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How long can glass eels be detected in the stomach of a Sea bass?

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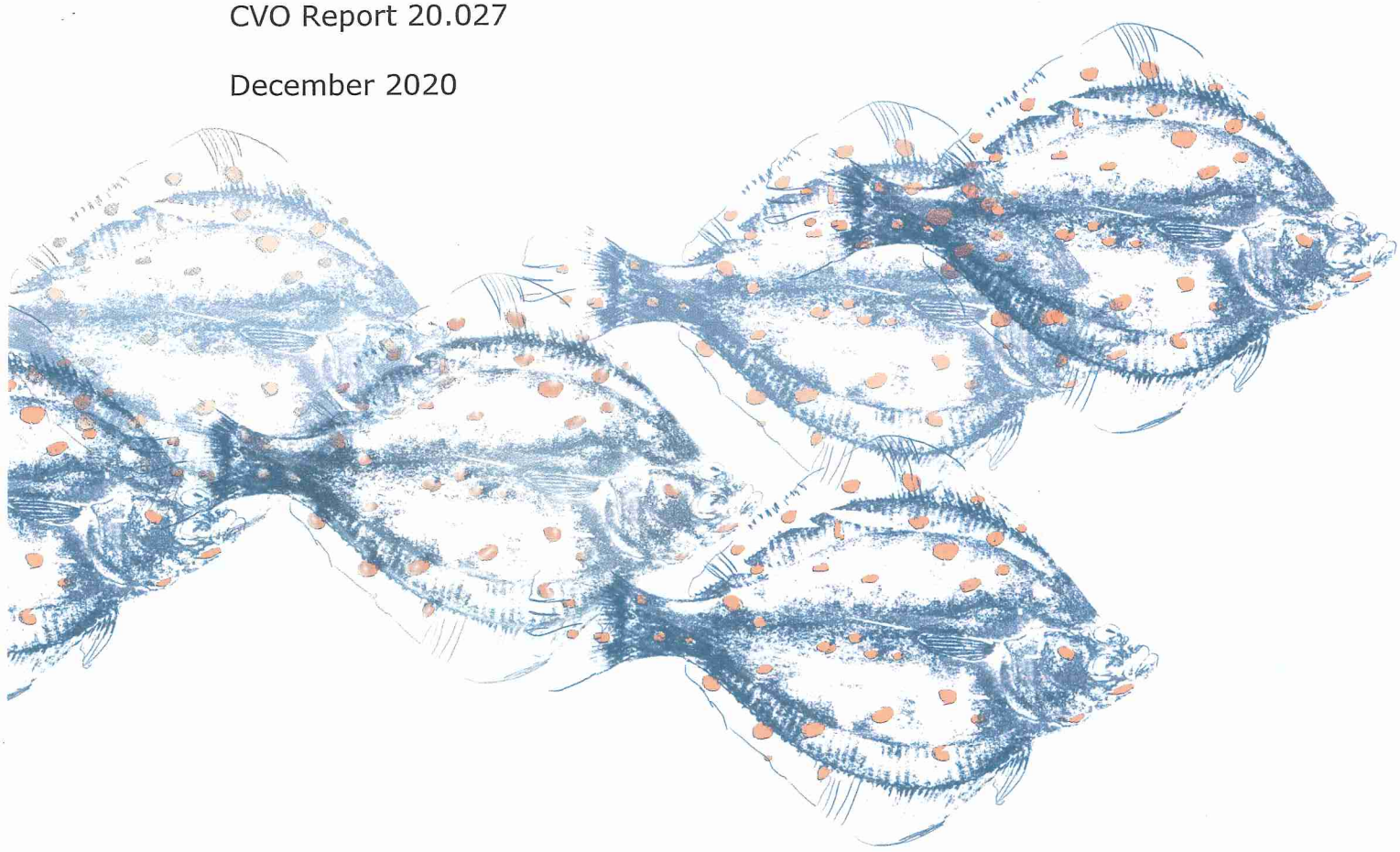
CVO Report 20.027

December 2020



Centrum voor Visserijonderzoek (CVO)

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Summary

The European eel (*Anguilla anguilla* L.) is an economic important species and is listed on the IUCN red list as critically endangered. Nonetheless, each year between late winter (February) and late spring (May) glass eels arrive at the various coastal areas attempting to migrate to freshwater habitat. As part of the statutory tasks (WOT) a yearly index of glass eels arriving at the sluices at Den Oever was created since 1938. The program consists of a monitoring with liftnets and traps and is commissioned by Wageningen Marine Research (WMR). In addition, WMR also equips glass eels with a Visible Implant Elastomer tag to determine abundance and differences in retention time between years since 2019 using (batch) mark- and recapture methods. However, effects of tagging in relation to predation risk are unknown. Tagged glass eels might have an increased risk for predation, which in turn would influence abundance and retention time estimates. This study aims to determine differences in predation risk when glass eels are equipped with a VIE tag and exposed to European sea bass (*Dicentrarchus labrax*). Also, this study aims to determine the feasibility of stomach content analysis of predatory fish for future field studies on predation of glass eels near barriers. To answer both questions a laboratory experiment was conducted using marked (red and blue) and unmarked glass eels which were exposed to sea bass for a 2-hour trial. If at least one glass eel was eaten, stomach content was analyzed by a hand pump. 48% of the trials showed successful predation and 13% showed clear attempts of predation but failed to eat glass eels. The other sea bass (39%) waited significantly longer to show some activity, and they moved significantly less. The study showed that no differences in predation risk for marked glass eels by European sea bass. Also, no difference was found in predation between red and blue marked glass eels. Stomach content analysis showed intact glass eel bodies after ending the 2-hour trial and glass eels could be clearly identified after 4 hours and parts of glass eels were found after 16 hours. This study shows that mark-recapture studies are not influenced by an increased predation risk of marked glass eels. Also, if European sea bass predates on glass eels in the field, it can be detected using a hand pump in field studies if conducted within 4-6 hours after predation.

The care and use of experimental animals (sea bass and glass eel) complied with the Dutch animal welfare laws, guidelines and policies as approved by the 'Central Committee Animal experiments' following protocol 2019.D-0050.

1 Introduction

The European eel (*Anguilla anguilla* L.) is an economic important species and is listed on the IUCN red list as a critical endangered status (Pike et al. 2020). The poor status of eel is confirmed by ICES. Based on multiple time-series across Europe, the ICES WGEEL report stated that recent recruitment series are only 2.1% in the North Sea series compared to 1960-1979 (ICES 2020). Many anthropogenic factors may have contributed to this decline such as overexploitation (Dekker 2000, 2003), migratory barriers resulting in mortality, habitat loss or degradation (Feunteun 2002, Tesch 2003, Griffioen et al. 2019) and climate change (Miller et al. 2016, Drouineau et al. 2018, Westerberg et al. 2018, Borges et al. 2019). Recruitment levels may also be negatively affected by changes in oceanic conditions and atmosphere regime shift (Knights 2003, Friedland et al. 2007, Bonhommeau et al. 2008a, Bonhommeau et al. 2008b). Nonetheless, each year between late winter (February) and late spring (May) large amounts of glass eels still arrive at the coastal areas trying to reach freshwater habitat (van Ginneken and Maes 2005).

After spawning in the Sargasso sea eel eggs hatch into leptocephali larvae which are transported by oceanic currents toward the European and North African coast (Schmidt 1923, Miller et al. 2019). During the oceanic drift the leptocephali larvae develop to glass eels (Schmidt 1923, van Ginneken and Maes 2005, Miller et al. 2019). Once the glass eels arrive at estuaries or coastal areas, they are attracted to freshwater flows giving multiple cues (e.g. organic matter, salinity difference) for the continuation of the migration (Creutzberg 1961, Mouton et al. 2011, Kroes et al. 2020). At the break point of tidal streams, glass eels switch from using tidal streams to counter-current swimming to colonize the upper parts of the river systems (Edeline et al. 2007). In estuaries this may lead to temporal accumulations of glass eels due to the loss of tidal advection and not all glass eels switch to counter-current swimming (Edeline et al. 2007). These temporal accumulations of glass eels may be negatively influenced by barriers causing prolonged accumulations. In turn, these high and prolonged accumulations may induce additional population losses due to predation risk. However, little is known about predation (risk) of glass eels (Miyake et al. 2018).

As part of the Dutch statutory task program (WOT) a yearly index of recruitment was created since 1938 (Griffioen et al. 2017). The program consists of monitoring with liftnets and traps and is commissioned by Wageningen Marine Research (WMR). In addition, WMR also equips glass eels with a Visible Implant Elastomer (VIE-)tag (North West Marine Technology) to determine abundance and differences in retention time between years since 2019 using (batch) mark- and recapture methods. However, effects of tagging in relation to predation risk are unknown. Tagged glass eels might have an increased risk for predation, which in turn would influence abundance and retention time estimates.

This study aims to determine differences in predation risk when glass eels are equipped with a VIE tag and exposed to European sea bass (*Dicentrarchus labrax*). Also, it is assumed that digestion of glass eels in stomachs of predators is rapid and therefore difficult to detect after a certain time period. This study aims to determine the feasibility of stomach content analysis for future field studies studying predation of glass eels near barriers. For that, we conducted a laboratory experiment using marked and unmarked glass eels which were exposed to a sea bass for a 2-hour trial. If glass eel was eaten, stomach content was analyzed by flushing (hand pomp, Kamler and Pope 2001) in fixed periods: 0.5 - 32 hour after finalizing a 2-hour trial. To our knowledge this study gives an unique insight in predation of glass eels in general, additional risks of marked glass eels and gives insight in the feasibility of field experiments using stomach content analysis by stomach flushing.

2 Material and Methods

Glass eels
Glass eels were collected along the Dutch coast using ELFI's (www.elverfinder.com) deployed for national monitoring of the WOT (statutory research tasks by Dutch government). An ELFI uses a continue attraction flow pumped from the hinterland to attract and catch glass eels. Contrary to a conventional glass eel ladder, an ELFI is a floating device and uses a coconut fiber on the 'ladder'. Also, it is equipped with a container where glass eels are trapped for monitoring purposes. In the national glass eel WOT monitoring, ELFI's are emptied twice a week during March until the end on June 2020. This ensured sufficient numbers of glass eels throughout the experimental period. Glass eels were transported in aerated tanks to WMR in IJmuiden for marking and testing.

Glass eels were kept in multiple 45l aquaria (lwh: 50x30x30cm) which were connected on a filtered saltwater (34‰) system, aerated and temperature controlled at 11°C. Glass eels were kept for a maximum of ten days including testing period before being released again at original catch location if not predated. No food was provided during the stay since previous studies have suggested that glass eels do not feed during estuarine migration (Lecomte-Finiger 1992, Kawakami et al. 1999, Tesch 2003). Cage enrichment was available in the form of multiple PVC-pipes (3-4cm diameter).

European sea bass
48 European sea bass were caught with a fishing rod near cooling water outlets of factories at Borssele and IJmuiden, on 24th and 29th of April 2020 respectively. Average weight at Borssele (n=24) was 91.4gr (min. 64.7gr, max. 123.8gr) and average length was 21.2cm (19.1-24.4cm). For the sea bass caught at IJmuiden (n=24) average weight was 467.4gr (312.6-733.3gr) and average length was 36.4cm (31.9-43.5cm). Fish were stored in 1000L (Borssele) and 2800L (IJmuiden) aerated tanks which were filled with seawater (34‰), temperature controlled at 11-13°C and a light regime of 13 hours light and 11 hours dark. Fish from different sites were kept separately. Food was offered daily in the form of bred sandworms *(Alitta virens)* and lugworms *(Arenicola marina)*. Fish were acclimatized for a minimum of 14 days. To exclude stress as interfering factor of the experiments (Beitinger 1990) sea bass were only used in the experiment when food intake was successful at least three days before a trial.

