

20 ABSTRACT

21

22 Fish develop morphological specializations in their trophic and locomotor systems as a

23 result of varying functional demands in response to environmental pressures at different

24 life stages. These specializations should maximize particular performances in

25 specialists, adapting them to their trophic and habitat niches at each ontogenetic stage.

26 As differential growth rates of the structural components comprised in the head are

27 likely to be linked to the diet of a fish throughout its development, we investigated the

28 ontogenetic development of two haplochromine cichlid species belonging to different

29 trophic guilds. We employed geometric morphometric techniques to evaluate whether

30 starting from morphologically similar fry they diverge into phenotypes that characterize

31 trophic guilds and locomotor types. Our examination of overall body shape shows that

32 certain specialized morphological features are already present in fry, whereas other

33 traits diverge through ontogeny due to differences in species-specific allometric

34 variation. Allometric shape variation was found to be more relevant for the biter

35 specialist than for the sucker morphotype. Our results confirm that phenotypic changes

36 during ontogeny can be linked to dietary and habitat shifts in these fish. Furthermore,

37 evidence for an integrated development of trophic and locomotor specializations in

38 morphology was observed.

39

40 **KEYWORDS:** functional morphology – allometry – ontogeny – cichlids – adaptive

41 radiation

42

43 INTRODUCTION

44

45 Cichlids are an excellent multidisciplinary model to investigate morphological
46 evolution considering functional morphology, ecological speciation, phenotypic
47 plasticity, and convergent morphotypes. In this context, East African cichlids exhibit a
48 large array of ecotypes in relation to selective pressures on foraging performance and/or
49 behavior, occupying a large range of habitats and trophic niches (Fryer & Iles, 1972;
50 Liem & Osse, 1975; Van Oijen et al., 1981; Witte, 1981; Hoogerhoud et al., 1983; Witte
51 & Van Oijen, 1990). In part, the characterization of these ecotypes is based upon the
52 functional pressures on their internal and external anatomy, which interrelate with
53 environmental factors that stimulate the expression of genetic and plastic responses in
54 their morphology (Sage & Selander, 1975). In accordance, these functional pressures
55 change ontogenetically (Osse, 1990; Zengeya et al., 2007), parallel to dietary and niche
56 shifts that many of these species experience (Galis & De Jong, 1988; Goldshmidt et al.,
57 1990; Galis, 1993). This results in a progressive modification of the locomotor and
58 trophic apparatus' morphology, making them more efficient towards a species-specific
59 diet and habitat during ontogeny (Adriaens et al., 2001; Holzman et al., 2008).

60

61 Trophic specialization is reflected in an array of internal and external
62 morphologies that can be situated along a biting/sucking functional continuum
63 (Albertson & Kocher, 2006). This has led to the description of numerous trophic guilds
64 (Greenwood, 1974). In the constructional sense, cichlid morphology can be divided into
65 different functional apparatuses that are integrated spatially. It has been documented
66 that certain morphological specializations in locomotor anatomical structures
67 reiteratively correspond to specific trophic guilds, advocating a connection between the
68 development of locomotor and trophic specializations in cichlid fish (Barel, 1983).

69 Of the functional systems known in cichlids, their oral apparatus is one of the
70 best documented. It generally reflects a trade-off between two mechanically different
71 functions involved in food acquisition: sucking and biting. Mechanically speaking, a
72 fish jaw consists of two opposing lever systems, one for jaw opening and the other for
73 jaw closing (Albertson & Kocher, 2006). The magnitude of how the lever system
74 transmits force or speed is calculated by two ratios that are determined from the
75 insertions of the interopercular mandibular ligament and adductor mandibulae muscle,
76 respectively, at the mandibular articulation. The first characterizes jaw opening, and is
77 the ratio of the retroarticular process (opening in-lever) and the length to the rostral
78 (tooth) tip of the lower jaw (out-lever). The second ratio is calculated as the ratio
79 between the length from the tip of the coronoid process (closing in-lever) and the length
80 of the out-lever, and characterizes jaw closing. These ratios reflect the mechanical
81 advantage of the system. A low mechanical advantage predicts rapid jaw rotation,
82 characteristic of sucking species, while a high mechanical advantage predicts powerful
83 jaw rotation, characteristic of biting species.

84
85 Feeding performance is influenced by locomotor ability in labrid fish (Higham,
86 2007a; Collar et al., 2008). Integration of locomotor behavior and feeding kinematics in
87 centrarchid fish (Higham, 2007b) and cichlids (Higham et al., 2006) has led to the
88 prediction that physiological, behavioral, and morphological aspects implicated in these
89 functions co-evolve in fish. More recently, certain locomotor morphotypes have been
90 associated to substrate type (Hulsey et al., 2013; Takeda et al., 2013), which is known to
91 be correlated with diet (Winemiller et al., 1995; Genner et al., 1999; Kassam et al.,
92 2004; Arbour & López-Fernández, 2013). In general, four locomotor types have been
93 related to body shape for fish (Webb, 1982): 1) fast steady swimming, 2) unsteady time-
94 dependent swimming, 3) unsteady acceleration plus turning swimming, and 4) place-

95 bound maneuverability. Following this classification, zooplanktivores would require
96 steady swimming, which is characterized by an efficient anterior streamline provided by
97 a relatively narrow head and high postcranial body; and benthic oral-shelling
98 molluscivores would require place-bound maneuverability, which is characterized by
99 round dorsal head profiles and a relatively deep body at the height of the paired fins
100 (Barel, 1983).

101 Whether this integration of trophic and locomotor specializations is already
102 present in fry morphology or develops later during ontogeny has not been documented
103 yet. Here, we survey the morphological variation throughout the ontogeny of two
104 haplochromine cichlids belonging to different trophic guilds to observe at what
105 developmental moment species develop morphological specializations belonging to
106 their respective trophic and locomotor ecological niches. Furthermore, we will discuss
107 the functional implications of morphological specialization at different stages in
108 ontogeny as predicted from literature.

109 The haplochromine species flock of Lake Victoria, the youngest of the African
110 rift lakes, has led to the appearance of ~300 endemic species in the last 200,000 years
111 (Fryer & Iles, 1972; Elmer et al., 2009). Species have occupied basically every available
112 niche and food resource, taking on a wide variety of morphotypes specific to the
113 functional demands imposed by their particular environments (Rainey & Travisano,
114 1998). Within these, *Haplochromis piceatus* and *H. fischeri* are two syntopic endemic
115 species from Lake Victoria (i.e. Mwanza Gulf). These two species are specialized
116 feeders located on opposite sides of the sucking/biting functional axis, with adult head
117 and body shape features representative of their respective trophic guilds: *H. piceatus* is a
118 pelagic zooplanktivore (fast and steady swimmer) and specialized in suction feeding
119 (Barel, 1983; Goldschmidt et al., 1990), and *H. fischeri* is a benthivorous, oral-shelling
120

121 molluscivore (place bound maneuverer) specialized in forceful biting (Greenwood, 1981
122 in: Katunzi, 1983). As such, they form an excellent case for comparing shape
123 differentiation reflecting morphological specializations during the different stages of
124 ontogeny since they belong to different trophic guilds along the sucking/biting
125 functional axis (Albertson & Kocher, 2001). However, the amount of shape variation
126 that corresponds to genetic factors or to plastic factors cannot be accounted for since
127 genetic relationships within the endemic Lake Victoria superflock are still under
128 discussion (Meyer, 1993; Verheyen et al., 2003; Wagner et al., 2012).

129 To analyze shape variation reflecting morphological specializations,
130 morphological features implicated in feeding and locomotion must be identified and
131 quantified, taking into account the homology of structures in both the head and body
132 (Kershbaumer & Sturmbauer, 2011). Since Lake Victoria cichlids are known to exhibit
133 low morphological variation albeit with important consequences for their ecology (Van
134 Oijen et al, 1981), we expect that morphological specializations will become more
135 pronounced in later stages of ontogeny given that functional requirements during larval
136 stages are more similar (Moser, 1981).

137

138 MATERIALS & METHODS139 *Specimens*

140

141 The *Haplochromis piceatus* (Greenwood & Gee, 1969) and *Haplochromis*142 *fischeri* [Seegers, 2008; formerly *H. sauvagei* (Pfeffer, 1896)] specimens that founded

143 the aquarium population stock used in this study were collected at the Mwanza Gulf in

144 southern Lake Victoria and shipped to the Haplochromis Ecology Survey Team (HEST)

145 (Van Oijen et al., 1981) laboratory at the University of Leiden during the 1980's. Since

146 then they have been tank bred and reared for 29 generations. In the aquarium facility of

147 the Royal Belgium Institute of Natural Sciences specimens were fed "ad libitum" with

148 commercial fish food (JBL Novostick and Hikari Cichlid Excel pellets) and a weekly

149 complement of frozen *Tubifex* and *Daphnia*. Carcasses were fixated in 80% non-150 denaturalized ethanol after an overdose of MS-222. A total of 34 specimens of *H.*151 *piceatus* and 37 specimens of *H. fischeri* were used. The samples for each species

152 comprised an ontogenetic series with individuals that had already absorbed their yolk

153 sac, spanning from 1 - 11.5cm standard length (SL) (Table 1). In order to account for

154 the influence of domestication on shape, three type specimens from Lake Victoria,

155 Tanzania were included for *H. piceatus* (RMNH 62769) and two for *H. fischeri*156 (formerly *H. sauvagei*; RMNH 70426), provided by the NCB Naturalis (the Netherlands

157 Centre for Biodiversity, National Museum of Natural history and Research Center on

158 Biodiversity in Leiden, The Netherlands).

159

160 Specimens were photographed with a Nikon D70 digital reflex camera using a

161 Sigma 105mm macro lens at five megapixels resolution. Fish were placed on a

162 20x15cm dissection board with a white paper background equipped with a scale bar.

163 Specimens were centered to avoid optical distortion of the images at the lens borders

164 (Arnqvist & Martensson, 1998). When needed, pins were placed in the tail and/or

165 pectoral fin region to minimize unnatural bending of certain structures due to the
166 fixation process.

167

168 To match the observed ontogenetic morphological changes to ecological data
169 found in literature specimens of both species were pooled into three size classes (1-4 cm
170 SL, 4-8 cm SL, ≥ 8 cm SL). We use size as a proxy for age, which has its pros and cons
171 (Godfrey & Sutherland, 1995), but whose use has been justified before in ontogenetic
172 studies (Zelditch et al., 2000). These size limits were established based on earlier work
173 on ontogenetic development in African cichlids (Van Oijen et al., 1981; Witte, 1981;
174 Hoogerhoud et al., 1983; Goldschmidt et al., 1990; Witte et al., 1990). Maternal
175 mouthbrooding care stops when fry reach an approximate length of 1 cm SL, and these
176 experience an increase of 4 cm SL during their first year (Witte, 1981). Witte et al.
177 (1990) noted changes in habitat, diet, and morphology at an approximate length of 7 cm
178 SL and observed an increased growth (1cm) of tank-bred specimens relative to wild
179 individuals of the same species (*H. piceatus*). Adjusting our data to these observations,
180 size ranges have been defined as 'I' (1-4 cm SL), 'II' (4-8 cm SL), and 'III' (>8 cm SL).

