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Do young on-grown eels, *Anguilla anguilla* (Linnaeus, 1758), outperform glass eels after transition to a natural prey diet?

By J. Dainys¹, H. Gorfine², E. Šidagytė³, E. Jakubavičiūtė¹, M. Kirka¹, Ž. Pūtys¹ and L. Ložys¹

¹ Laboratory of Marine Ecology, Nature Research Centre, Vilnius, Lithuania; ² School of BioSciences, University of Melbourne, Parkville Campus, Victoria, Australia; ³ Laboratory of Evolutionary Ecology of Hydrobionts, Nature Research Centre, Vilnius, Lithuania

Summary

Survival rates among European eels, *Anguilla anguilla* (Linnaeus, 1758), on-grown using a formulated diet in a commercial aquaculture facility, were compared with glass eels from the same cohort following their transition to a natural prey diet in the laboratory. Treatments included zero, 42-day, and 196-day periods of grow-out prior to 30-day experimental periods when eels were fed *Chironomus* spp. larvae (10 tanks, each containing 240-L water and 40 glass or 10 on-growing eels; 12:12 h photoperiod; water temperature 18 °C). All glass eels survived, compared to 87% (42-day) and 99% (196-day) for on-grown eels. Although the eels on-grown for 196 days had a high survival rate, they did lose weight. Farm-reared eels may have accumulated sufficient resources over the 196-days to survive the first 30 days after weaning from a formulated diet, but not for an

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29 additional 30 days (84% survival). Lack of superior survival rates among on-grown eels challenges
30 the presumed benefits of releasing on-grown eels for population restoration.

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31 Introduction

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34 Rapid population declines have been observed since the 1980s over the entire distribution
35 range of the European eel (*Anguilla anguilla* L.) (Moriarty and Dekker, 1997; Feunteun,
36 2002; Dekker, 2004; ICES, 2015). As a consequence, in 2007 the European Union (EU)
37 issued the Regulation of European Council No. 1100/2007, which lays down measures for
38 the restoration of European eel stocks. This regulation obliges member states to develop
39 and implement national Eel Management Plans. The aim of these plans is to allow 40% of
40 spawners (silver eels) from a pristine stock to migrate towards the spawning area in the
41 Sargasso Sea; restocking inland waters is one means to accomplish this (Dekker and
42 Beaulaton, 2015). Nowadays, after capture in the wild, glass eels are often on-grown in
43 aquaculture facilities prior to translocation and release. During the on-growing phase, eels
44 are weaned from a natural diet and fed with specially formulated food. Their weight
45 increases up to ten-fold during this period; consequently, mortality among on-grown eels
46 released back into the wild was believed to be lower when compared to mortality rates
47 among eels liberated at the glass eel stage. This construct is speculative and in need of
48 testing (ICES, 2007, 2015). Moreover, there is evidence that stocking of hatchery-reared
49 fish does not always deliver significant improvements (Larscheid, 1995; Blaxter, 2000).
50 White and Knights (1994) suggested that any initial growth advantage from releasing on-
51 grown eels is lost after about 5-6 years. Simon and Dörner (2014) found over a 7-year
52 study period following re-stocking, that glass eels exhibited superior growth and condition
53 compared to eels on-grown in an aquaculture facility, and had caught up in body size
54 within three to four years post-release. There is also evidence that stocking with on-grown
55 eels may result in poorer survival rates than for glass eels (Berg and Jorgensen, 1994;
56 Pedersen, 2000; Wickström, 2001; ICES, 2007). Exposure of different fish species to an
57 artificial environment during on-growing can produce phenotypic changes that give rise to
58 impaired migratory and anti-predator behaviour, thereby reducing chances of survival
59 (Brown and Laland, 2001; Svåsand, 2004). There has also been speculation that on-
60 growing in aquaculture facilities adversely affects the capacity for the weaning of eel to
61 natural food during the post-release period (Smirnov et al., 1994; ICES, 2013), resulting in
62 increased mortality. In addition to these negative aspects of farm-reared eels, glass eel on-
63 growing in aquaculture facilities poses risks of spreading parasites and diseases, genotypic

64 population alteration, release of slower growing individuals caused by size-dependent
65 sorting, as well as increasing the cost per stocked eel (Egusa, 1979; ICES, 2011).