VIE tagging
Glass eels used for the experiment were visually selected on transparency rejecting pigmented individuals. Glass eels were anesthetized using 0.4ml/l 2-phenoxyethanol before implanting double tagging them with a 2-4mm VIE tag using a 0.3mm needle. After implanting VIE tags, glass eels were transferred to an aerated container to recover from the treatment and checked for normal behaviour. Normal behaviour was defined as regular swimming behaviour or hiding in the PVC-pipes offered as cage enrichment. Control groups followed the same procedure except for tagging. Glass eel were equipped with a double red or double blue tag representing two extremes of the light spectrum.

Test procedure
To test differences in predation and behavioural response of 40 individual sea basses, 2-hour trials were run: 20 trials with 10 double tagged blue glass eels (test group) and 10 untagged glass eels (control group) and 20 trials with 10 double red tagged glass eels (test group) and 10 untagged glass eels (control group). Each trial had one sea bass and was only used once during the experimental period. Sea bass were randomly netted from their holding tank and were held without food for 48 hours before being moved to a test tank. The sea bass placed in a test tank was offered 18 to 24 hours acclimatization time (without food) before introducing 20 glass eels for a 2-hour trial. In total sea bass was held without food for 66-72 hours before the experiment to trigger food intake interest during the experiment. Glass eels were randomly selected from a holding tank, and could be used multiple times to reduce numbers of total glass eels used

for the experiment. Each day, two trials were conducted simultaneously in two 2800l (lwh: 200x200x70cm) tanks each equipped with one LED floodlight (4500 lumen, 4000K and 380-780nm wavelength). Tanks had one vertical outlet pipe at the center of the tank that was used for recirculating before and after the trial. During the trials all recirculating pumps were switched off. Also, all outlet pipes were covered to prevent glass eel escapement. Trials with sea bass that showed unresponsive behaviour related to stress or other unknown reasons during the trials were indicated as invalid for predation evaluation. Those tests were repeated with new sea bass to support statistical analysis for predation analysis. Trials were observed visually by observers (behind sheets) for the first 15 minutes during the trial and filmed for behaviour analysis by a GoPro7 centered above the tank for the full 2-hour period (Photo 1 and Photo 2). After the 2-hour trial, the sea bass was removed and kept separate for stomach content analysis. The (remaining) glass eels were caught and counted for predation analysis. The care and use of experimental animals (sea bass and glass eel) complied with the Dutch animal welfare laws, guidelines and policies as approved by the 'Central Committee Animal experiments' following protocol 2019.D-0050.

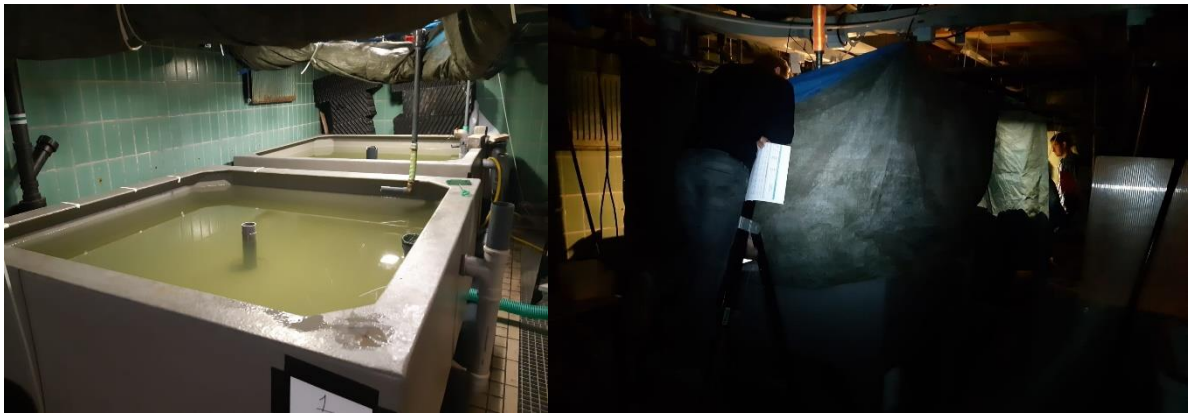

Photo 1. Experimental setup of two tanks (left photo) and students (right photo) T. Menke (front) and W. Janssen (back) observing during the first 15 minutes of a trial behind sheets.

Sea bass behaviour

To correlate successful predation to specific swimming behaviour a video analysis was done for each trial. In each trial it was checked whether there was at least one clear predation attempt. This 'predation attempt' was defined as a (clear) rapid movement towards a glass eel. Moreover, to test for activity of the sea bass and validity of the trial, each sea bass was tested for (1) grid change (2) spatial use of the tank (Spread of Participation Index, SPI) and (3) latency. To do so, the tank was virtually divided into a grid of 9 squares (3x3) and movement of the sea bass was analyzed using Cowlog (Hanninen and Pastell 2009). Each time a sea bass moved between grid cells it was noted as 'grid change' (1) and expressed as grid change per minute. Spatial use of the tank (2) was defined as the time spend in each grid cell and expressed as a (SPI) (Dickens 1955, Plowman 2003, Rose and Robert 2013). The SPI is an index of how an animal uses its available space. This index ranges from an SPI of 0 to 1, where a 0 stands for equal or maximum use of all the grids and a 1 indicates that only one single grid was used. Latency (3) was defined as the time (seconds) until the sea bass changed grid for the first time ignoring the first ten seconds after release of the glass eels.

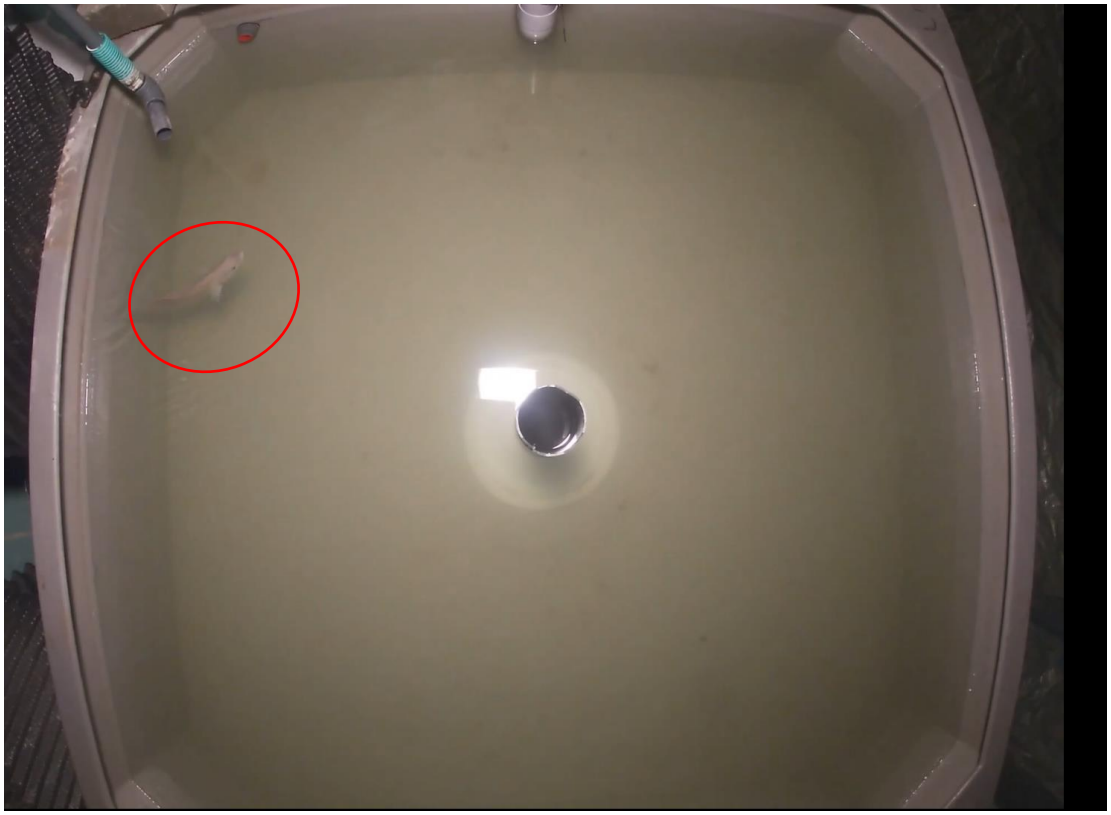

Photo 2. Experimental setup captured from above (still from video). Red circle: sea bass (35.4 cm) approaching a glass eel swimming at the surface of the tank.

Stomach content analysis

Sea bass that showed successful predation of glass eel were kept separately and the stomach content procedure by a hand pump was undertaken (Kamler and Pope 2001). The handpump consisted of a 30cm silicon tube connected to a bulb filled with seawater to flush the stomach with seawater. At the end of the tube a second tube with larger diameter was attached parallelly to allow water to return out of the fish including stomach content. Water and stomach content was collected in a tray for analysis. During the procedure fish was anesthetized using 0.4ml/l 2-phenoxyethanol, weighted and measured. This procedure was repeated for three sea basses after 0.5, 1, 2, 4, 8, 16 and 32 hours after finalizing the 2-hour trial. If flushing did not successfully return (parts of) glass eel the fish was euthanized and stomachs (and intestines) were analyzed manually by dissection to evaluate the hand pump procedure. If no glass eels were found by dissection it was assumed that glass eels were digested and no hand pump procedure was conducted in subsequent sea basses.

Data analysis predation

A binomial GLM (logit link function) was run with the number of eaten glass eels per trial as response variable and multiple covariables. Highly correlated covariates were excluded from the model: Length and weight were highly correlated; weight was chosen to be in the model. Also, grid change per minute and SPI were highly correlated; grid changes per minute was chosen to be in the model. The full model had the following covariates: latency, the time of the experiment, weight of the sea bass, grid change, days in the lab, catch site of the sea bass, color of the tag and if the glass eel was marked or unmarked. From this full model, backward elimination by AIC was performed to select covariates. A restriction was set-up in the model selection that the variable marked/unmarked was not to be eliminated. Separate analysis for comparison between covariates was done with Monte Carlo Permutation Tests (using 10^5 simulations). P-values lower than 0.05 were considered to be significant.

3 Results

Predation

In 18 out of the initial 40 trials with 40 individual sea basses, one or more glass eel was eaten. Eight trials were no glass eels were predated were repeated with new sea basses (4 trials using red marked glass eels and 4 using blue marked glass eels) which added 5 trials with successful predation. In total 23 (48%) out of 48 trials one or more glass eel was eaten. In 6 trials (12%) a clear attempt was seen by video analysis and in 19 trials (40%) no glass eel was eaten or an attempt was made. A total of 164 glass eels (17% of total 960 glass eels that were exposed to predation) were eaten. Of those 77 (16% of 480) were marked and 87 (18% of 480) were unmarked glass eels. When predation occurred an average of 7.1 ± 5.6 (Mean ± SD) (36%) glass eels were eaten. In one trial all 20 glass eels were consumed. Eight out of 23 successful sea bass ate over 50% of the glass eels within 2 hours.

In the trials using VIE-tag-red 11 out of 23 sea bass showed successful predation and 66 glass eels were eaten 30:36 (control:marked). In the trials using VIE-tag-blue 12 out of 25 sea bass showed successful predation and 98 glass eels were eaten 57:41 (control:marked).

Backward elimination performed on the binomial GLM resulted in a model with only the covariate marked/unmarked kept in; this model revealed no significant difference between marked and unmarked glass eels (p=0.27, Table 1).

No differences in predation were found between different tag-colors, neither between control and marked groups, if analyzed separately using Monte Carlo Permutation Tests (p=0.67).