181

182

183 *Morphological Data Acquisition*

184

185 To analyze shape variation in head and body morphology, 32 homologous
186 landmarks (LMs) (Fig. 1) were digitized: 13 in the head region, 11 outlining the exterior
187 and denoting the base of the fins, two for the pectoral fin, and six indicating the lateral
188 line and central longitudinal axis. The landmarks denoting the longitudinal axis were
189 not included in the shape analysis, but were used as reference to apply the unbending
190 procedure in TPS Util v1.38 (Rohlf, 2006a), in this way circumventing shape variation
191 caused by unnatural bending during the fixation process.

192

193 The following LMs were used for the shape analysis: **LM1**: dorso-caudal tip of
194 the preopercular bone; **LM2**: dorsal origin of the opercular slit; **LM3**: ventral
195 intersection point between opercular and interopercular bone; **LM4**: caudal tip of lower
196 jaw at the level of retroarticular process; **LM5**: inferior rostral tip of the upper jaw;
197 **LM6**: superior rostral tip of upper jaw at the intersection between premaxillary and
198 upper lip; **LM7**: dorso-caudal bending point of the upper lip tissue at the extremity of
199 the lip fissure; **LM8**: unbending landmark: anterior margin of rostral tip of the rostrum;
200 **LM9**: dorso-caudal margin of the supraoccipital crest; **LM10**: base of dorsal fin leading
201 edge; **LM11**: posterior end of the base of the last spinous dorsal fin ray; **LM12**: base of
202 dorsal fin trailing edge; **LM13**: base of caudal fin at the dorsal edge; **LM14**: unbending
203 landmark: caudal end of lateral line in caudal fin peduncle; **LM15**: base of caudal fin at
204 the ventral edge; **LM16**: base of anal fin trailing edge; **LM17**: posterior end of the base
205 of the last spinous anal fin ray; **LM18**: base of anal fin leading edge; **LM19**: base of
206 pelvic fin trailing edge; **LM20**: base of pelvic fin leading edge; **LM21**: center of the
207 orbit; **LM22**: anterior-most point of the orbital margin; **LM23**: ventral-most point of
208 the orbital margin; **LM24**: base of ventral edge of the pectoral fin; **LM25**: base of
209 dorsal edge of the pectoral fin; **LM32**: point of maximum curvature at dorso-caudal side
210 of the operculum.

211
212 Landmark coordinates were digitized on the photographs using TPS Dig2 v2.10
213 (Rohlf, 2006b). Digitization error (3.3%) (1 ind./size class *2 species * 3 replicas) and
214 orientation error (6.3%) (5 inds. * 2 species * 3 replicas) in the sample were quantified
215 according to the protocol available on: [http://www.fun-](http://www.fun-morph.ugent.be/Miscel/Methodology/Morphometrics.pdf)
216 [morph.ugent.be/Miscel/Methodology/Morphometrics.pdf](http://www.fun-morph.ugent.be/Miscel/Methodology/Morphometrics.pdf).

217 In order to incorporate variation in head width in the analysis (as biter
218 morphotypes tend to have wider heads), two measurements were taken on the head

219 using an electronic caliper (0.1 mm accuracy). ‘Snout width’ was measured at the height
220 of the posterior extremity of the gape (LM7) and ‘head width’ was measured at the level
221 of the preopercular bone (LM1). In addition, standard length and interlandmark
222 distances (calculated in Past v1.81 (Hammer et al., 2001)) were included as variables in
223 the regression analysis.

224

225

226 *Analysis of Shape*

227

228 Shape data was analyzed statistically by means of Geometric Morphometrics
229 (Zelditch et al., 2004). The correlation between Procrustes and tangent distances
230 between specimens was tested using TPS Small v1.2 (Rohlf, 2003). A Principal
231 Component Analysis (PCA) was performed on shape variables in MorphoJ v1.05b
232 (Klingenberg, 2011) to search for the axes that maximize shape variation within the
233 ontogenetic sample. Multivariate analysis of variance (MANOVA) was performed on
234 shape variables in IBM SPSS Statistics v19 (SPSS, Inc.) to test for significant
235 differences between species’ ontogenetic trajectories. Ontogenetic growth vectors were
236 calculated and their directions and lengths compared. To estimate the range of angles
237 between growth vectors, the residuals from the regression of shape on size (CS and
238 lnCS) were paired with predicted shape values and bootstrapped (2500 iterations) with
239 replacement in IMP-VecCompare8 (Sheets, 2003-2014) to obtain significance values
240 under the null hypothesis of parallel vectors. Ontogenetic growth vector lengths were
241 calculated as Procrustes distances in IMP-Regress8 (Sheets, 2003-2014) using as a
242 reference the consensus shape from the twelve smallest specimens (i.e. the six smallest
243 specimens of each species). These distances were then regressed on size (lnCS) for each
244 sample, and the slopes’ mean, confidence intervals (95%), and p-values calculated
245 through a Monte Carlo resampling procedure (1000 iterations) with replacement

246 (Zelditch et al., 2004) using the PopTools v3.2 (Hood, 2011) plugin in Microsoft Excel
247 2010. Next, the common allometric trajectory was calculated for both species and a
248 novel PCA performed on the residuals to extract species-specific allometric shape
249 variation. Shape changes are visualized by means of the deformation-based thin-plate
250 spline interpolating function (Bookstein, 1991; Bookstein et al., 1996) and illustrated as
251 wireframe grids.

252
253 To discern what structures were developing divergently at each ontogenetic
254 stage and whether or not their development was correlated with size(lnCS), an ANOVA
255 was performed to test for differences between group means in log-transformed
256 biometric variables (SL, snout width, head width, and interlandmark distances).
257 Afterwards, variables were corrected for size (lnCS) to eliminate ontogenetic size
258 variation using General Linear Models (GLM) in IBM SPSS Statistics v19 (SPSS, Inc.).
259 To explore the differences between factor levels in GLM models with two categorical
260 variables (i.e. SPECIES and SIZE_CLASS), in the absence of post-hoc significance
261 tests when the homogeneity of slopes assumption is violated, we compared the
262 estimated marginal means plots. The level of statistical significance was set at a p-value
263 < 0.05 . The Bonferroni and Tamhane's T^2 (when variables present unequal error
264 variances across groups) adjustment for multiple comparisons were applied where
265 necessary.

266

267 **RESULTS**268 *Ontogenetic Shape Trajectories*

269

270 A MANCOVA was performed on shape variables using ‘size’ (lnCS) as the
271 covariate to test the null hypothesis of isometric growth and remove the effect of size
272 differences between individuals within the ontogenetic series (Table 2). Again Wilk’s λ
273 resulted significantly greater than expected by chance, indicating that species differ in
274 their ontogenetic shape trajectories irrespective of differences in size. The multivariate
275 distribution parameter was also significant for lnCS, leading us to reject the null
276 hypothesis of isometric growth. This means that shape is allometric, so that it changes
277 as a function of size. The interaction effect ‘species*lnCS’ also resulted significant,
278 which violates the homogeneity of slopes assumption in the MANCOVA. However, in
279 biological terms this implies that each species has a different allometric trajectory in the
280 shared ontogenetic shape space. ‘Size’ explains a larger proportion of the variance
281 (~10%) in the model than ‘species’ in view of the partial ETA squared values (ETA =
282 0.994 (size) vs 0.864 (species)). In units of Procrustes distance (d^2) this corresponds to
283 0.072 vs. 0.057 of 0.155, respectively.

284

285 The magnitude of the difference between species’ ontogenetic shape trajectories
286 was tested under the null hypothesis of parallel directions in the shared morphospace.
287 The angle between species’ ontogenetic vectors is of 34.4°, and the 95th percentile of the
288 ranges of the within-species angles are 30.7° for *H. piceatus* and 24.2° for *H. fischeri*.
289 The interspecific angle exceeds both within-species ranges, so we can conclude that the
290 two species differ significantly in the direction of their ontogenies of shape.

291

292 To test for differences in the ontogenetic rate of amount of shape variation
293 relative to increase in size between species, we calculated the Procrustes distance from
294 each specimen to a consensus configuration calculated using the 6 smallest specimens

295 of each species (1-2cm SL). The Procrustes distances were plotted on size (CS) and the
296 slope of the regression bootstrapped (1000 iterations) to obtain the confidence intervals
297 for each species (*H. piceatus*: 0.0024-0.0042; *H. fischeri*: 0.0026-0.0039). No
298 significant differences were observed between species in the length of their ontogenetic
299 vectors.

300

301

302 *Ontogenetic Shape Variation*

303

304 The PCA analysis maximized between individual shape differences, revealing
305 two trends in the shared ontogenetic morphospace: PC1 (37%) shape variation reflects
306 similar shape changes for both species in relation with size increase, while PC2 (15%)
307 reflects a component of shape variation that discriminates species (Fig. 2). Since PC1
308 shape variation is frequently considered a size axis in geometric morphometric studies,
309 we calculated how much of PC1 and PC2 shape variation are correlated with size in our
310 sample by regression. We observed that 64% ($p < 0.0001$) of PC1 shape variation is
311 predicted by size, while 17% ($p < 0.0001$) is predicted for PC2.

312

313 Shape changes associated with the PC1 axis from smaller to larger individuals
314 (positive to negative values) (Fig. 2) involve *i*) a relatively shorter head, snout and oral
315 jaws, *ii*) a dorsally shifted and reduced orbit, *iii*) a relatively longer ascending arm of the
316 preopercular and larger opercular area, *iv*) a relatively deeper body and straightening of
317 the dorsal outline, *v*) a rostral displacement and inclination of the pectoral fin, and *vi*) a
318 steeply angled transition from the caudal peduncle towards the anal fin.

319 Shape changes associated with the PC2 axis from positive (*H. fischeri*) to
320 negative (*H. piceatus*) values (Fig. 2) reflect *i*) a proportionally deeper head and cheek
321 depth, *ii*) relatively longer snout, oral jaws and ascending arm of the preopercular, *iii*) a
322 steeper angled transition from the neurocranium towards the dorsal fin, *iv*) a relatively

323 deeper anterior body with a steeper angled transition towards the caudal peduncle, and
324 v) a relatively shorter caudal peduncle.

