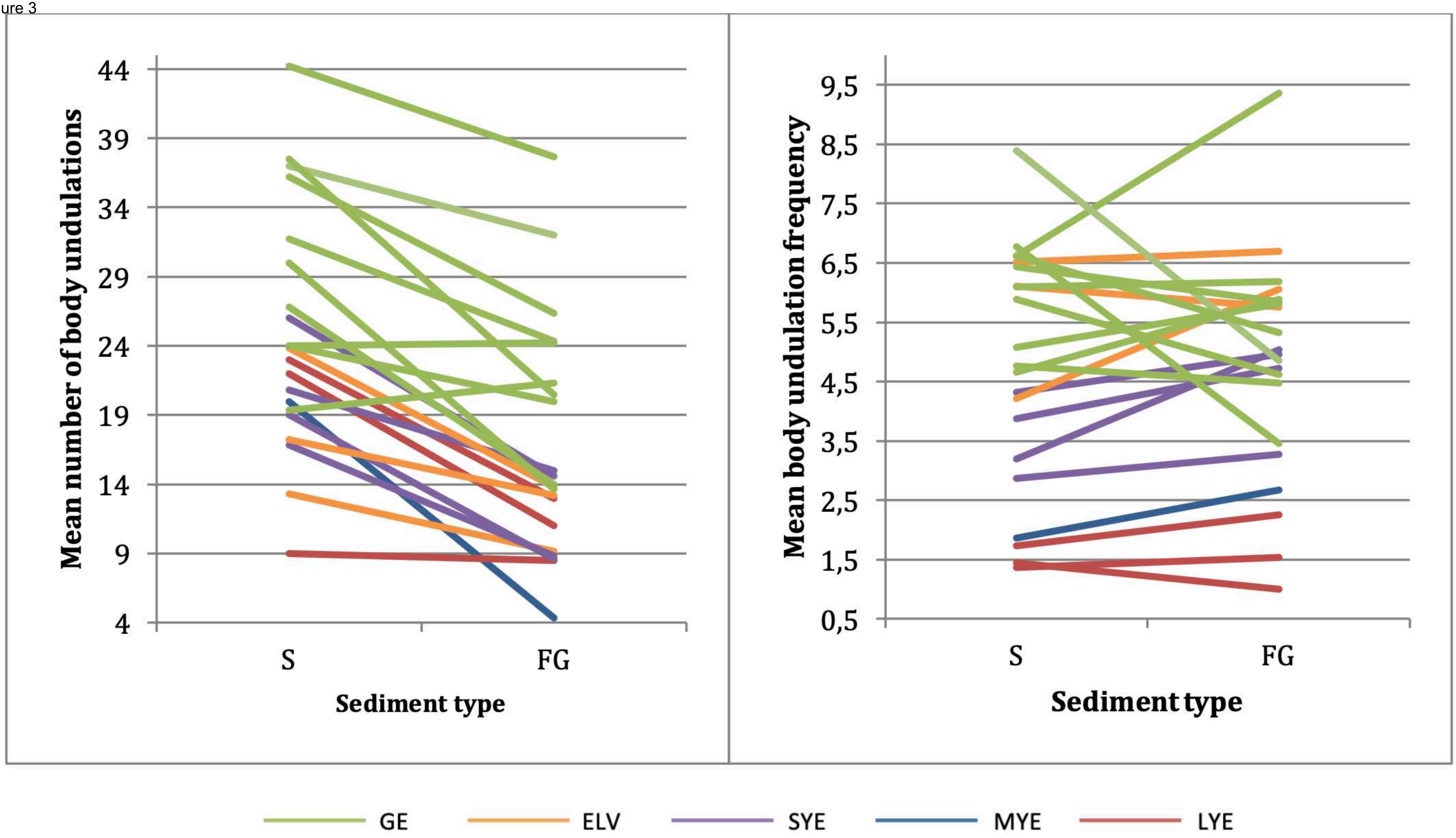
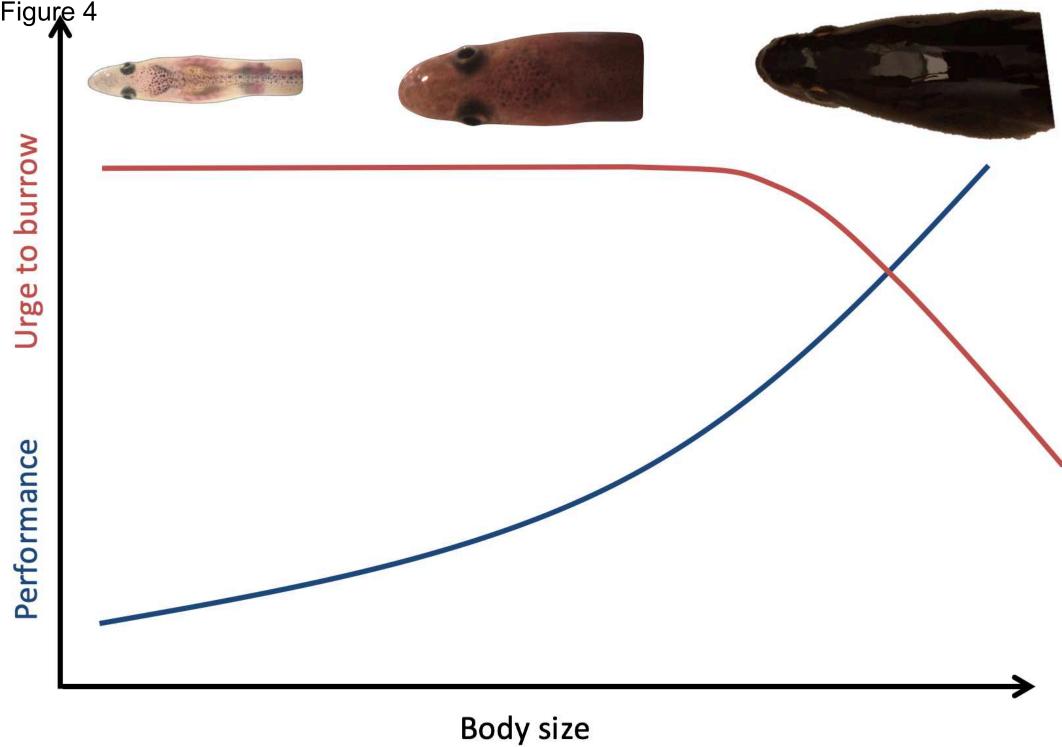

sand
fine gravel
Time (s)
5
10
15
20
25
30
35
GE
ELV
SYE
MYE
LYE
GE
ELV
SYE
MYE
LYE

Figure 3



Mean number of body undulations
44
39
34
29
24
19
14
9
4
S
FG
Sediment type
Mean body undulation frequency
9,5
8,5
7,5
6,5
5,5
4,5
3,5
2,5
1,5
0,5
S
FG
Sediment type
GE
ELV
SYE
MYE
LYE

Figure 4



Supporting information: Video burrowing glass eel

Supporting information: Video burrowing small yellow eel

Click here to access/download

Supporting information: Video

Supplementary_smallyelloweel.mp4

Supporting information: Video burrowing large yellow eel

Supporting information: Video

Supplementary_largeyelloweel.mp4