66 To our knowledge, there have been no laboratory studies undertaken to compare
67 survival rates among on-grown European eels and glass eels under controlled conditions.
68 The primary aim of the present study was to test how well on-grown eels could be weaned
69 from a formulated diet to feed on *Chironomus* spp. larvae that are typically consumed by
70 eels in natural water bodies (de Nie, 1982), and how this transition affects their survival in
71 comparison to glass eels under the controlled conditions provided by an indoor laboratory
72 facility.

75 **Materials and methods**

76
77
78 The effects of three pre-release options on the survival of translocated wild-caught glass
79 eels were tested during 30-day sequential laboratory experiments when the eels were fed
80 *Chironomus* spp. larvae. These treatments were: Group A, glass eels not on-grown; Group
81 B, eels fed a specially formulated diet whilst on-grown in an aquaculture facility for 42
82 days; and Group C, fed for 196 days. The experiments were run sequentially to ensure that
83 eels for each treatment were sourced from the same cohort of imported glass eels, to
84 control for inherent differences in growth and survival.

85 After 30 days in the Group C treatment, it was evident that weight loss and poor
86 physical condition prevailed among some individuals, although not to the point of obvious
87 morbidity. To allow time for these eels to regain condition, this experiment was extended
88 for an additional 30 days (Group C₁).

90 **Source of eels for experimental treatments**

91 Glass eels were shipped from the United Kingdom and upon arrival in Lithuania were
92 transported to an aquaculture facility in the Vilnius district for rearing. Initially, the eels in

93 this facility were fed cod roe at 10% of their body weight for the remainder of the glass eel
94 phase. At the beginning of the post-glass eel on-growing phase the eels were transitioned
95 to a specially formulated fish food (ALLER FUTURA EX, 00-0 GR., [http://www.aller-](http://www.aller-aqua.com/introduction-to-fish-feed/warm-freshwater-species)
96 [aqua.com/introduction-to-fish-feed/warm-freshwater-species](http://www.aller-aqua.com/introduction-to-fish-feed/warm-freshwater-species)). This meant that prior to the
97 transfer of eels from the aquaculture facility to the experimental laboratories at Lithuania's
98 Nature Research Centre (NRC), the Group A glass eels were fed only cod roe, whereas
99 both the B and C groups of on-grown eels were progressively transitioned and reared
100 exclusively on formulated fish feed. Water temperature in the rearing system at the
101 aquaculture facility was maintained within the range of 25–27°C. Immediately after
102 transportation to NRC, the eels were acclimated to a lower water temperature of 18°C in
103 the laboratory experimental system. No mortality was observed during this acclimation
104 period.

106 **Experimental system**

107 Ten experimental semi-square fiberglass tanks (length = 0.6 m, width = 0.6 m and depth =
108 0.8 m), each containing 240-L water were connected to the same recycling system
109 (mechanical filter Hydrotech drum, USA, mesh size = 40 µm; biological filter Bio-Blok
110 200, Denmark, volume = 4.5 m³, biofilter material inner surface = 200 m²/m³). The
111 experimental system was exposed to simulated day–night cycles of artificial light (Hagen
112 Sun Glo T8 4200K lamps; 12:12 hour photoperiod). Water flow rate from the filter to each
113 experimental tank was 5 L min⁻¹ (50 L min⁻¹ through a biological filter) and monitored
114 twice a day. After gradual adaptation, eels were randomly allocated among 10 replicate
115 tanks each containing 40 individuals in the Group A experiment (N = 400), and 10
116 individuals in each of Group B (N = 100) and Group C (N = 100). During all experiments
117 the eels were fed *ad libitum* with live *Chironomus* spp. larvae. Eels in all replicates were
118 fed at the same time and all uneaten larvae were removed within two hours after feeding.
119 Throughout the experiments accumulated faeces were removed every two days before
120 feeding. Total wet weights of eels within each experiment replicate, i.e. ten replicates per
121 group, were weighed at the beginning and end of each experiment. Individual
122 measurements were not taken in order to minimise the risk of handling-induced mortality.
123 Weights of a sample (N=40) of individual eels retained in the aquaculture facility were
124 measured periodically and their means calculated over duration of the study. Fish health