325

326

327 *Allometric Shape Variation*

328

329 The multivariate regression of shape on size revealed that 28% ($p < 0.0001$) of
330 ontogenetic shape variation is explained by size (Fig. 3). This allometric shape variation
331 from positive to negative values is similar to PC1 shape variation, but differs in that *i*)
332 there is no relative shortening of the head, *ii*) the leading edge of the dorsal fin shifts
333 more dorso-rostrally, *iii*) there is no relative change in the inclination of the dorsal
334 outline of the caudal peduncle, and *iv*) the bases of the leading edges of the anal and
335 pelvic fins display a less important ventral shift.

336

337 Deriving from the significant interaction effect between species and size in the
338 MANCOVA that indicated different allometries of shape between species, we regressed
339 species' allometries separately, but within the same Procrustes superimposition. For *H.*
340 *piceatus* 28% of shape variation could be predicted by size and 42% for *H. fischeri*. The
341 interspecific angle between them was of 35° ($p < 0.0001$). To test for allometric shape
342 variation discriminating species we performed a new PCA on the residuals from the
343 shared allometric regression to maximize shape differences between individuals.
344 Species were clearly discriminated (Wilk's $\lambda = 0.031$; $F = 14.333$; $p < 0.001$; $ETA =$
345 0.969) along residPC1 (30%) (Fig. 4); *Haplochromis piceatus* individuals have positive
346 residPC1 scores, while *H. fischeri* individuals have negative ones (with two exceptions).
347 residPC1 axis shape variation predicted 34% of PC1 shape variation (with vectors at an
348 angle of 40°) and 48% of PC2 (with vectors at an angle of 55°).

349 Shape changes described by the residPC1 axis (Fig. 5) from *H. fischeri* to *H.*
350 *piceatus* comprise *i*) a significant increase in head length, oral jaw length, snout height,

351 and body height, *ii*) a more terminal positioned mouth, *iii*) a dorso-rostral shift of the
352 origin of the first soft and hard dorsal fin rays, creating a steep transition towards the
353 dorsal caudal peduncle, *iv*) a caudal shift of the pectoral fin, *v*) and a dorso-rostral shift
354 of the origin of the first soft and hard anal fin rays, resulting in a steep transition
355 towards the ventral caudal peduncle.

356

357

358 *Biometric Variables*

359

360 Interlandmark distances were chosen from the landmark configuration
361 considering that they covered anatomical structures known to be implicated in
362 sucking/biting performance and/or in other functions (Fig. 6). The linear measurements
363 employed are defined in Table 3. All variables were transformed to their natural
364 logarithm to linearize allometric relationships for regression analysis (Mascaro et al.,
365 2013). A preliminary GLM was performed using $\ln CS$ as covariable to test what
366 variables were correlated with an increase in size (Table 4). Variables not correlated
367 with an increase in size were OpW, GH, LJ, PDA, BH, AF2, and PcF-PvF. It is
368 noteworthy to mention that CS did not show significant differences between species,
369 indicating a similar growth rate (as quantity of shape change per increase in size) (Fig.
370 S1).

371

372 To observe what variables differed between species, a distinct ANOVA with
373 SPECIES as the categorical variable was performed for each variable to avoid
374 correlation interactions between variables in a multivariate GLM model (Table 4).
375 Species had significantly different means for the variables BL, OpW, GH, HL, HH, and
376 AF2. Since species samples consist of an ontogenetic series, an ANCOVA was
377 performed to correct for size ($\ln CS$). Additionally, the variables SL, HW, LJ, ChD, SnL,
378 PDA, and PcF-PvF resulted significant, however violating the homogeneity of slopes

379 assumption (except for SL). This indicates that the relationship between these variables
380 and the covariate differ between species, suggesting different ontogenetic trends of
381 these variables for each species.

382
383 To observe differences in our biometric variables between size classes through
384 ontogeny, ANOVA was performed as before with SIZE_CLASS as the categorical
385 variable (Table 4). Size classes presented significantly different means for the variables
386 CS, SL, SW, HW, BL, HL, ChD, SnL, and NL. However, after correcting for
387 differences in size (lnCS) through ANCOVA, only the variables HW, HH, BH, and
388 PcF-PvF resulted significantly different between size classes. Of these, only BH
389 violated the homogeneity of slopes assumption, suggesting a change in the ontogenetic
390 trend of this variable at a determined size range for both species.

391
392 To further elucidate differences between species' ontogenetic series in biometric
393 variables, a GLM was performed including both SPECIES and SIZE_CLASS as
394 categorical variables in the model (Table 4). Once again, size correction was executed.
395 Size classes had significantly different means for the variables SL, SW, HW, HL, LJ,
396 ChD, SnL, PDA, and PcF-PvF. All of them violated the homogeneity of slopes
397 assumption indicating differences in variable values between species, depending on the
398 size range of individuals during ontogeny. Estimated marginal means plots were
399 generated to estimate the timing of these ontogenetic shifts in variable values between
400 species' size categories (Fig. 7).

401

402 DISCUSSION403 *Evolution of Morphological Allometry*

404

405 Shape variation associated to a common allometric trajectory and that from
406 species-specific allometry were examined separately to observe what shape changes
407 were correlated solely to a common allometric trajectory from those that involved
408 species-specific development (Fig. 5). Species-specific allometric shape variation
409 accounted for a larger percentage of the shape variation within the ontogenetic sample
410 (30%) than the common allometric component (28%). Both allometric components
411 contribute to the shape differences associated to our PC1 and PC2 axes that maximize
412 individual differences (Fig. 2), and an interaction between them in ontogenetic shape
413 space is patent. Together they predict 98% (64% and 34%) of PC1 shape variation and
414 65% (17% and 48%) for PC2.

415

416 Allometric changes discriminating our species coincide with shape variation
417 associated to their respective locomotor and trophic specializations, similar to what has
418 been observed in other Lake Victoria specialists (Bouton et al., 1999). Since species did
419 not display differences in relative growth rates, allometric differences in biometric
420 variables between species must improve some species-specific function at a certain
421 moment in ontogeny (Pelabon et al., 2014). In a constructional context, this may be
422 achieved by different spatial arrangements of the respective apparatuses between
423 species and/or size classes (Strauss, 1984; Barel et al., 1989; Liem, 1991; Barel, 1993)
424 originated by the reallocation of resources to meet functional demands at different
425 ontogenetic moments (Ruehl & DeWitt, 2005; Taborsky, 2006; Von Bertalanffy, 1957).
426 This seems to be the case with the oral jaws and the interpectoral-pelvic fin length
427 (Table 4). In relation to the recent literature on cichlid shape divergence along the
428 benthic-limnetic axis (Hulseley et al., 2013; Takeda et al., 2013), the sucker morphotype

429 apparently may be allocating more resources to increase in body length along the
430 anterior-posterior axis during ontogeny, while the biter morphotype to increase lengths
431 along the dorso-ventral axis and head width.

432
433 We expected that species' shape differences would become more pronounced
434 through ontogeny starting out from morphologically similar fry. We found that even
435 though larvae were morphologically very similar, they already displayed differences in
436 morphological characters uncorrelated with size that are implicated in
437 trophic/respiratory (gill height and opercular width), a larger size of the gill arches
438 enlarges the volume of the buccal cavity during suction feeding (Osse, 1990), and
439 locomotor functions (soft anal fin region length). The former variables had larger values
440 in the sucker morphotype, whereas the latter was larger in the biter morphotype. Hence,
441 functionally relevant morphological differentiation between species is already present at
442 the beginning of ontogeny for these characters, but is later magnified due to species-
443 specific allometries that arise at specific moments in ontogeny (size classes). This
444 implies that the developmental program of morphological specializations is decoupled
445 in modular genetic programs throughout ontogeny, which may allow for phenotypic
446 plastic adjustments at each ontogenetic stage (Atchley, 1984). In view of the
447 morphologic (Barel et al., 1977) and genetic irresolution (Elmer et al., 2009; Wagner et
448 al., 2012) of the Lake Victoria *Haplochromis* genus (including the species studied), we
449 lack the phylogenetic framework to make any conclusions on the divergence in the
450 evolutionary direction of species' allometric trajectories. Nonetheless, the most recent
451 common ancestor of the entire Lake Victoria Region haplochromine species flock was
452 estimated to have existed at 4.5 million years ago (Elmer et al., 2009).

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454
455

456 *Trophic and Locomotor Functional Significance of Shape Variation*

457

458 Species-specific allometric shape variation discriminating species (Fig. 5) agrees
459 with similar comparisons relating to convergent sucker and biter morphotypes in all
460 three East African Lake cichlid assemblages (Young et al., 2009): elongate bodies are
461 typical of planktivorous suction feeders, whereas deep bodies with short down-turned
462 heads are associated with diets comprised of harder prey items.

463 The functional implications of morphological specializations that facilitate more
464 powerful biting have been evaluated in cichlids before (Barel, 1983; Van Leeuwen &
465 Spoor, 1987; Galis, 1992; Bouton et al., 1998). It is agreed that in molluscivores, the
466 jaw apparatus is more adapted to forceful biting. To this we have to add the intraspecific
467 differences in muscle recruitment and possible patterns of jaw movement (Liem, 1978;
468 Galis, 1992). However, intraspecific shape variation due to phenotypic plastic
469 adaptations to diet items (Bouton et al., 1999) can be ignored in our results because
470 species were fed the same food regime. The pattern of morphological variation observed
471 in *H. fischeri* in overall body shape predicts certain internal anatomical variation
472 (Sanderson, 1990). In the head, the ample dorso-caudal shift of the eye and the
473 substantial increase in length of the ascending arm of the preopercular bone and in
474 height of the suspensorium, enlarges the space in this region, providing a larger
475 insertion area and available volume for the adductor mandibulae muscle implicated in
476 forceful biting (Barel, 1983). In the oral jaw lever system, we observe a relative increase
477 in length of the coronoid process (closing in-lever) relative to the lower jaw (out-lever),
478 which grants a higher mechanical advantage to the system (Albertson & Kocher, 2006).
479 Both of these changes mechanically lead to a progressively stronger biting force
480 (Bouton et al., 2002), which can thus be expected in *H. fischeri*.

481 In the constructional context (Barel et al., 1989), the development of structures
482 implicated in the trophic core functions of biting and sucking (Barel, 1983) is also
483 constrained by that of adjacent apparatuses. All these apparatuses (oral jaw apparatus,
484 expansion apparatus, gill apparatus, and locomotor apparatus) share spatial demands,
485 resulting in morphological constraints reflected in functional trade-offs. The different
486 arrangements between apparatuses determine the range of form-features allowed
487 architectonically. Based on these arrangements, Barel (1983) identified associated
488 morphologies between the oral jaw apparatus and remaining apparatuses that either
489 optimize one core trophic function or the other.