Table 1. *Estimated coefficient parameters, standard errors, z-value and P-values for the binomial GLM (n=46 observations based on 23 trials in which glass eel was eaten)*

term	*estimate*	*Std. error*	*z-value*	*p-value*
(Intercept)	*-0.1221*	*0.1565*	*-0.7804*	*0.4352*
Marked/unmarked	*0.2442*	*0.2213*	*1.1036*	*0.2698*

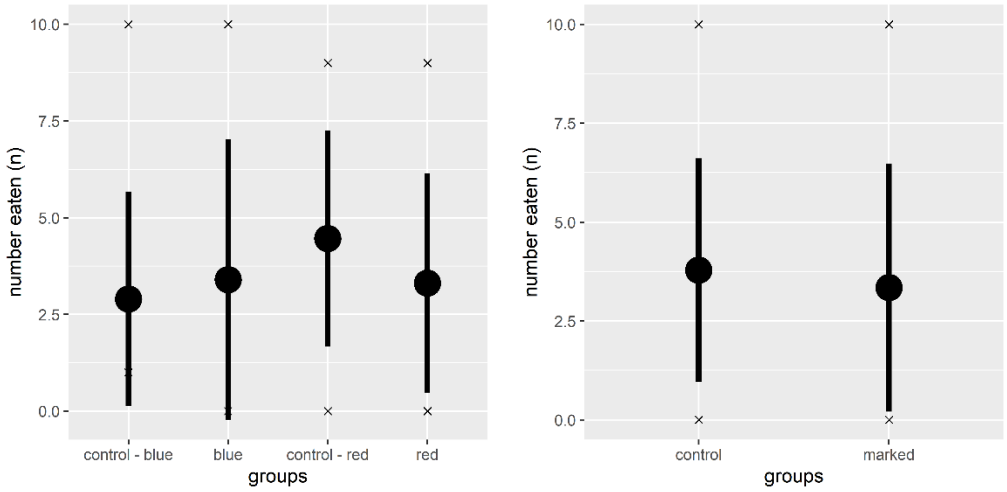

Figure 1*. Number of marked and unmarked glass eels eaten by sea bass (n=23 trials). Left: average number of glass eels eaten in trials with different colors. Right: average numbers of glass eels eaten in the control groups and in the marked (red and blue combined) groups. Figure shows means (dot), s.d. (lines) and x=min and max value.*

Difference between large and small sea bass in predation

In the experiment two length classes of sea bass were used (small: 21.1 cm range 19.1-24.4 cm and large: 36.4 cm range 31.9-43.5cm) and the larger sea bass ate more glass eels during a 2-hour trial compared to small sea bass ($p<0.01$). However, successful predation independent to the total number of glass eels eaten was slightly higher for small sea bass: thirteen out of 24 trails (54%) using small (n=13, mean length 21.4 cm) sea bass showed successful predation in comparison to ten out of 24 trials (42%) using large (n=10, mean length 35.6 cm) sea bass. In general large sea bass showed slightly less interest in eating glass eels, but when it ate, it ate more compared the small sea bass.

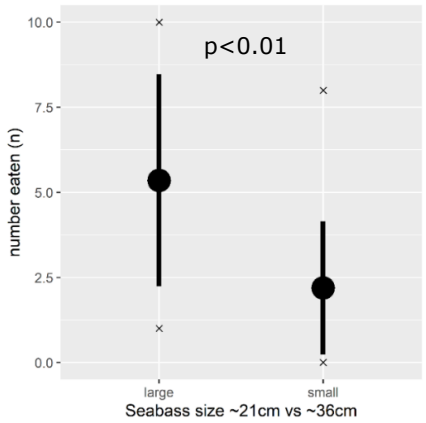

***Figure 2**. Results of predation between large (n=10) and small (n=13) sea basses. A significant difference in number of glass eels eaten was found between large and small sea bass (p<0.01) following Monte Carlo Permutation Tests. Figure shows means (dot), s.d. (lines) and x=min and max value.*

Sea bass behaviour

To verify the sea bass behaviour three groups were identified during the experiment. The first group showed active and successful predation (n=23), the second group was unsuccessful but showed clear attempt(s) for predation during the trial (n=6) and the third group showed no response / no interest (n=19). For further analysis the first and second group were combined (n=23+6) and identified as 'predation behaviour'. The other, and third, group was identified as 'no attempt'. A significant difference was found for sea bass that showed predation behaviour and sea bass that showed no attempt (grid change $p<0.03$ and latency $p<0.01$). Sea bass that showed no clear response to the glass eels ('no attempt') were less active and more restricted to a specific location in the tank. In other words, unresponsive sea bass waited longer to show some activity (latency), and if they moved, it was significantly less (grid change) compared to sea bass that showed a predation attempt. However both groups did not show a statistically significant difference in SPI (SPI, $p<0.09$).

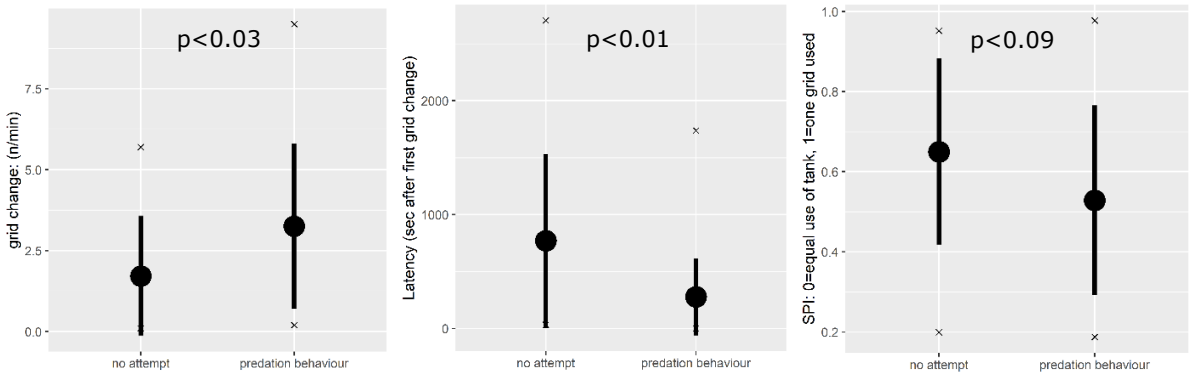

***Figure 3**. Results of behavior comparisons (grid change, latency and SPI) of sea bass showing no attempt of predation (n=19), compared to sea bass that showed a clear attempt to predate on glass eels during the experiment either successful (n=23) of unsuccessful (n=6). A significant difference was found for grid change and latency following Monte Carlo Permutation Tests. Figure shows means (dot), s.d. (lines) and x=min and max value.*

Stomach content analysis

Stomach contents were analyzed using a hand pump and showed intact glass eels bodies after 4-6 hours after predation (Table 2). After 16-18 hours parts of glass eels were found (Photo 3) and after 32 hours no glass eels were found using a hand pump or dissection.

***Table 2.** Results of European sea bass stomach content analysis by flushing using a hand pump and dissection. Stomach flushing has been conducted after a 2-hour trial in fixed time intervals 0.5-32 hours after trial by 21 sea basses.*

time after trial (hour)	glass eels eaten marked (n)	unmarked (n)	N glass eels retreived after flushing	remarks
0.5	1	1	all	Body intact, rigid
0.5		1	all	Body intact, rigid
0.5		3	all	Body intact, rigid
1	2	6	all	Body intact, rigid
1	2		all	Body intact, rigid
1		2	all	Body intact, rigid
2	3	5	all	Body intact, rigid
2	1	3	all	Body intact, rigid
2	1	3	all	Body intact, rigid
4	3	2	all	Body soft. From one glass eel the tail was removed
4	7	4	all	Body soft. Some heads show clear signs of digestion process (e.g. eyes removed from socket)
4	6	8	all	Body soft. Bodies show clear signs of digestion process: falling apart easily.
8	7	4	incomplete number, pieces	5 partially digested, but recognizable, glass eels. 3 pieces of glass eels
8	5	7	incomplete number, pieces	11 partially digested, but recognizable, glass eels and multiple pieces of glass eels
8	1	1	None -> dissection	One glass eel found in intestines
16	10	10	Pieces	17 pieces of tissue of glass eels of 1-4 cm. 4 heads
16	9	9	incomplete number, pieces	9 pieces of tissue of glass eels of 1-4 cm. 2 heads and one digested but recognizable glass eel (which was found in the holding tank after being spit out earlier)
16	8	4	pieces	12 pieces of tissue of glass eels of 1-4 cm. 6 heads of which 2 attached to part of body
32	0	2	None -> dissection	Nothing found also not after dissection
32	5	7	None -> dissection	Pieces of tissue found in intestines. Multiple tags found in tanks among feces.
32	2	1	none	

Photo 3. Pictures of stomach content analysis by flushing using a hand pump. From top to bottom: 1 hour, 2 hours, 4 hours, 8 hours and 16 hours after ending the 2-hours trial. (See appendix for detailed photos)

4 Discussion

Predation

This study showed that there was no increased predation risk for marked glass eels by European sea bass. The lack of a statistically significant result could be caused by low statistical power because the number of successful replicates was low (n=23). However, because the experimental setup was conducted in an environment where marked glass eels are relatively highly exposed compared to unmarked glass eels due to the light conditions, an increased risk for marked glass eels under field condition is suggested to be absent. In addition a sample size calculation was conducted to quantify the number of required trials (each trial required 1 sea bass and 20 glass eels) to find a significant difference between predation of marked and unmarked fish given the current results. Within the experiment, the average difference in proportion of eaten glass eels was found to be approximately 0.166 (0.417:0.583). The sample size calculation for proportions (power = 0.80, significance level = 0.05, difference in proportion = 0.166) revealed that the number of required trials was approximately 107. Considering that only 23 out of 48 showed successful predation (48%), independent of using small or large sea bass, up to 223 trials may be required. If possible difference in predation between marked and unmarked eel is so small that it needs such large amounts of trials, this difference is not expected to influence results in the field and might therefore not be very interesting to conduct in the laboratory.

In addition to our conclusions, other studies found similar results for other predatory fish with other prey: largemouth bass (LMB), channel catfish (CCF), blacktail shiner (BTS) and small mouth bass (Roberts and Kilpatrick 2004, Reeves and Buckmeier 2009). Reeves and Buckmeier followed a similar experimental setup using 10 marked and 10 unmarked fish (Hatchery-produced age-0 LMB, CCF and BTS). Predator fish (LMB, flathead catfish and white bass) were held without food for 3 days. However their trials continued for 24 hours or until 50% of the prey had been consumed and 10 repetitions per predator species were used. They did not find significant differences in predation of all three predator fish between marked (three tags of different colors in each prey fish) and unmarked fish. Another study used yellow (VIE -tag), green (VIE -tag) and unmarked fantail darters which were exposed to rock bass and smallmouth bass during 6-8 days trials or after 50-75% of the prey were eaten (Roberts and Kilpatrick 2004). They used eight marked and 8 unmarked fish per treatment and found no significant difference within 12 repetitions of rock bass and 4 repetitions of smallmouth bass.

Our trials showed 48% successful predation and 13% clear attempts of predation. The other sea bass (39%) waited significantly longer to show some activity (latency), and if they moved they moved significantly less (grid change). This could be the results of no interest in the glass eels, but it could also be a stress response. Based on the results and experimental setup it cannot be distinguished what determined sea bass to ignore the glass eels. Although based on significant different behaviour (latency and grid change) between the two groups, stress might be the crucial factor on ignoring the glass eels. To exclude stress as interfering factor of the experiments sea bass were only used in the experiments when food intake was successful (Beitinger 1990). However, some sea bass may have been stressed during the experiments explaining lack of interest in the glass eels. In addition, an increased expression of freezing behaviour is a main indicator of stress in Sea bass (Cerqueira et al. 2020). Therefore it can be concluded that stress is a factor to be taken into account during predation experiments with sea bass.