125 was monitored in each tank daily; no external symptoms of any diseases were noted during
126 this series of experiments. Tests for ammonium and nitrite concentrations were performed
127 at 2-day intervals and for nitrate at 5-day intervals (Aquamerck Nitrite Test 14658,
128 Aquaquant Ammonium Test 14400; Aquamerck Nitrate Test 11170; Merck, Germany).
129 Samples for water quality tests were taken from outlet pipes of each tank.

130

131 **Data analysis**

132 Specific growth rates (SGR) of each replicate were calculated in addition to the survival
133 rates (S). The SGR was calculated as described in Wootton (1990) and used to monitor eel
134 condition.

135 As survival data were non-normal (Shapiro–Wilk’s W tests, $P < 0.05$), both non-
136 parametric and parametric approaches were used to test for group effect (A, B, C) on eel
137 survival after one month in laboratory conditions. Kruskal-Wallis ANOVA was followed
138 by Multiple Comparison tests of mean ranks, and One-Way ANOVA performed on
139 arcsine-transformed data was followed by Tukey HSD post-hoc tests. Statistical analysis
140 was conducted with StatSoft STATISTICA® 8 software.

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143 **Results**

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146 During the experiments water temperature was maintained at $18.3 \pm 0.4^{\circ}\text{C}$. Concentrations
147 of ammonium and nitrite were $<0.05 \text{ mg L}^{-1}$ and the concentration of nitrate was $<38 \text{ mg}$
148 L^{-1} for the duration of all experiments.

149

150 **Growth**

151 Average initial weight of Group A was 0.46 ± 0.01 g (N = 400), while the weights of groups
152 B and C were 1.14 ± 0.1 g (N = 100) and 8.31 ± 1.9 g (N = 100), respectively. Although
153 the absolute average growth rate (abs) of Group A (abs 0.13 ± 0.02 g) was lower than
154 Group B (abs 0.19 ± 0.1 g) over 30 days, its relative growth rate (rel) as a proportion (%) of
155 initial weight (rel $26.2 \pm 5.9\%$; SGR 0.8 ± 0.1) was higher than Group B ($16.8 \pm 8.6\%$;
156 SGR 0.5 ± 0.2). In contrast, both absolute and relative growth rates of Group A were larger
157 than Group C (abs -0.5 ± 0.7 g; rel $-5.9 \pm 7.7\%$; SGR -0.2 ± 0.3) (Fig. 1).

158

159 **Survival**

160 Survival within the first 30 days after transfer from aquaculture to experimental conditions
161 differed significantly between groups A and B as well as between groups B and C (Table
162 1). Non-parametric and parametric tests gave congruent results. Group A had 100%
163 survival compared to 13% and 1% mortalities for groups B and C, respectively, at the end
164 of their 30-day laboratory periods. There were no statistically significant differences in
165 survival rates between groups A and C during the 30-day experimental periods.

166

167 **Extension of experiment with Group C**

168 The limited duration and sequential conduct of experiments with groups A and B
169 precluded comparison of how each treatment would affect survival over the longer term.
170 Nevertheless, the SGR of these two experimental groups was positive and physical
171 condition was evidently good, suggesting that these eels were feeding and gaining weight
172 in contrast to those in Group C.