490 The head shape of *H. fischeri* has a more rounded profile resulting from the
491 rostral-ventral shift of the anterior edge of the dorsal fin. The rostral-ventral shift of the
492 leading edges of the anal and pelvic fins create a flat ventral margin, which is
493 complementary to this shape of the head profile in providing rotation maneuverability
494 characteristic of benthic feeders (Aleyev, 1977). In more recent investigations (Drucker
495 & Lauder, 2001; Chadwell & Ashley-Ross, 2012), functional studies of locomotor
496 specialization have revealed certain aspects of fin development that were also apparent
497 in our results. Differences in the soft anal fin region length affect the
498 generation/resistance of hydrodynamic forces during swimming. This is because the
499 posterior region of the anal fin is functionally decoupled from the anterior region and
500 provides roll and/or yaw stability while generating additional thrust forces during slow
501 turning maneuvers (Chadwell & Ashley-Ross, 2012). The development of these
502 locomotor specializations associated with the biter trophic morphotype advocate a
503 certain integration of feeding and locomotor functions (Collar et al., 2008; Franchini et
504 al., in press), although it may just be a species-specific pattern. However, preliminary
505 tests of shape covariation between head and postcranial body landmark configurations
506

507 for this species display shape changes related to trophic and associated locomotor
508 specializations with PLS2 paired axis explaining 15% of the total shape covariation
509 between modules, supporting the former outcome (pers. obs.).

510
511 The relative elongation of the lower jaw (out-lever) in our *H. piceatus* sample
512 results in a smaller mechanical advantage and consequently in an improved kinematic
513 efficiency. The dorsal shift at the ventral intersection point between opercular and
514 interopercular bone alters the inclination of the head occasioning an upturned mouth
515 characteristic of pelagic feeders. The increase in size of the snout and increasing
516 horizontal dorso-ventral orientation of the ventral head profile provide a more
517 rectangular lateral head profile that when expanded results in a larger and more
518 cylindrical buccal cavity with an increased buccal volume characteristic of suction
519 feeders (Barel, 1983; Muller & Osse, 1984).

520 Associated changes in the locomotor apparatus are an efficient anterior
521 streamline and a minimum body area reflected in relatively small widths and depths in
522 outer head shape. In the constructional context, the increase in body height and the
523 caudal displacement of the pectoral fin in *H. piceatus* leave more space adjacent to the
524 head for the epaxial and hypaxial musculature, which coincides with the necessity of an
525 increased need of power for head expansion in slow-swimming suckers (Barel, 1983;
526 Wainwright et al., 2001; Carroll et al., 2004). In accordance with *H. fischeri*, the
527 development of these locomotor specializations associated to the sucker morphotype
528 advocate a certain integration of feeding and locomotor functions in Lake Victoria
529 haplochromines.

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535 *Ecomorphological Implications of Morphological Specialization*

536

537 The existence of differences between species in biometric variables correlated
538 with size that are implicated in trophic and locomotor function advocate a benefit of
539 increased growth considering that fish mortality is usually an inverse function of size
540 (Galis & De Jong, 1988). Growths of characters in the head are especially important for
541 food uptake. In the biter morphotype, the increase in head width allows individuals to
542 feed upon larger prey items through ontogeny. Such a functional ontogenetic shift has
543 been put forward for *H. fischeri* (Katunzi, 1983), and our observations corroborate that
544 morphological specializations produced by its species-specific allometry facilitate a
545 behavioral food-partitioning between individuals of different ontogenetic stages based
546 on prey size in this species (Katunzi, 1983; Ferry-Graham et al., 2002). However, this is
547 not the case for the sucker morphotype since Galis and De Jong (1988) observed during
548 its ontogeny by means of optimal foraging models equal *Chaoborus* prey uptake and
549 decreasing uptake of *Daphnia* prey with increasing size. We observed that variables in
550 the head implicated in trophic specialization in this species do not begin to increase
551 significantly in length until size class II, suggesting that a relatively larger buccal
552 volume isn't a constraint in food uptake until size class III, which coincides with the
553 optimal foraging model of Galis & De Jong (1988). And on the contrary, oral jaw length
554 increases in value through all of ontogeny, continuously potentiating suction feeding
555 (kinematic efficiency and jaw protrusion) as *H. piceatus* individuals get bigger.

556

557 The benefits of increased growth in size class II are less obvious in view of
558 biometric variables implicated in locomotor performance. Both body height and
559 interpectoral-pelvic fin length display a similar increase in value at this size class for
560 both species (*H. piceatus* displaying higher absolute values), but neither were correlated
561 with size. The increase in interpectoral-pelvic fin length for the size class II biter

562 morphotype results in enhanced maneuvering capacities and force generation at the
563 pectoral girdle (Drucker & Lauder, 2002). This morphological specialization can be
564 linked to an ontogenetic habitat shift towards deeper waters (Goldschmidt et al., 1990)
565 where larger forces are necessary for benthic locomotion due to higher pressures.
566 Similarly, the dorsal head profile at size class II becomes higher and more rounded
567 which in addition to the flat ventral outline provided by the increase in interpectoral-
568 pelvic fin length, provides an adaptation to pitch over the bottom more effectively
569 (Aleyev, 1977). These observations in our biter morphotype advocate an integrated
570 development of the trophic and locomotor apparatus through ontogeny due to changing
571 functional demands (Higham, 2007).

572 The development of locomotor specializations described by an efficient
573 streamline in the sucker morphotype due to increased values in their body height and
574 interpectoral-pelvic fin length is also more pronounced at size class II. However, body
575 length displayed a significantly increased growth rate at size class I for this species.
576 These observations support that morphogenetic programs are decoupled at different
577 ontogenetic stages (Atchley, 1984), and coincide with the ontogenetic niche shift this
578 species undergoes from shallow littoral nurseries to deeper waters when becoming III
579 since predator avoidance and prey capture depend more on speed in pelagic waters
580 (Witte, 1981; Goldschmidt et al., 1990).

581
582 In the context of the adaptive radiation of East African cichlids, more ecological
583 studies surrounding the biomechanics of the ontogenetic dietary and niche shifts that the
584 two species studied undergo are necessary to evaluate whether the here observed
585 morphological differentiation corresponds directly to differences in performance that
586 can influence their survival at different moments in ontogeny. Although the species are
587 syntopic in Lake Victoria, they shouldn't compete with one another since they have

588 different depth distributions (Van Oijen et al., 1981; Goldschmidt et al., 1990), and
589 differences in breeding strategies concerning timing, spawning, and brooding sites that
590 are likely to contribute to the partitioning of resources (Goldschmidt & Witte, 1990).
591 Thus that the ontogenetic patterns of morphological specialization observed should be
592 more the product of independent selective pressures for each species. The integration
593 during ontogeny of shape variation patterns involving morphological features
594 implicated in trophic and locomotor specializations does not agree with a three stage
595 model of adaptive radiation in which habitat and trophic niche adaptation are considered
596 independent of one another (Streelman & Danley, 2003), but puts forward an
597 integration of these two stages in the adaptive radiation process.
598

599 AUTHOR CONTRIBUTIONS

600

601 The geometric morphometric analyses were performed by J. H. S-S. under

602 supervision of D. A. The acquisition and rearing of the fish specimen used were

603 performed by L. A. under the supervision of E. V.

604

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606

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615

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- 822

823 FIGURE & TABLE LEGENDS

824

825

826 **Table 1.** Sampling design with the number of individuals per species and species' size

827 class. Size classes are designated based on intervals of standard length (SL): 'I' (1-4 cm

828 SL), 'II' (4-8 cm SL), and 'III' (>8 cm SL).

829

830 **Fig. 1.** Landmarks digitized. Landmarks 1-25 and 32 were used for the morphological

831 analysis. Landmarks 8, 14, and 26-31 were used for the unbending procedure.

832 Landmark definitions are explained in the text.

833

834 **Table 2.** Multivariate analysis of covariance results for size-dependent shape variables.835 All effects in the model had significance values under $p < 0.05$. Partial ETA squared

836 values reflect the relative contribution of each effect in explaining the total variance in

837 the model.

838

839 **Fig. 2.** PCA scatterplot showing the first two principal components. PC1 explains 37%

840 of the total ontogenetic variation, and PC2 15%. Species samples are divided into three

841 groups defined by size class. Confidence ellipses denote 90% mean value intervals for

842 groups. Wireframe deformation grids are included to illustrate the shape variation

843 ranging from -0.1 to 0.1 units in PC value from the consensus configuration for each

844 axis. The black outline approximates shape variation for the biter morphotype (more

845 negative PC1 values and more positive PC2 values), while the grey outline for the

846 sucker morphotype (more positive PC1 values and more negative PC2 values) for each

847 axis.

848

849 **Fig. 3.** A plot of the regression scores of ontogenetic shape on size (lnCS). Confidence

850 ellipses denote 90% mean value intervals for species' size classes.

851

852 **Fig. 4.** PCA scatterplot of ontogenetic shape variation described by the residuals of the
 853 multivariate regression of shape on size (lnCS). residPC1 explains 30% of the total
 854 variation. A clear separation between species can be observed along the residPC1 axis.
 855 *H. piceatus* (grey) individuals are located on the positive values, and *H. fischeri* (black)
 856 individuals are located on the negative values. Ellipses denote the species' 90% mean
 857 confidence intervals.

858
 859 **Fig. 5.** Wireframe deformation grid of residPC1. The grey outline (*H. piceatus*) defines
 860 a change in the PC score of 0.1, and the black outline (*H. fischeri*) a change in the PC
 861 score of -0.1 from the consensus configuration.

862
 863 **Fig. 6.** Illustration of the wireframe used to describe body shape. Interlandmark
 864 distances calculated from the landmark configuration (in grey) constitute the variables
 865 used in the regression analysis that are described in Table 3.

866
 867 **Table 3.** Definition of the interlandmark distances used in the regression analyses.
 868 Interlandmark distances were calculated in Past v1.81 (Hammer et al., 2001).

869
 870 **Table 4.** Results of the GLM analyses on interlandmark distance variables. Each
 871 column represents a separate GLM model with its corresponding categorical variable(s).
 872 (*) denotes a significant effect between the dependent variable and the
 873 covariate/categorical variable in each column. (†) denotes the violation of the
 874 homogeneity of slopes assumption for a dependent variable in each column. [For more
 875 explanation see the *Biometric Variables* section in Material and Methods.]

876
 877 **Fig. S1.** Estimated marginal means of covariates (SL corrected by lnCS). The
 878 horizontal axes denote size classes and individual lines represent each species (black: *H.*

879 *fischeri*, grey: *H. piceatus*). Line segments that are parallel indicate that there is no
880 interaction between the categorical variables at that ontogenetic interval.