No preference for blue or red marked glass eels was found during the experiments, which might be in accordance with other studies using same marking technique but other colors and other fish (Reeves and Buckmeier 2009). The advantage of VIE-tags over external group dye methodology or photonic dyes, is that marks retain longer and are better visible in the fish for recapture purposes (Catalano et al. 2001, Griffioen and Winter 2018). Moreover VIE-tags illuminate by UV-light for clear identification by

professionals or volunteers. The question is whether this illumination induce an increased predation risk during sunlight conditions. First, the optimum wavelength for fluorescing the VIE-tags is deep violet (405 nm) which falls within range of current experimental set up and light conditions (380-780nm). However under natural circumstances glass eels migrate after sunset and in dark conditions (Tesch 2003). During that period, light conditions are far from optimal to be illuminated due to the absence of sunlight. Therefore, contrast (tag in transparent fish) may cause an increased predation risk above color distinction. In this study, only transparent glass eels were selected and test groups were tagged with double marks to increase contrast differences. Since no significant differences was found between test and control groups under light conditions, both color and contrast seemed not to induce additional predation risk.

Contrary to our findings, a study with photonic dyes and largemouth bass showed preference for brightly marked fish (pink and blue) were preferred over colorless (cryptic) marks (Catalano et al. 2001). No difference was found between pink and blue, but, on average, largemouth bass consumed over 13% more fish with brightly colored marks. Besides using other fish and other marking techniques, this study used tank coverage of 12% (natural vegetation) and substrate inside the tank which could lead to different results according to Reeves et al (2009). Also, contrary to our experiments, less predator fish were used (n=13) and experiments ended after 20-30% of the original number of fish remained in the tanks (3-7 days). The length of our experiments might have been too short for some of the sea bass to get used to the experimental tanks. As previous described other experiments continued over 24-hours up to multiple days (Catalano et al. 2001, Roberts and Kilpatrick 2004, Reeves and Buckmeier 2009). Besides duration of the experiments, percentage of successful predation is another restriction of ending the experiment (Catalano et al. 2001, Roberts and Kilpatrick 2004, Reeves and Buckmeier 2009). However, our experiments showed that eight out of 23 sea bass ate over 50% of the glass eels within 2 hours and longer experiments do not necessarily mean better results to analyze predation preference. If all glass eels are eaten no distinction can be made between marked or unmarked as food preference. A solution can be to quantify glass eel predation during the experiment continuously and end the trial if 50-75% of the glass eels are eaten. However, it is expected that stress of the sea bass was a factor influencing predation during the experiments. Therefore, continuously and visually counting the glass eels during the experiments will influence test results since the presence of observants could induce stress to at least some of the sea bass. Another option is to increase the number of glass eels offered to the sea bass and subsequently increase the duration of the experiment. To do so a balance between number of glass eels, tank size and size of the sea bass should be taken into consideration.

Stomach content analysis

Stomach content analysis showed intact glass eels bodies after ending the 2-hour experiment and glass eels could be clearly identified after 4 hours and parts of glass eels were found after 16 hours. This suggests a high probability to encounter glass eels using a hand pump for stomach content analysis if glass eels are eaten by a predator fish. Field experiments should therefore be conducted preferably within 4 hours after predation. Therefore, field studies should be conducted during the evenings and dark periods, assuming that glass eels are predated in this period, to observe glass eels predation by stomach analysis and to quantify glass eel mortality. In addition to stomach contact analysis, DNA analysis of the stomach contents can qualitatively determine whether glass eels were eaten by a predator fish (Miyake et al. 2018).

Justification

CVO Report: 20.027
Project number: 4311300081

The quality of this report has been peer reviewed by a colleague scientist and the head of CVO.

Approved by: Dr. T. van der Hammen
Colleague scientist

Signature: 

Date: December 2nd 2020

Approved by: Ing. S.W. Verver
Head Centre for Fisheries Research

Signature: 

Date: December 2nd 2020

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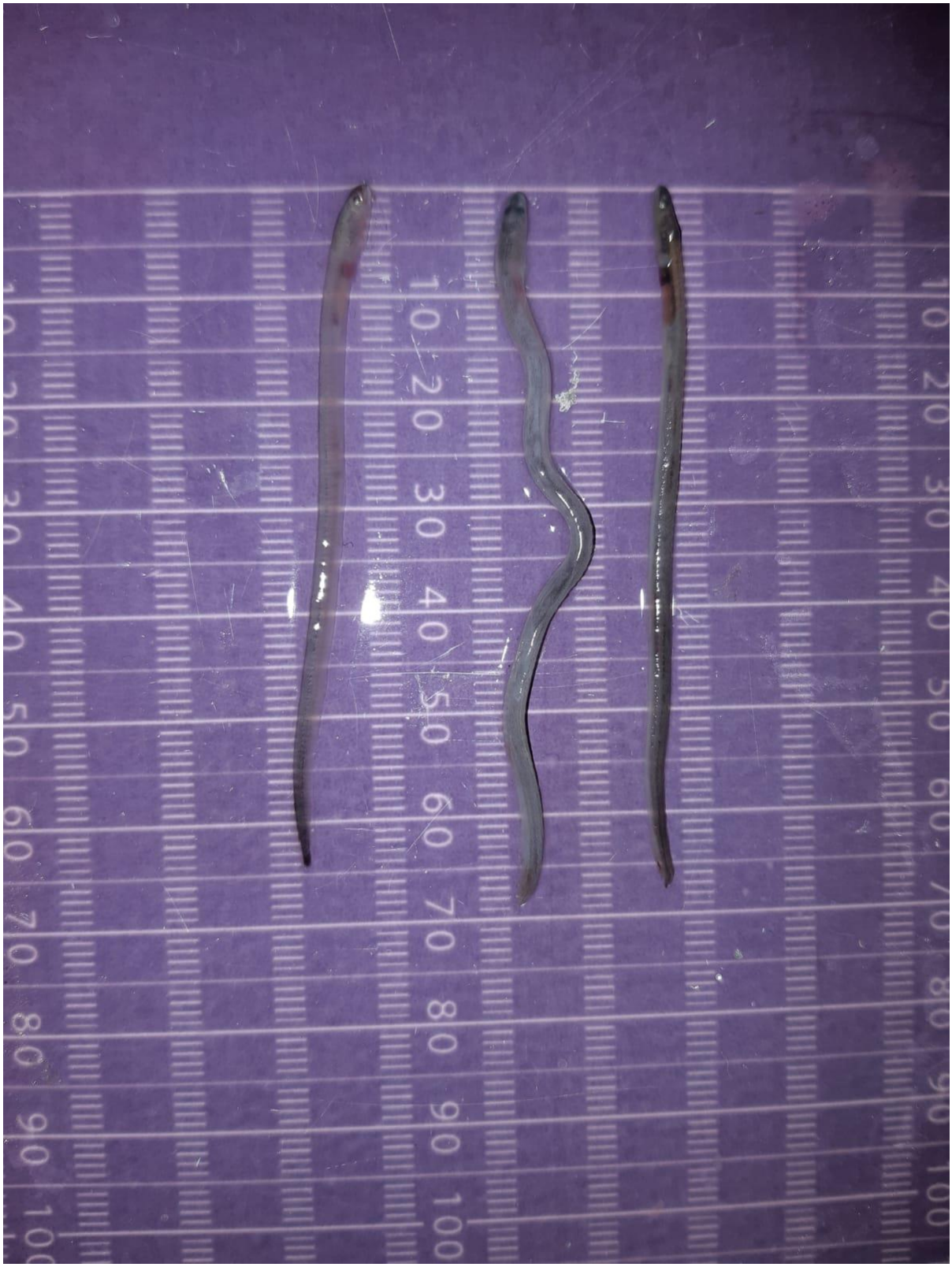
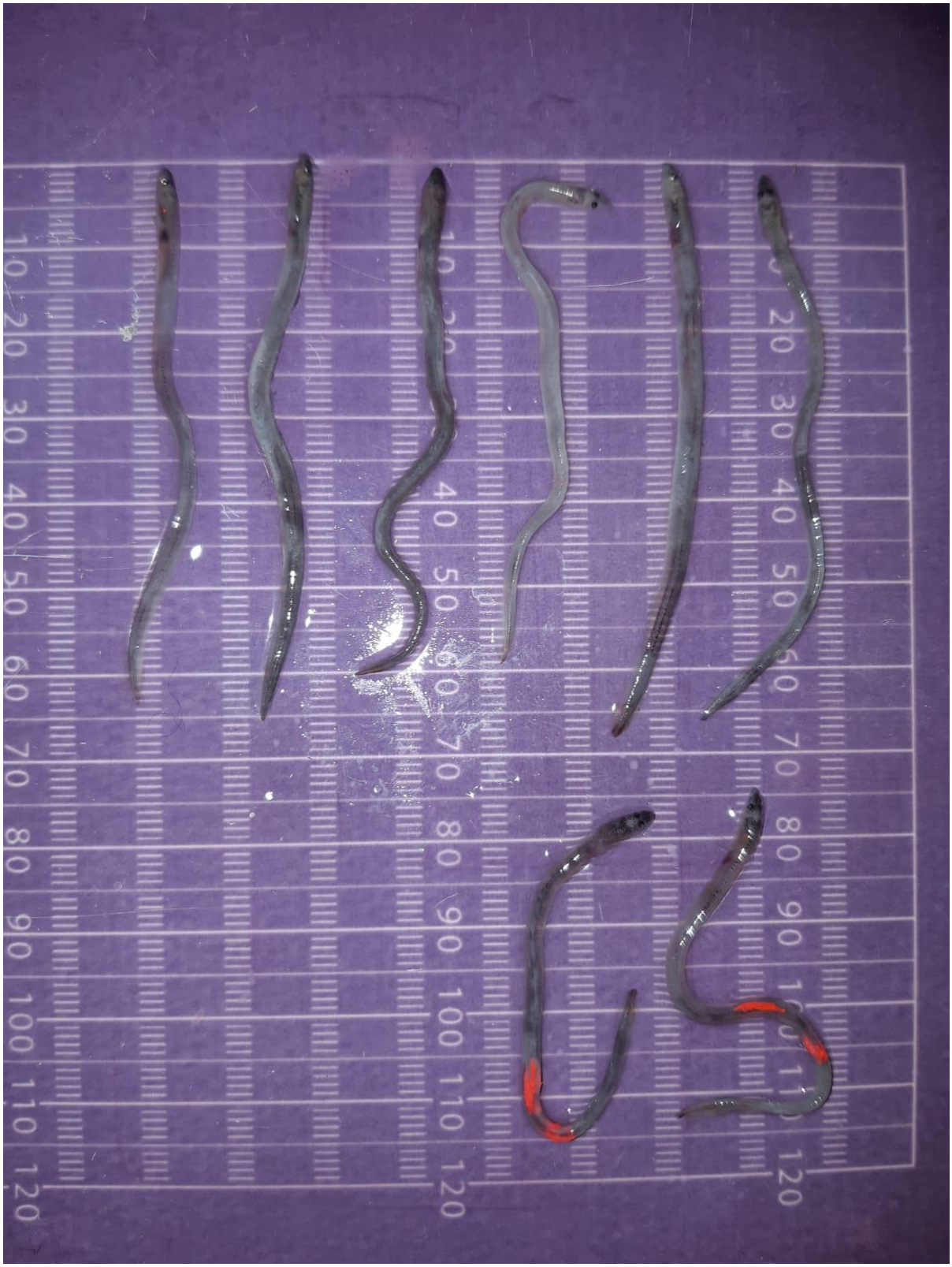
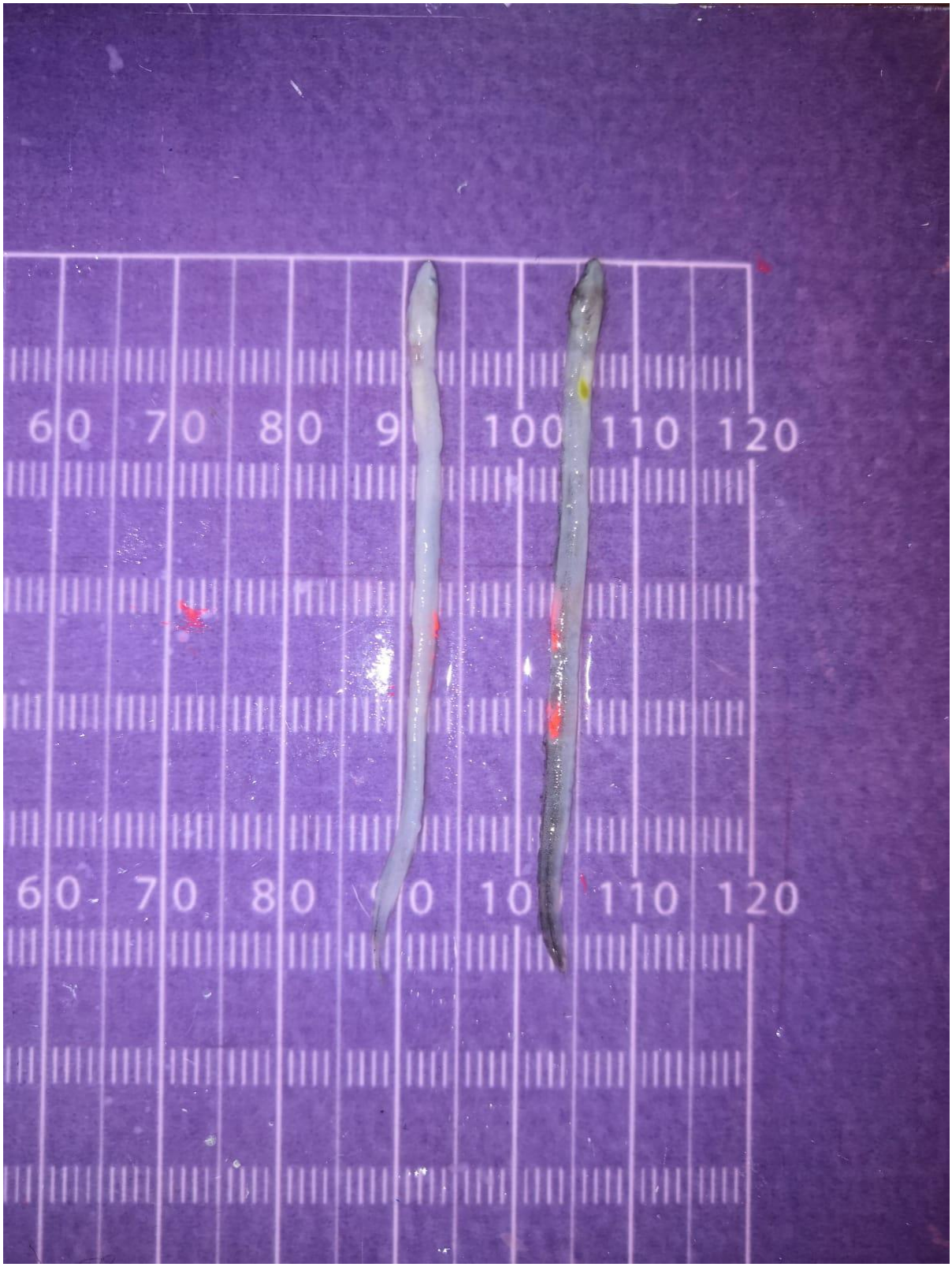
Appendix