173 After the 30-day period of the experiment with Group C the mortality rate was
174 negligible (only 1 individual died), but weight decreased by 0.5 ± 0.7 g per eel. It was
175 evident that weight loss and poor physical condition prevailed among some individuals,
176 although not to the point of obvious morbidity. Accordingly, this experimental treatment
177 was extended for an additional 30 days (Group C₁) to allow sufficient time for these eels to
178 regain condition. The average weight of eels at the beginning of the second phase of this
179 experiment was 7.81 ± 1.9 g. After an additional 30 days the mortality rate of the eels
180 increased and varied substantially among tanks ($15.0 \pm 20.1\%$) yet with a weight gain per

181 surviving eel of 0.6 ± 1.1 g ($7.6 \pm 12.5\%$ of initial body weight; SGR 0.2 ± 0.4). All
182 mortalities in Group C₁ occurred within the first 11 days of the second phase, with none
183 thereafter. The total mortality rate after the first and second phases of the experiment was
184 estimated to be 16%, similar to the mortality rate observed for Group B.

185

186

187 Discussion

188 Results of this study are consistent with field studies that showed survival rates to be worse
189 among farmed eels released into the wild when compared to wild eels and stocked glass
190 eels (Pedersen, 2000; Wickström, 2001; Simon and Dörner, 2014; Pedersen and
191 Rasmussen, 2016). This also calls into question current recommendations for stocking
192 glass and on-grown eels into Lithuanian water bodies at a ratio 4:1. After 30 days of
193 feeding on *Chironomus* spp. under laboratory conditions there were no significant
194 differences in survival rates between groups A and C, but the survival rate of Group B was
195 significantly lower. The negative SGR in Group C after the initial 30-day period was due
196 to weight loss and apparent poor physical condition among some individuals. Positive SGR
197 values for groups A and B indicated that these eels were able to gain weight on the
198 *Chironomus* spp. diet. Chironomids have been reported to be one of the main food items
199 among eel stomach contents, even when the aquatic environment had only low abundance
200 of larvae (de Nie, 1982). The survival rate for the eels in the Group C₁ treatment decreased
201 by an additional 15%, but the SGR in the survivors increased to become positive. It is
202 plausible that if Group B had also been extended for a further 30 days as the Group B₁ and
203 no further mortalities had occurred, then there would have been no difference between the
204 two on-grown treatments and both would have differed significantly from Group A,
205 thereby further strengthening support for an hypothesis of superior performance of glass
206 eels.

207 Kearney et al. (2011) suggested a link between initial energy reserves in the shortfin
208 glass eel (*A. australis*) and its survival in captivity. This might explain the observations in
209 our study where larger eels that were well-nourished in the aquaculture facility had
210 accumulated enough energy reserves to cope with an impaired ability to adapt to feeding
211 on *Chironomus* spp. or had an insufficient food intake during the first 30 days, thereby

212 delaying the onset of mortality as observed during the beginning of the second 30-day
213 phase of the experiment. Such phenomena might be related to a sudden change in food
214 from dry formulated feed to a naturally occurring live prey. This is a hypothesis also
215 supported by Bohlin et al. (2002) and Sundström et al. (2004), who suggested that decline
216 in condition and growth among brown trout (*Salmo trutta*) was often observed after sudden
217 transition from formulated to wild food. The decrease in the Group C₁ survival rate of
218 15.2%, compared to only 1% during the first 30-day interval of Group C, we attribute to
219 insufficient food intake. These losses may have contributed to the increase in the final
220 average SGR during the second phase with the elimination of weaker eels from the group.

221 Although this experimental study was conducted in a laboratory that cannot emulate the
222 environmental conditions encountered by eels following their release into natural water
223 bodies, the overall results imply that the ability of eels to wean from formulated food to
224 natural prey is to some extent limited by the duration of time spent consuming a
225 formulated diet. We acknowledge that the 30-day experimental period may have been less
226 than optimal for testing, and that growth rates on specially formulated feed under farm
227 conditions were better than what was achieved with *Chironomus* spp. larvae during this
228 study. Our study suggests that although on-growing with formulated food provides
229 reserves against starvation, this cannot fully compensate for reduced capability to adapt to
230 feeding on wild prey. This inference is supported by Smirnov et al. (1994), who concluded
231 that hatchery-reared fish used for stocking might be unable to adapt to natural food
232 consumed by wild fish and as a result, stocked fish are often considered to be inefficient
233 feeders unable to adapt to the consumption of natural prey (Paszkowski and Olla, 1985;
234 Maynard et al., 1996; Olla et al., 1998). As a result, released fish can show substantial
235 weight loss (Baer, 2009). This suggests that fish fed with formulated food might in some
236 cases suffer from post-release starvation caused by impaired ability to locate or correctly
237 identify food items in the wild.