881

882 **Fig. 7.** Estimated marginal means of variables with a significant
883 SPECIES*SIZE_CLASS interaction effect, and, that violate the homogeneity of slopes
884 assumption. The horizontal axes denote size classes and individual lines represent each
885 species (black: *H. fischeri*, grey: *H. piceatus*). Line segments that are parallel indicate
886 that there is no interaction between the categorical variables at that ontogenetic interval.

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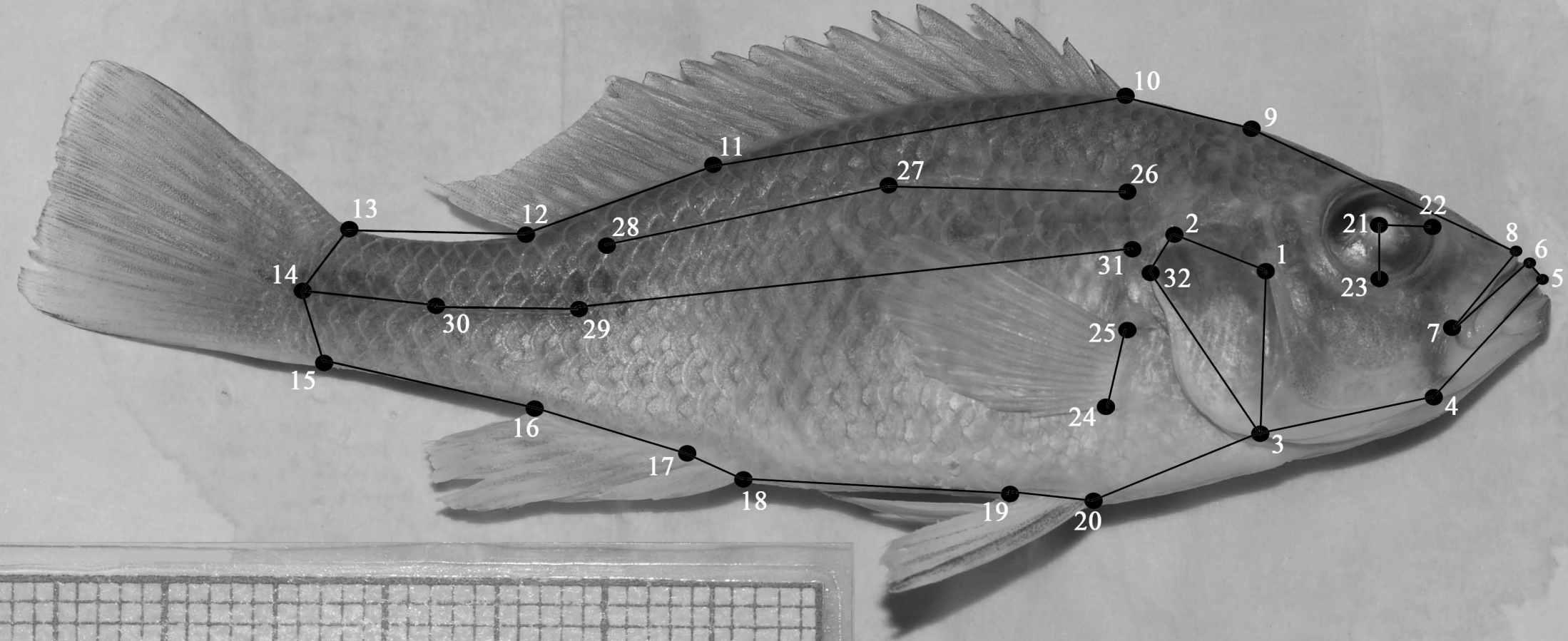
SPECIES	Individuals	I	II	III
<i>H. piceatus</i>	34	9	14	11
<i>H. fischeri</i>	37	10	11	16
TOTAL	71	19	25	27

Effect		Value	F	Sig.	Partial ETA squared
Intersección	Pillai's Trace	,993	59,895	,000	,993
	Wilk's λ	,007	59,895	,000	,993
	Hotelling's Trace	143,747	59,895	,000	,993
	Roy's Major Root	143,747	59,895	,000	,993
species	Pillai's Trace	,864	2,650	,010	,864
	Wilk's λ	,136	2,650	,010	,864
	Hotelling's Trace	6,360	2,650	,010	,864
	Roy's Major Root	6,360	2,650	,010	,864
lnCS	Pillai's Trace	,994	64,365	,000	,994
	Wilk's λ	,006	64,365	,000	,994
	Hotelling's Trace	154,476	64,365	,000	,994
	Roy's Major Root	154,476	64,365	,000	,994
species*lnCS	Pillai's Trace	,894	3,509	,002	,894
	Wilk's λ	,106	3,509	,002	,894
	Hotelling's Trace	8,421	3,509	,002	,894
	Roy's Major Root	8,421	3,509	,002	,894

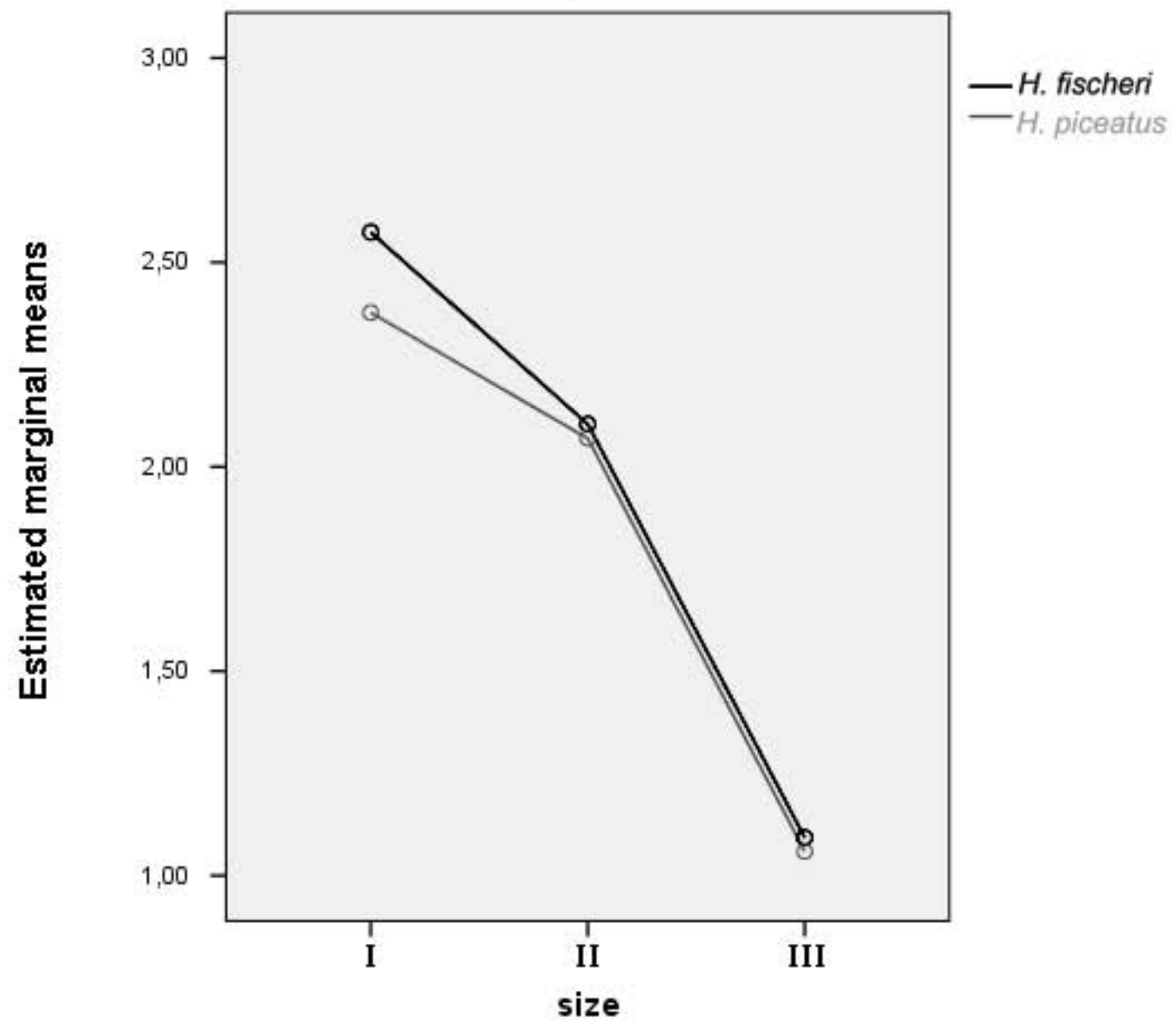
Design: Intersection + species + lnCS + species * lnCS

Variable	Landmarks	Definition	Apparatus
SL		Standard length	
CS		Centroid size	
HW		Head width	trophic
SW		Snout width	trophic
HH	LMs 3—9	Head height	trophic
NL	LMs 8—9	Neurocranium length	locomotor
HL	LMs 2—5	Head length	trophic
SnL	LMs 5—22	Snout length	trophic
ChD	LMs 4—23	Cheek depth	trophic
LJ	LMs 4—5	Lower jaw length	trophic
PDA	LMs 6—7	Premaxilla dentigerous arm length	trophic
GH	LMs 2—3	Gill height	respiratory, trophic (Osse, 1990)
BH	LMs 10—20	Body height	locomotor
BL	LMs 1—14	Body length	locomotor
AF2	LMs 16—17	Soft anal fin region	locomotor
PcF-PvF	LMs 20—24	Interpectoral-pelvic fin distance	locomotor
OpW	LMs 1—26	Opercular width	respiratory, trophic (Osse, 1990)