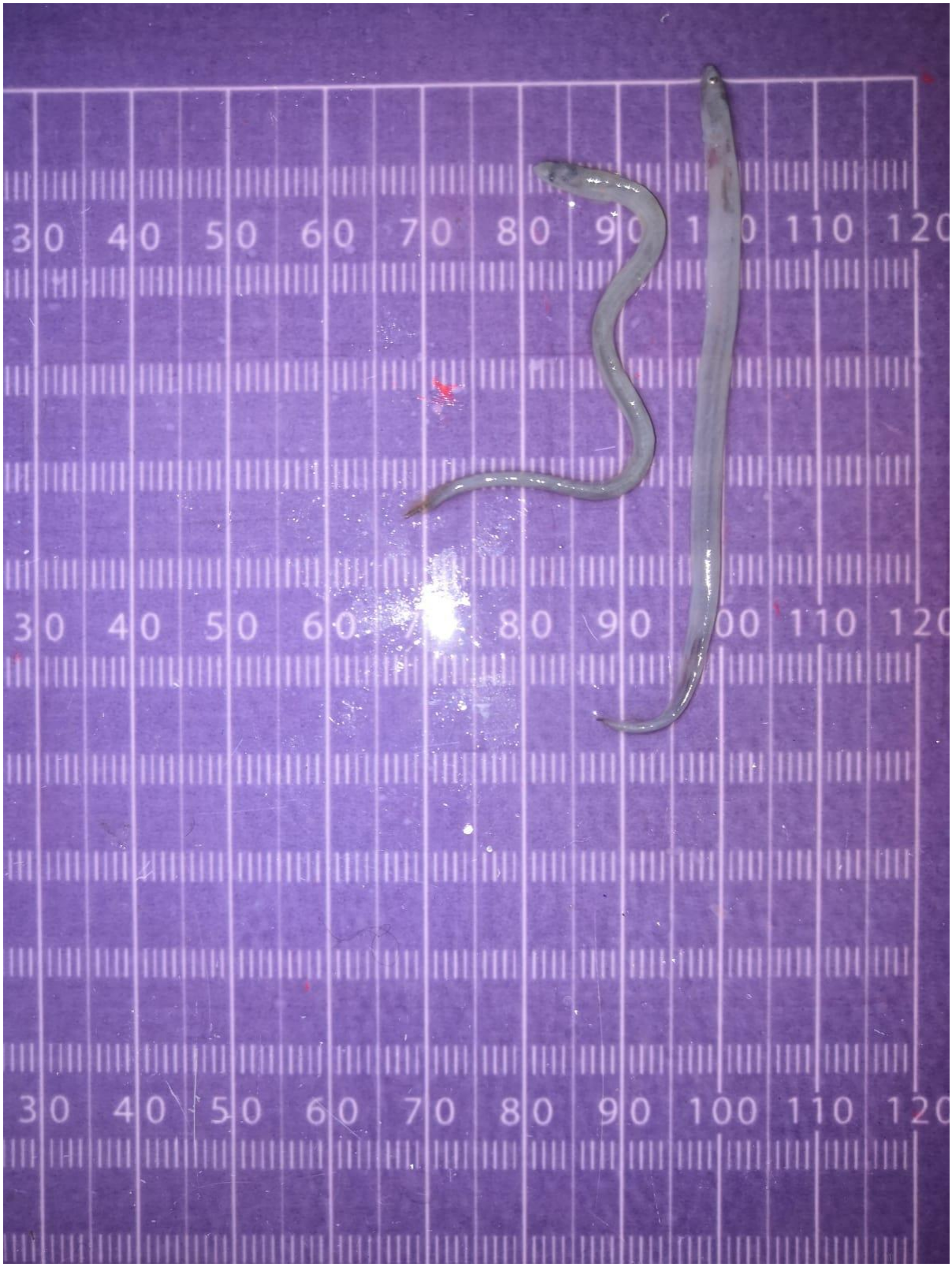
Fish #11 - 30 minutes: 3 unmarked



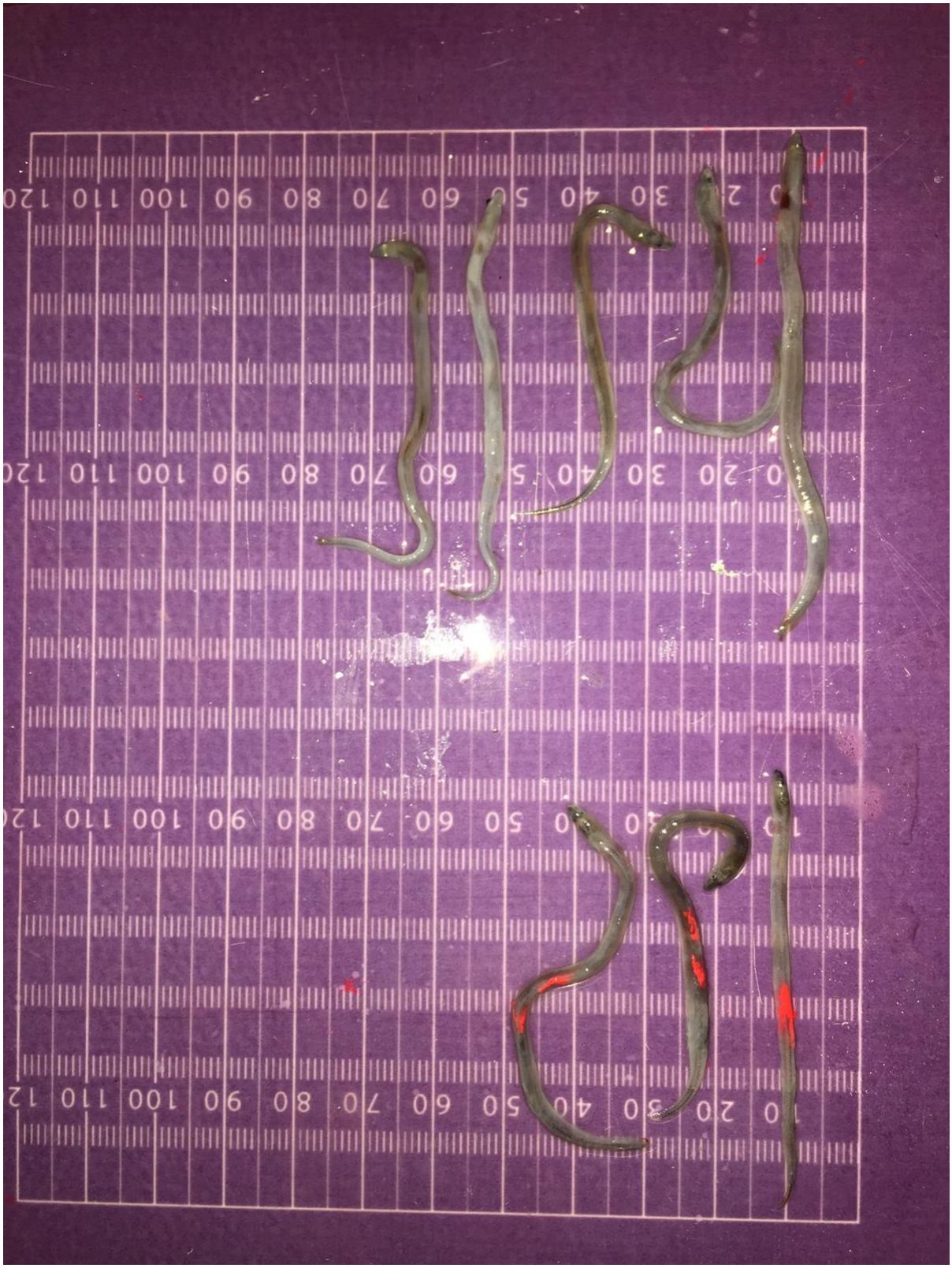
Fish #12 – 1 hour: 2 marked 6 unmarked



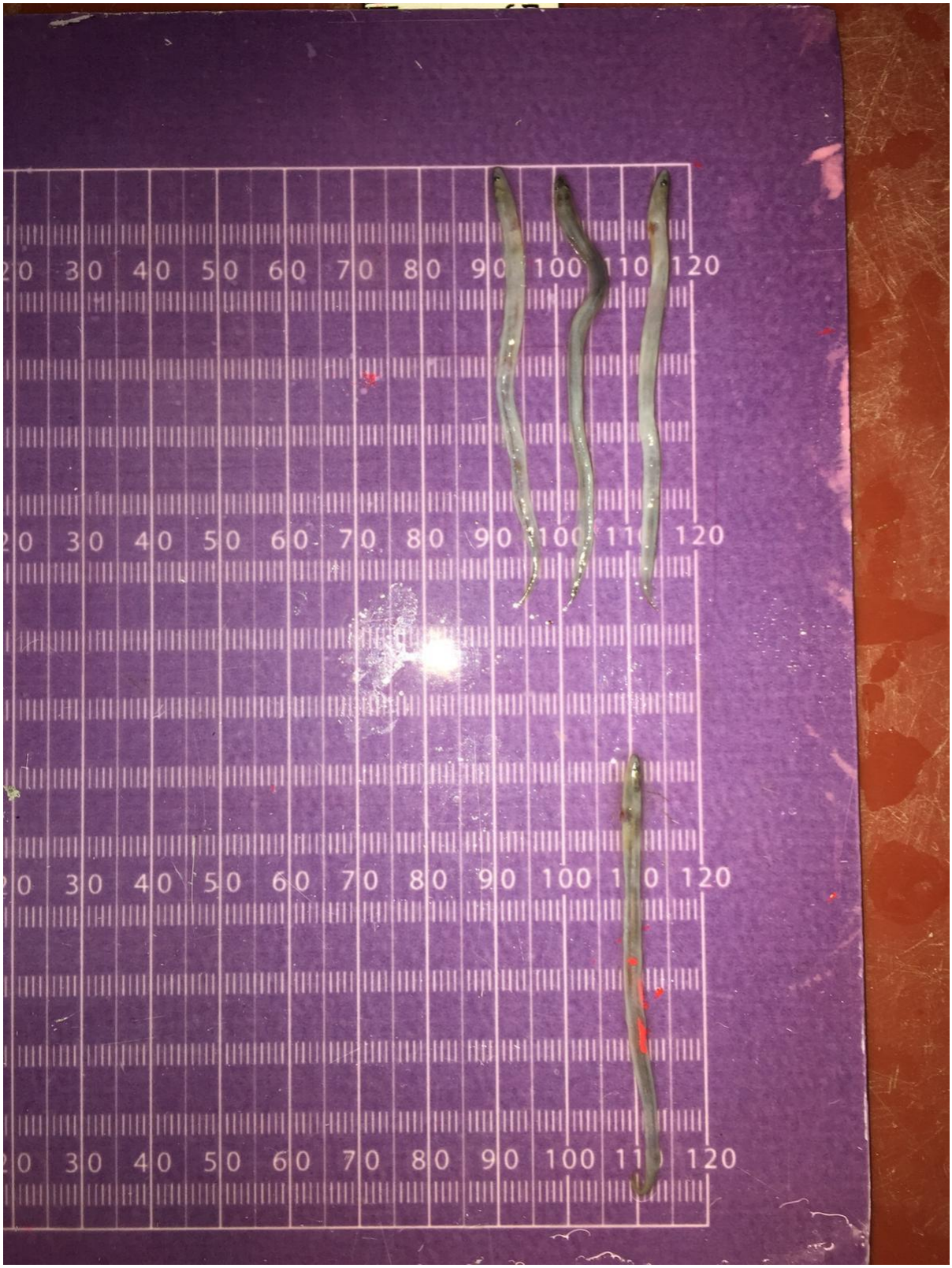
Fish #13 – 1 hour: 2 marked



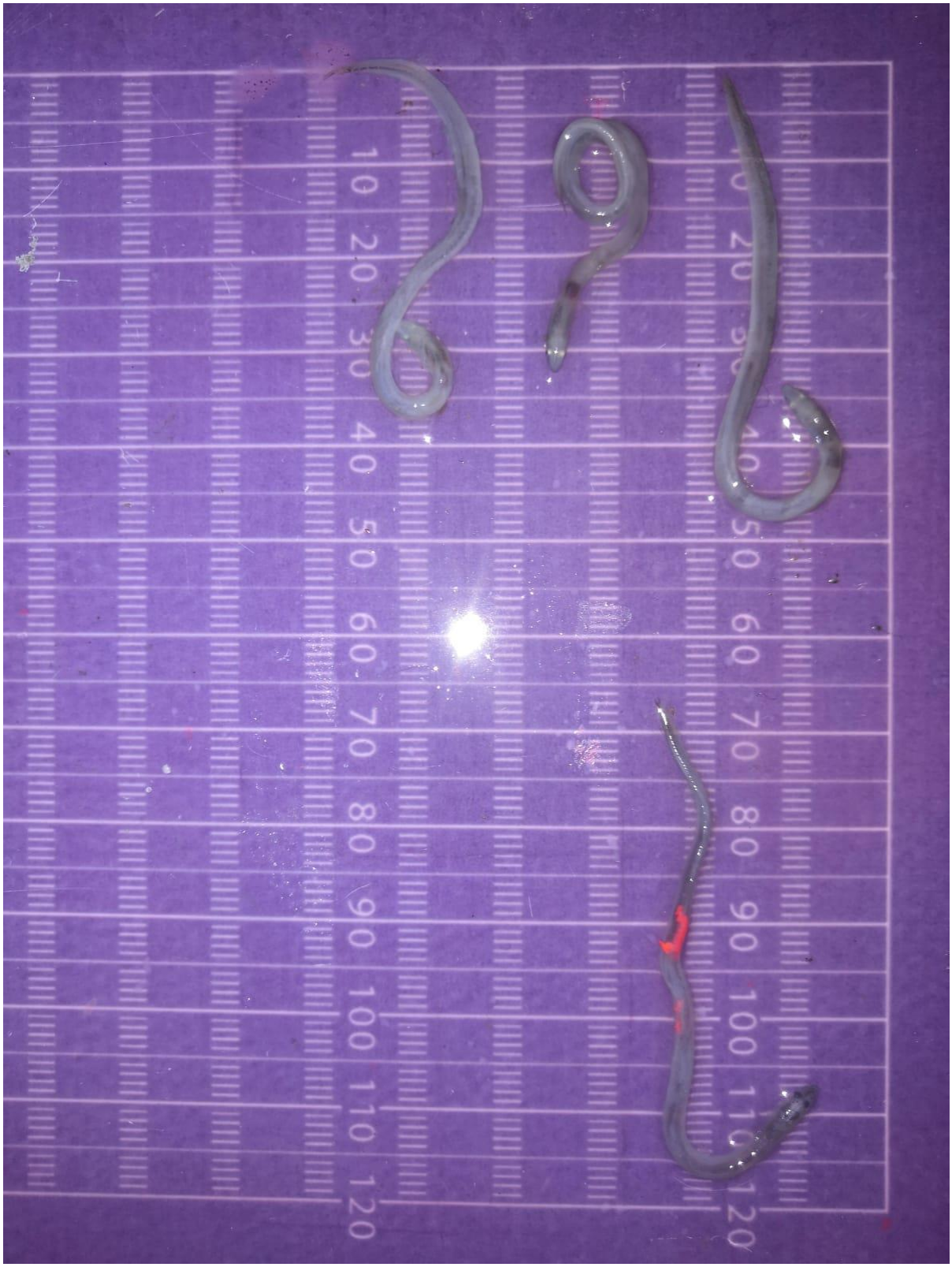