238 Simon et al. (2013) showed that eels released into the wild at the glass eel stage
239 exhibited a superior performance after four years than those sourced from commercial
240 farms where they had been on-grown to a larger size. Simon et al. (2013) concluded that
241 eels stocked as farm eels require more time to adapt to natural prey and to develop new
242 foraging strategies than eels stocked as glass eels, whereby during this adaptation period
243 the eels rely on stored energy reserves.

244 Survival rates in the wild are likely to be lower than those in this study. Hatchery-reared
245 fish are known to show poorly developed predator-avoidance behaviour (Olla et al., 1998;
246 Youngson and Verspoor, 1998; Einum and Fleming, 2001). Larger eels may suffer less
247 predation in the wild due to their size, but if time spent in farm tanks affected their ability
248 to evade or escape predators, then their survival rates would likely be lower. In any event,
249 poorer survival among larger eels retained in captivity for longer will translate into reduced
250 cost effectiveness.

251 Group A, exposed to aquaculture conditions for a relatively short time, exhibited
252 superior ability to switch from cod roe to *Chironomus* sp. larvae compared to those on-
253 grown on formulated feed for periods of 42 days or longer. Despite implications from this
254 study, the use of on-grown eels for stocking might be unavoidable because glass eels are
255 often available only at the end or beginning of the year, when the surface waters of most
256 lakes in Northern Europe are frozen. ICES (2013) recommends stocking on-grown eels as
257 a better option than glass eels in situations where water temperatures are very low, which
258 may lead to only modest glass eel survival. Considering the results of the current study and
259 similar findings in field studies, it could be argued that eels should be stocked as soon as
260 possible after capture, or at least kept under aquaculture farm conditions for the shortest
261 duration feasible, and, when possible, fed – or at least weaned – with natural food prior to
262 release to improve their ability to adapt to the natural conditions found in the wild. The
263 lack of superior survival rates among on-grown eels also suggests that a policy of releasing
264 glass eels at a four-fold greater rate than on-grown eels on the basis of weight equivalency
265 and presumed poorer survival may be ill founded. If future studies of glass and on-grown
266 eel survival in the wild display similar survival patterns to what we observed in the
267 laboratory, then a revision of recommendations for stocking densities should be
268 considered.

269

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271 Conclusion

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Results of the current study suggest that on-grown eels had no survival advantage in comparison to glass eels when fed *Chironomus* spp. in the laboratory, likely because eels must switch their diet from formulated to natural food, a transition with which at least some on-grown eels appear unable to cope.

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- 366
- 367
- 368 Corresponding author: Justas Dainys,

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372

Laboratory of Marine Ecology, Nature Research Centre,
Akademijos st. 2,
LT-08412 Vilnius, Lithuania
Email: dainys@ekoi.lt

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Table 1. Pair-wise comparisons of survival rates after 30-day experiments among Groups A, B and C. P-values of Tukey HSD tests following One-Way ANOVA ($F_{2,27} = 14.1$, $P < 0.001$) given above diagonal; P-values of Multiple Comparison tests of mean ranks following the Kruskal-Wallis ANOVA ($H_{2, N=30} = 14.8$, $P < 0.001$) given below diagonal. **Bold** = significant probabilities ($P < 0.05$)

Treatment Group	A	B	C
A	-	<0.001	0.870
B	0.019	-	<0.001
C	1.000	0.048	-

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Author/s:

Dainys, J;Gorfine, H;Sidagyte, E;Jakubaviciute, E;Kirka, M;Putys, Z;Lozys, L

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