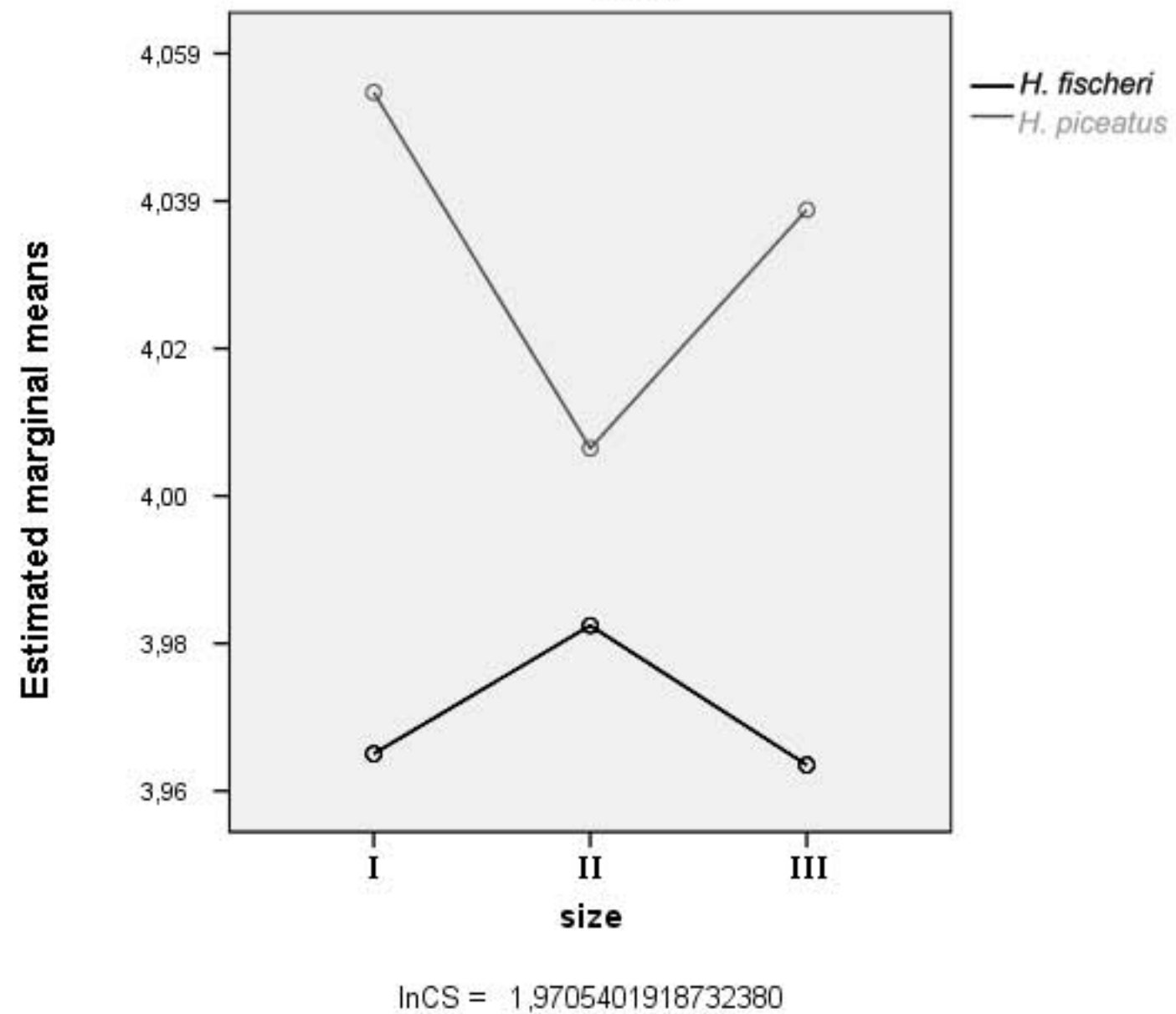
VARIABLE	COVARIATE	SPECIES	SIZE_CLASS	SPECIES*SIZE_CLASS
SL	*	*		*†
HW	*	*†	*	*†
SW	*			*†
HH	*	*	*	
HL	*	*		*†
SnL	*	*†		*†
ChD	*	*†		*†
LJ		*†		*†
PDA		*†		*†
GH		*		
BH			*†	
BL	*	*		
AF2		*		
PcF-PvF		*†	*	*†
OpW		*		