Fish #14 – 1 hour: 2 unmarked



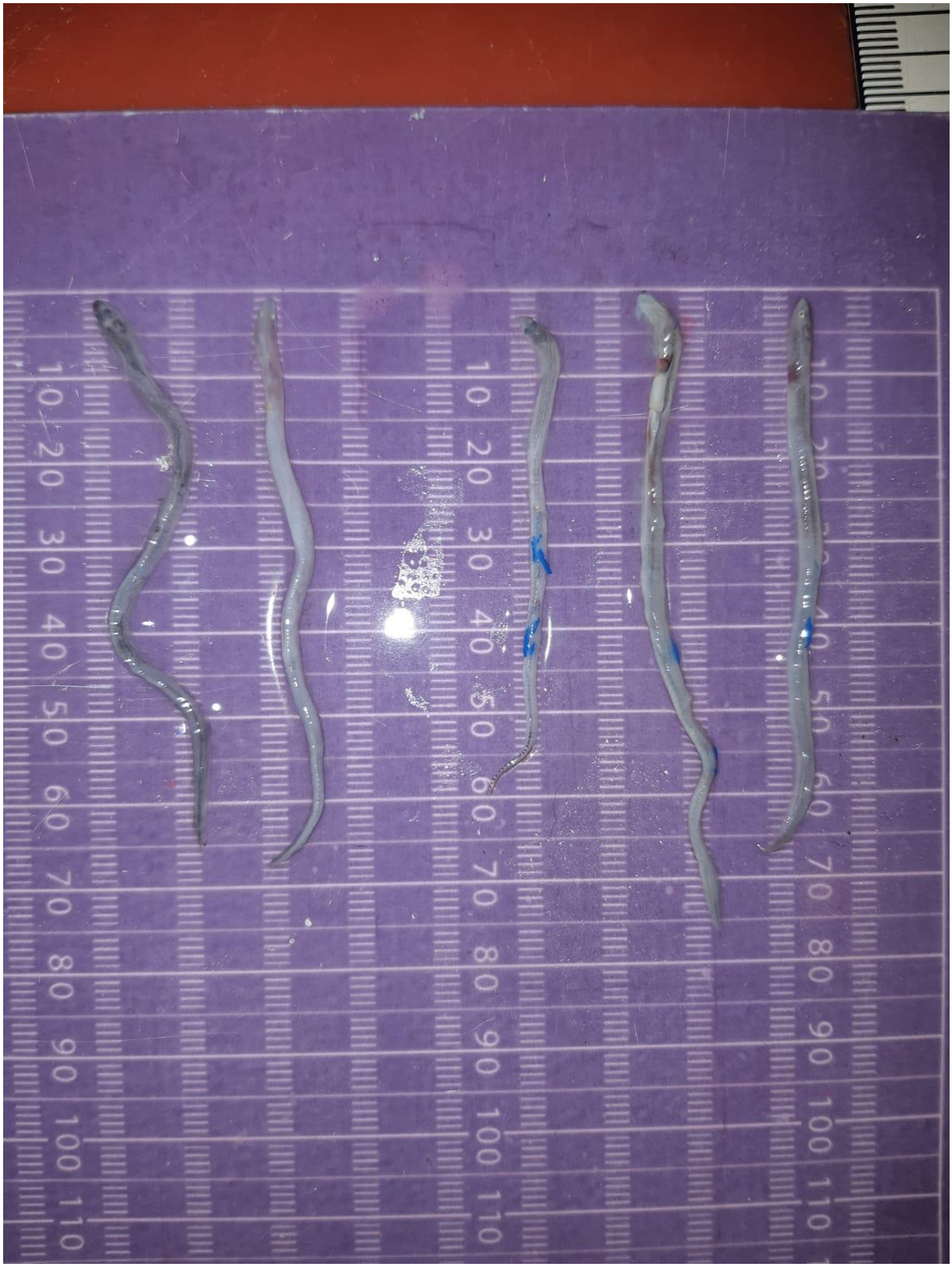
Fish #15 – 2 hour: 3 marked 5 unmarked



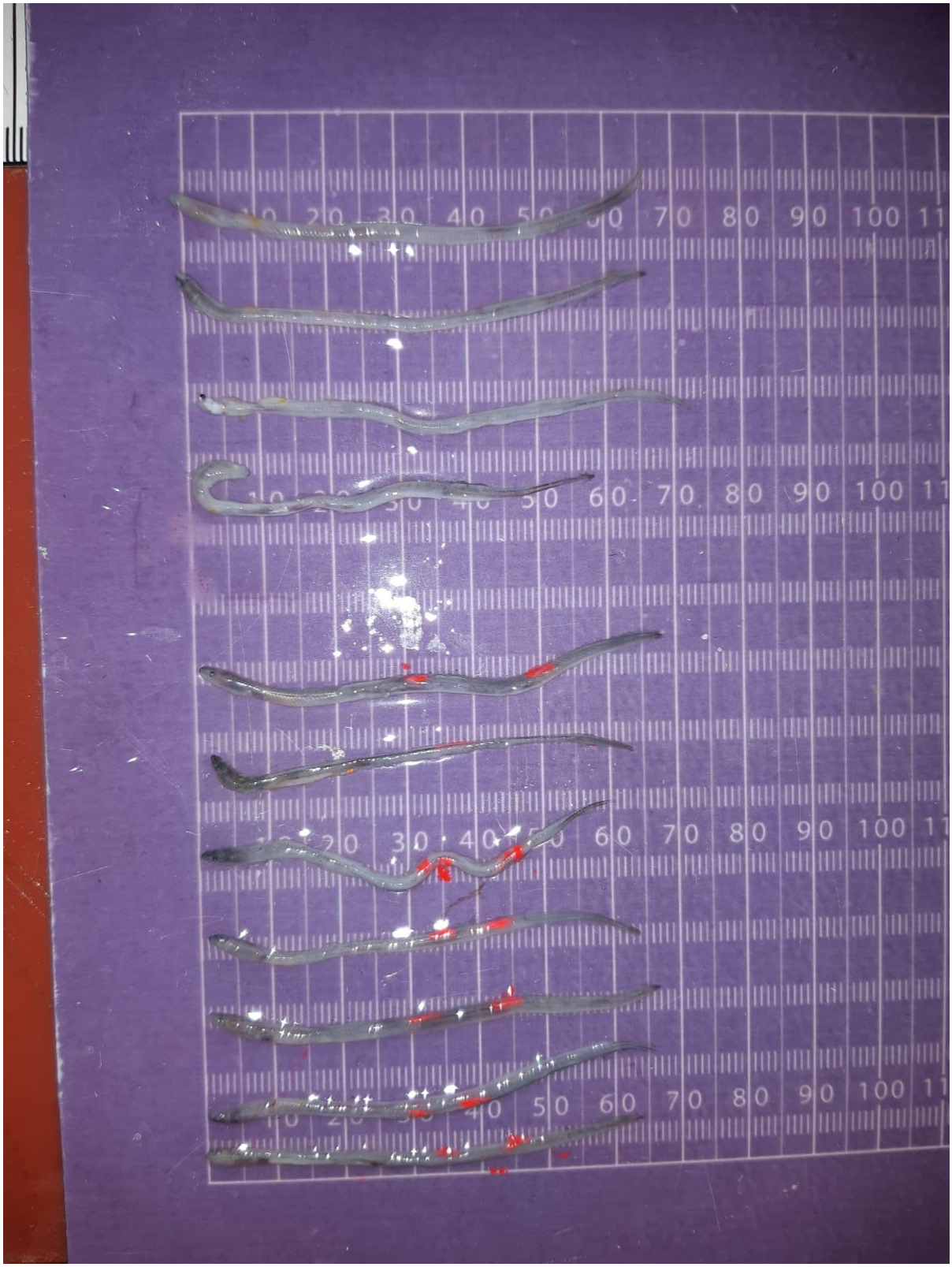
Fish #16 – 2 hour: 1 marked 3 unmarked



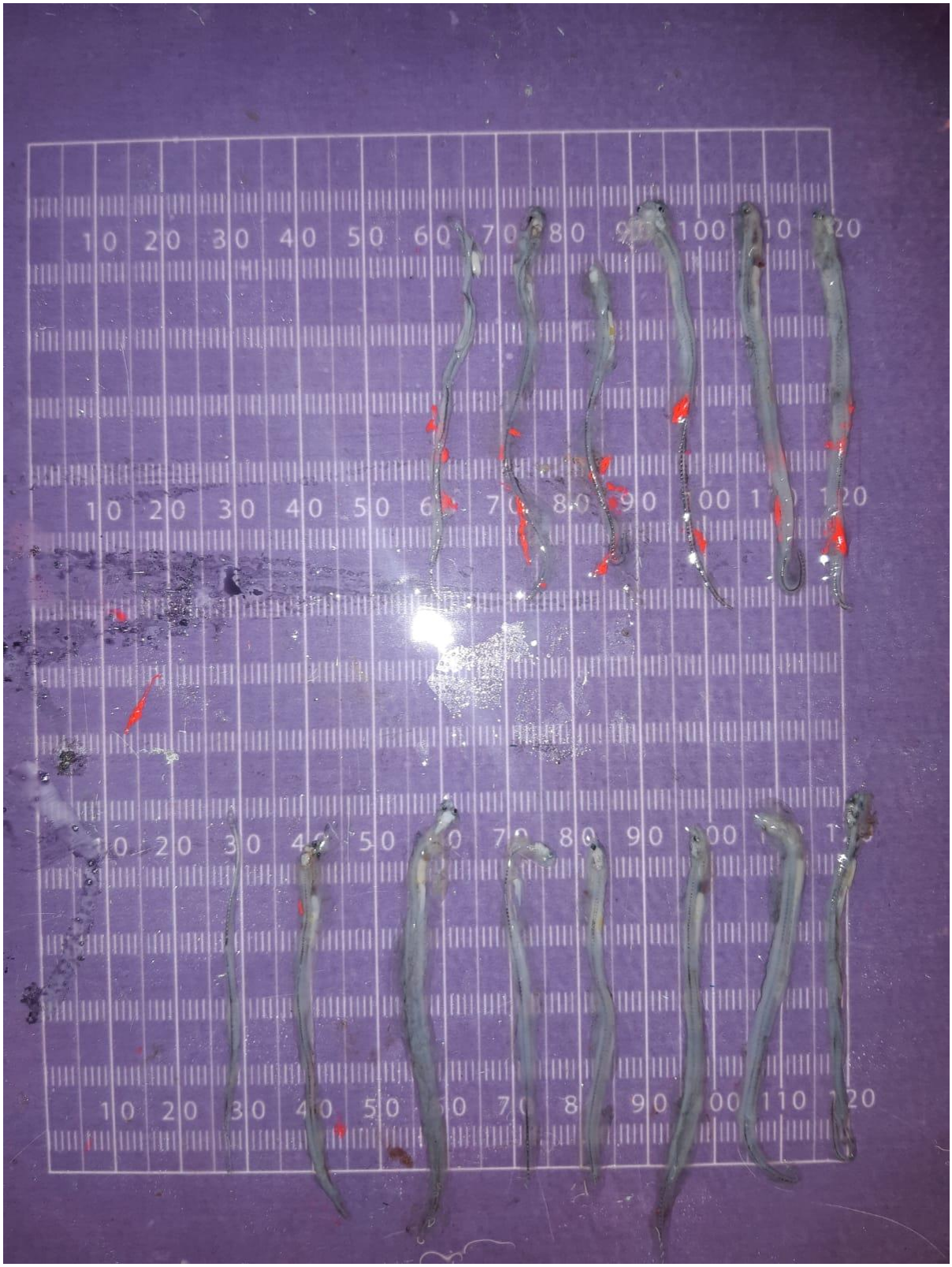
Fish #23 – 2 hour: 1 marked 3 unmarked