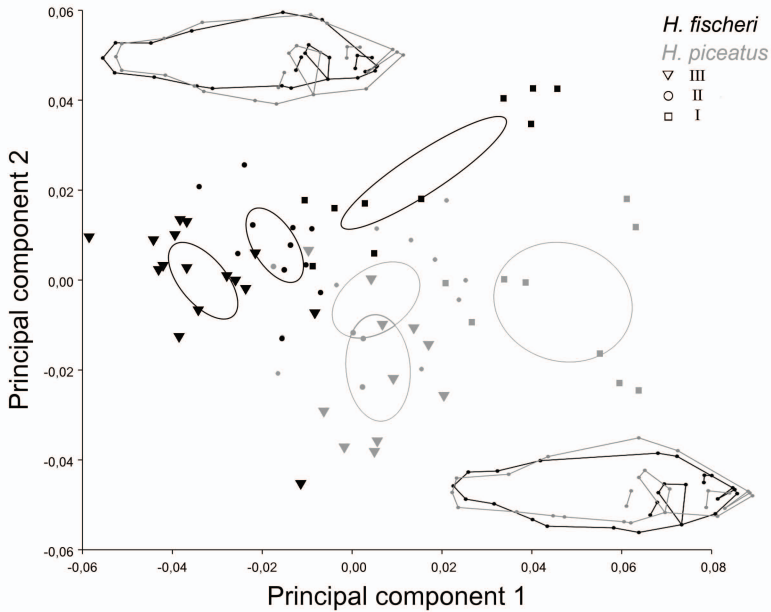


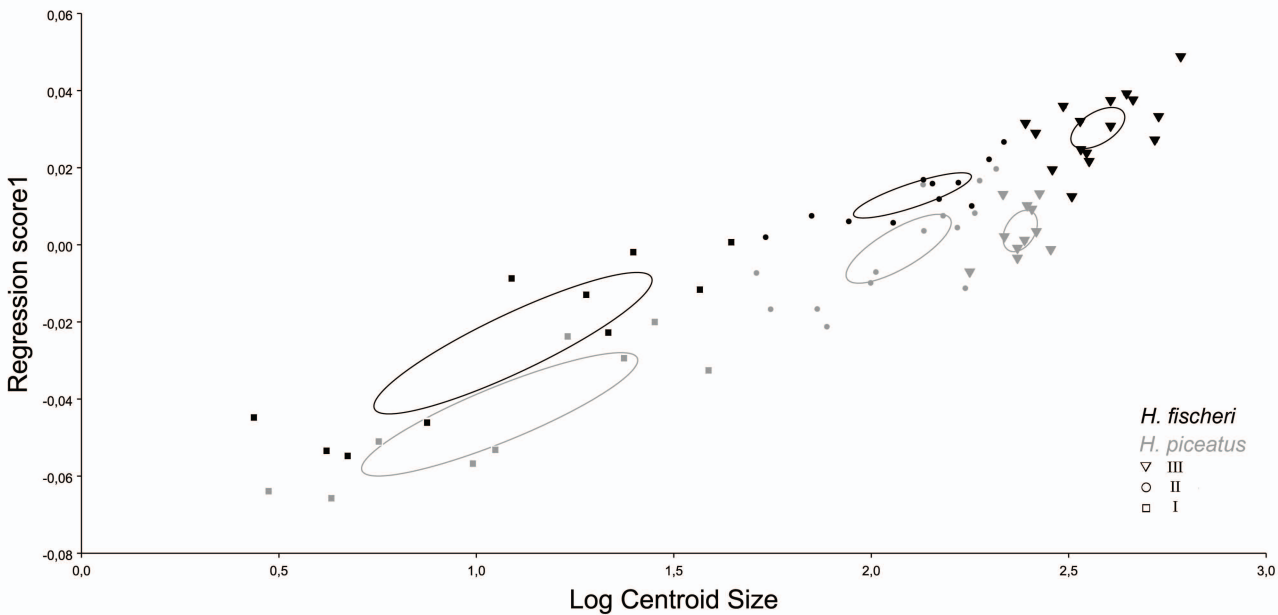
InCS

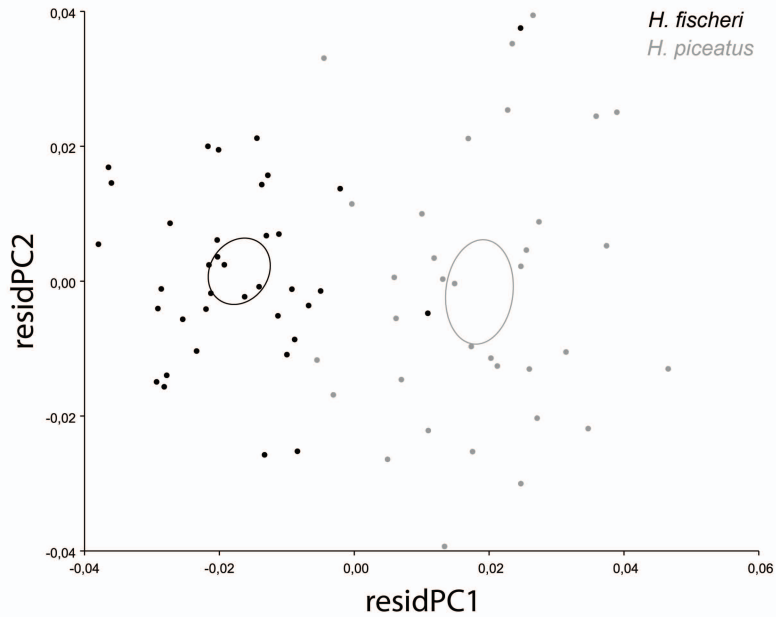


InSL



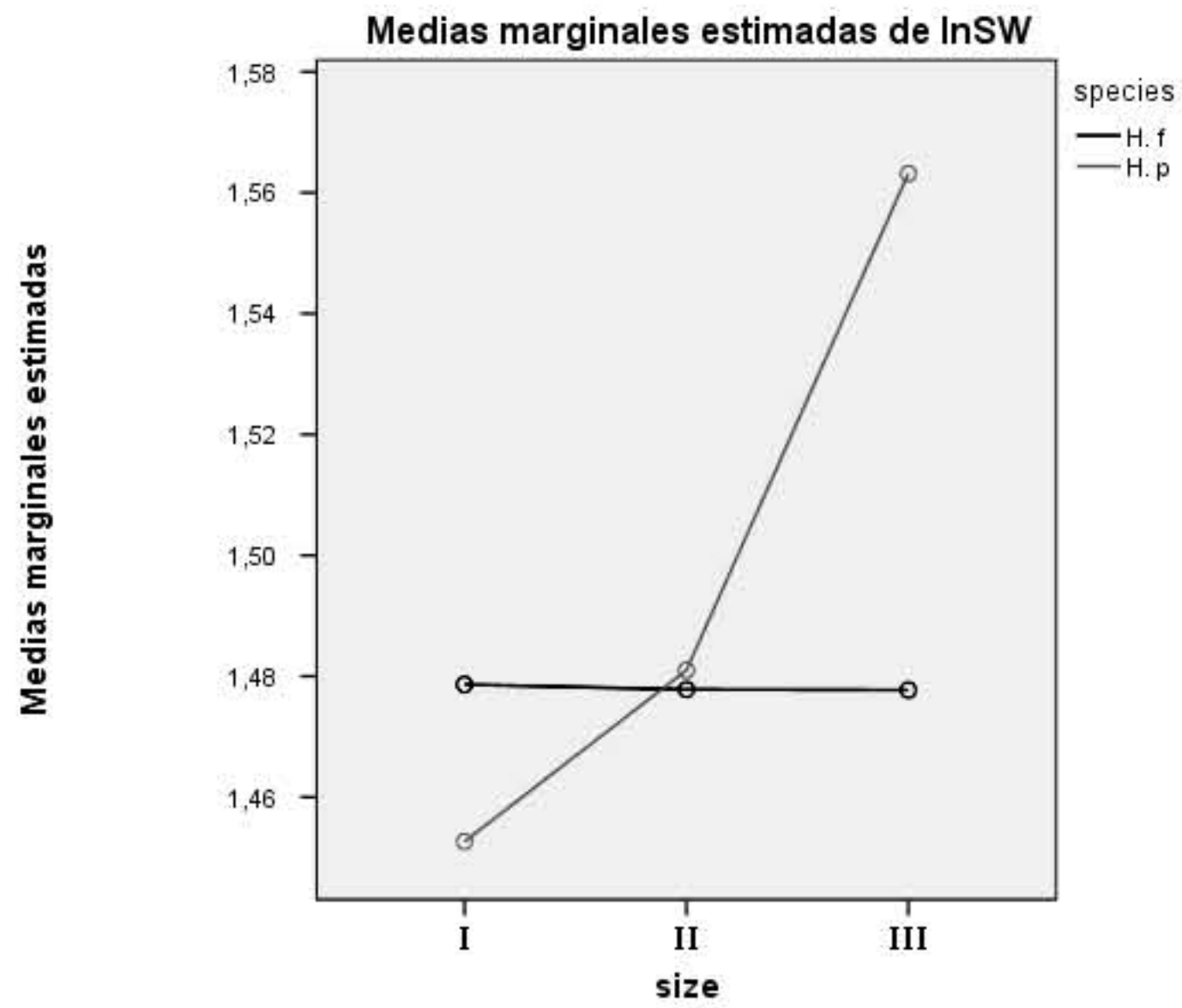




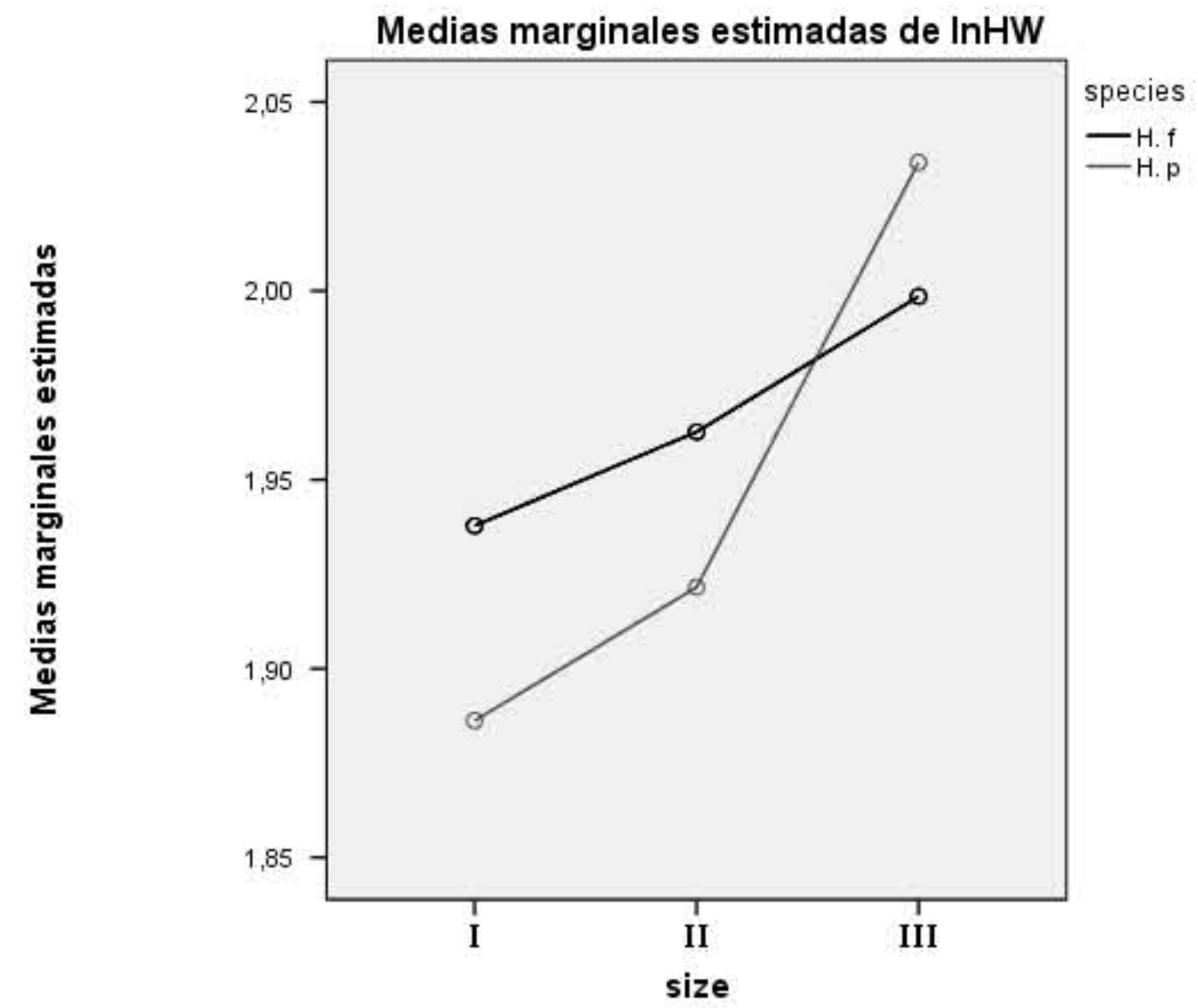




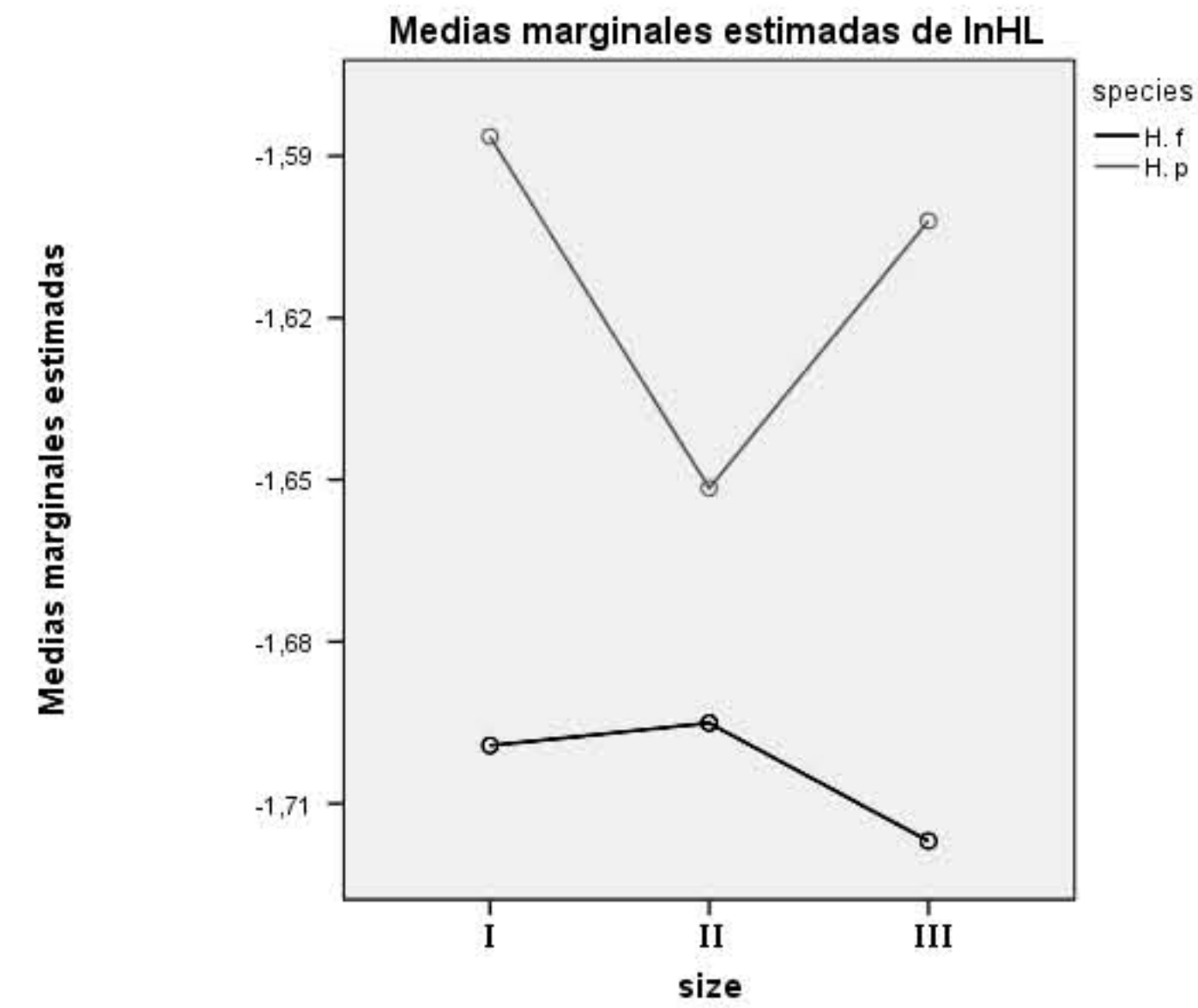




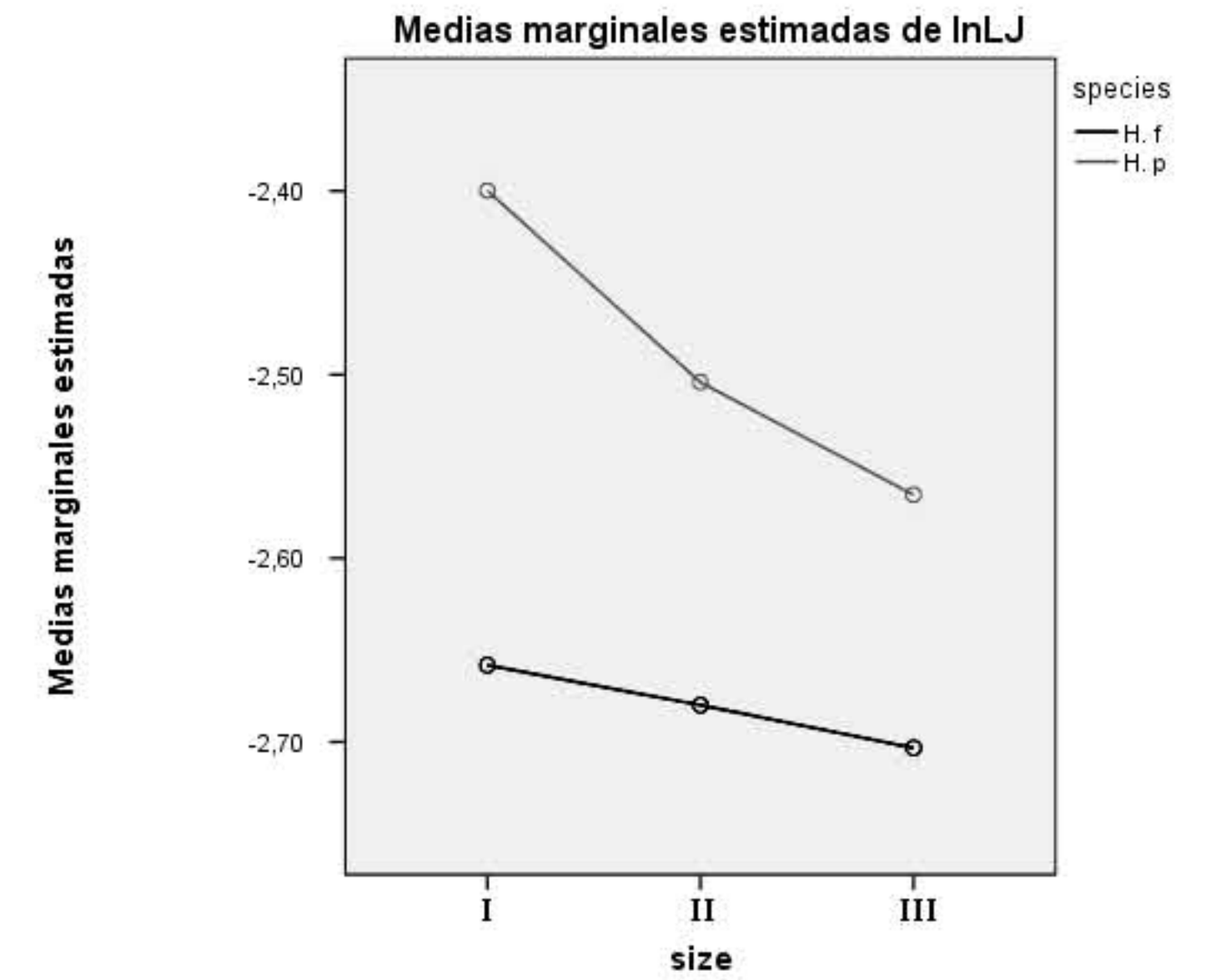