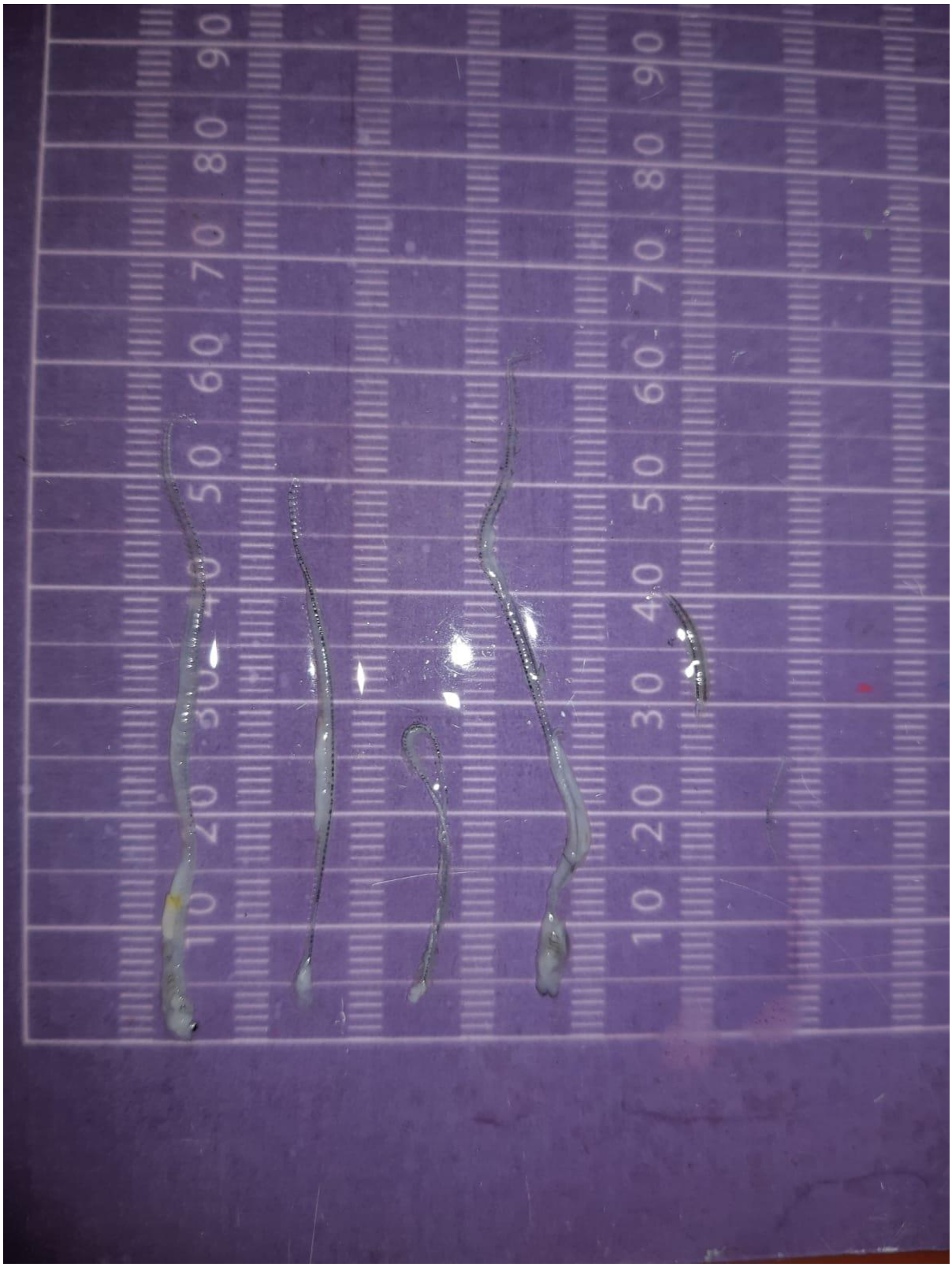
Fish #27 – 4 hour: 3 marked 2 unmarked



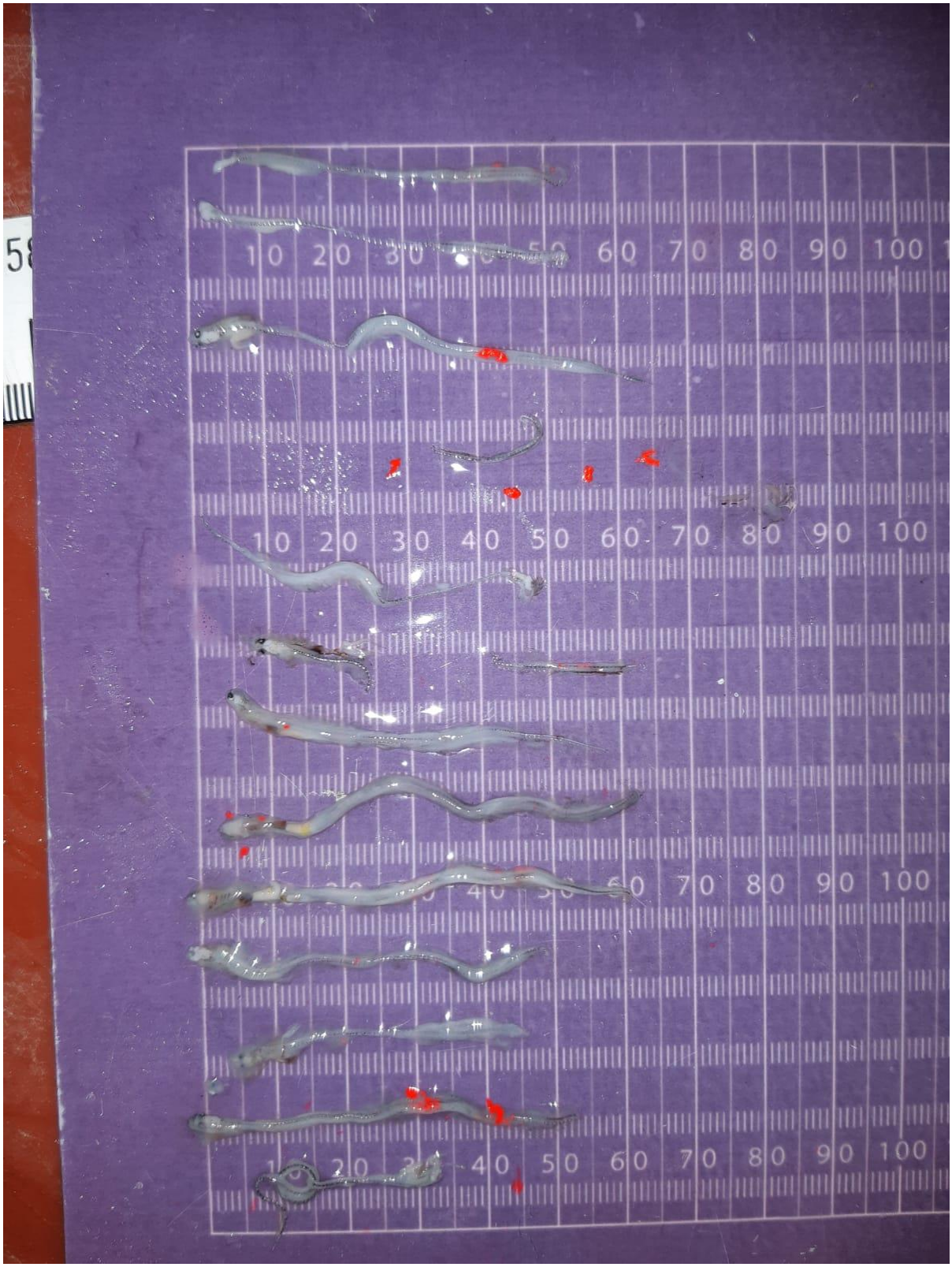
Fish #28 – 4 hour: 7 marked 4 unmarked



Fish #36 – 4 hour: 6 marked 8 unmarked

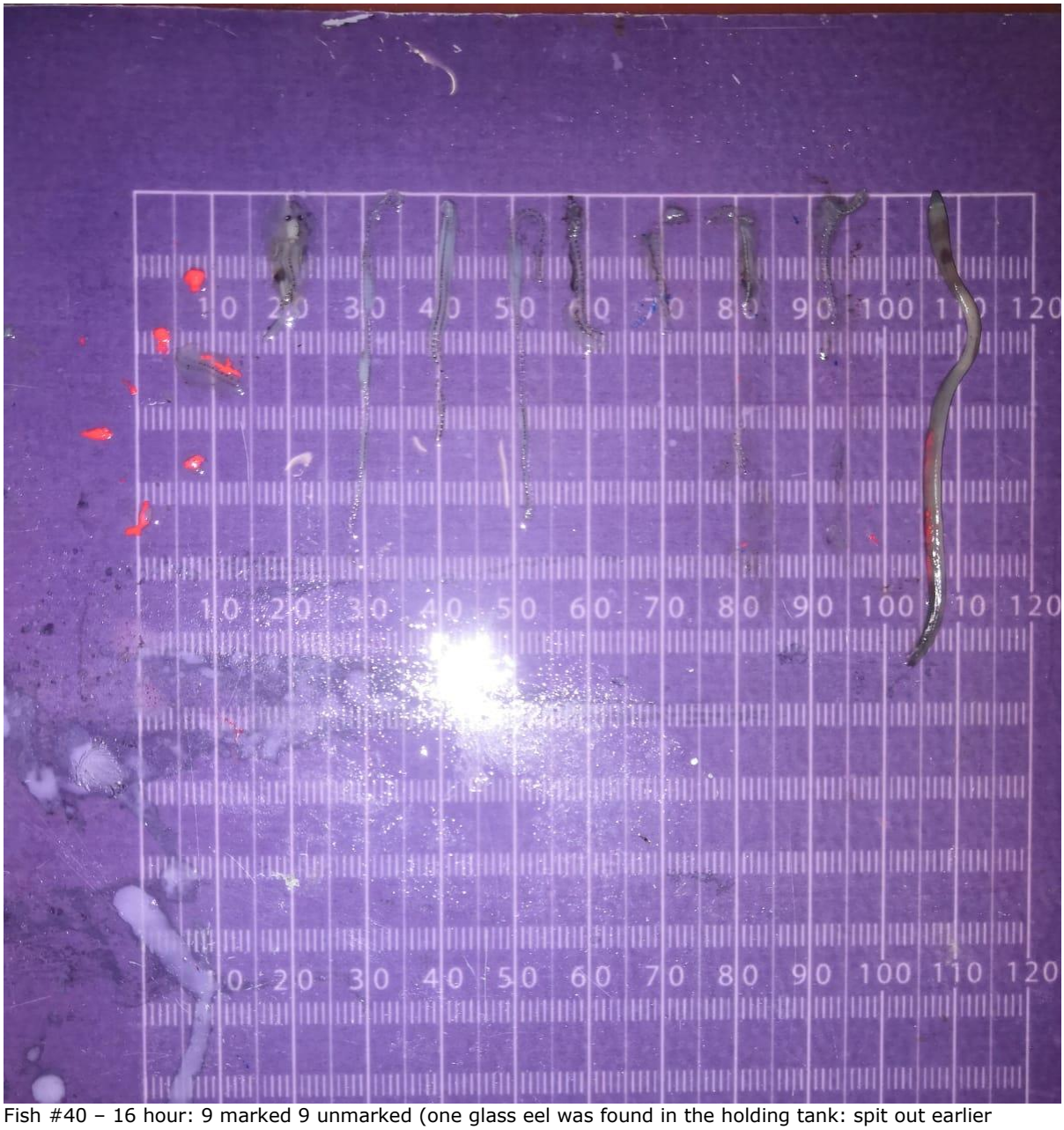


Fish #30 – 8 hour: 7 marked 4 unmarked

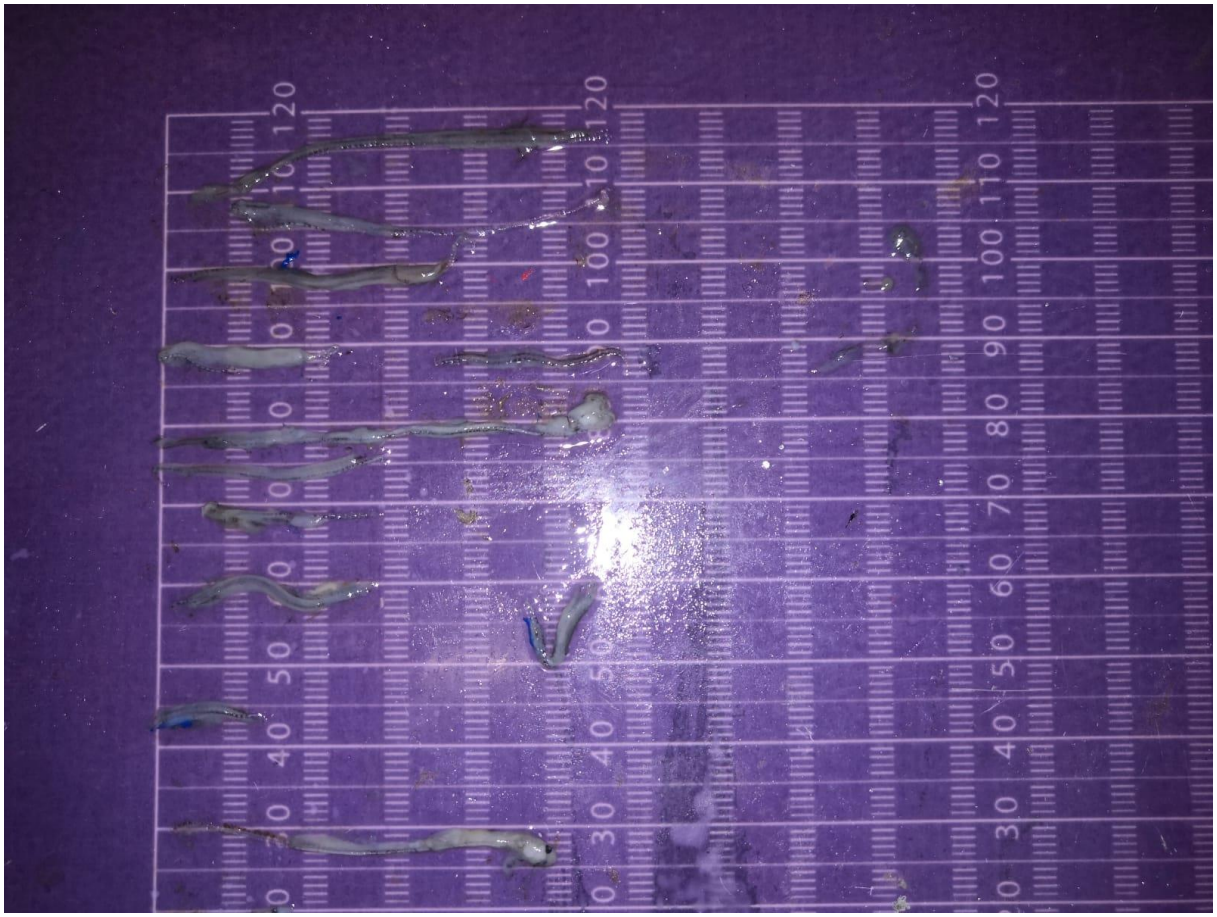


Fish #32 – 8 hour: 5 marked 7 unmarked

Fish #38 – 16 hour: 10 marked 10 unmarked



Fish #40 – 16 hour: 9 marked 9 unmarked (one glass eel was found in the holding tank: spit out earlier unnoticed)


Fish #45 – 16 hour: 8 marked 4 unmarked