Las covariables que aparecen en el modelo se evalúan en los siguiente valores: lnCS = 1,9705401918732380



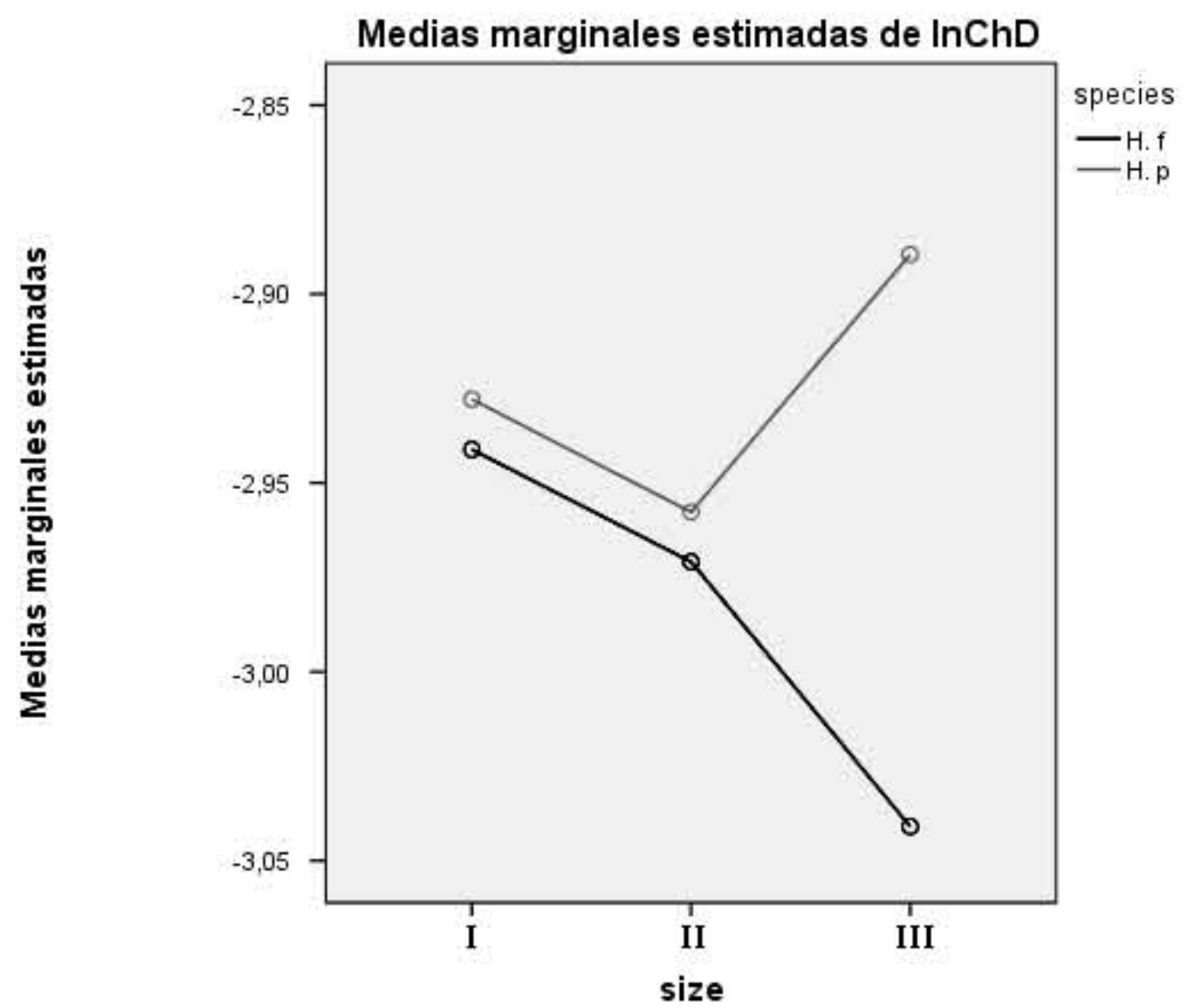
Las covariables que aparecen en el modelo se evalúan en los siguiente valores: lnCS = 1,9705401918732380



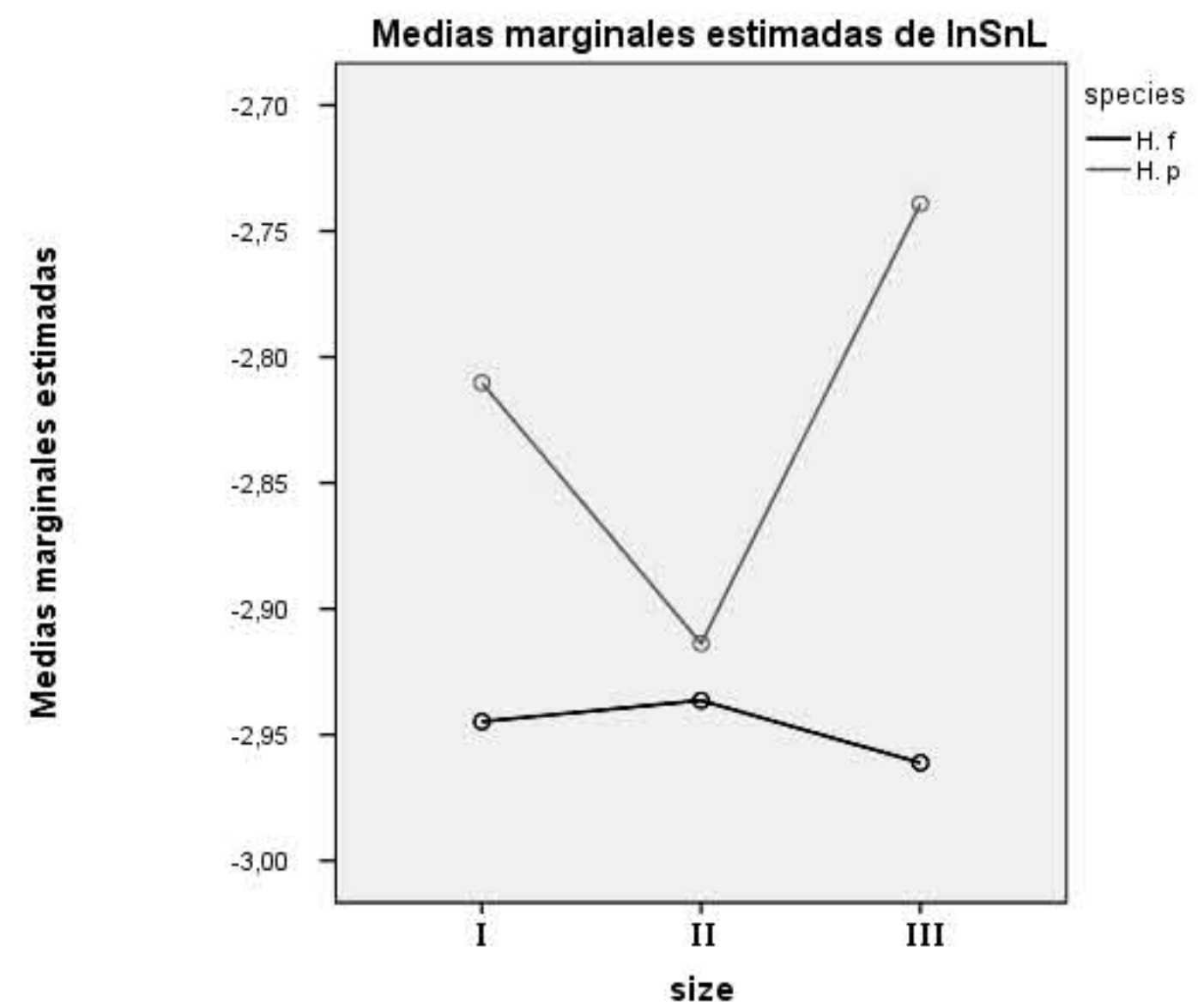
Las covariables que aparecen en el modelo se evalúan en los siguiente valores: lnCS = 1,9705401918732380



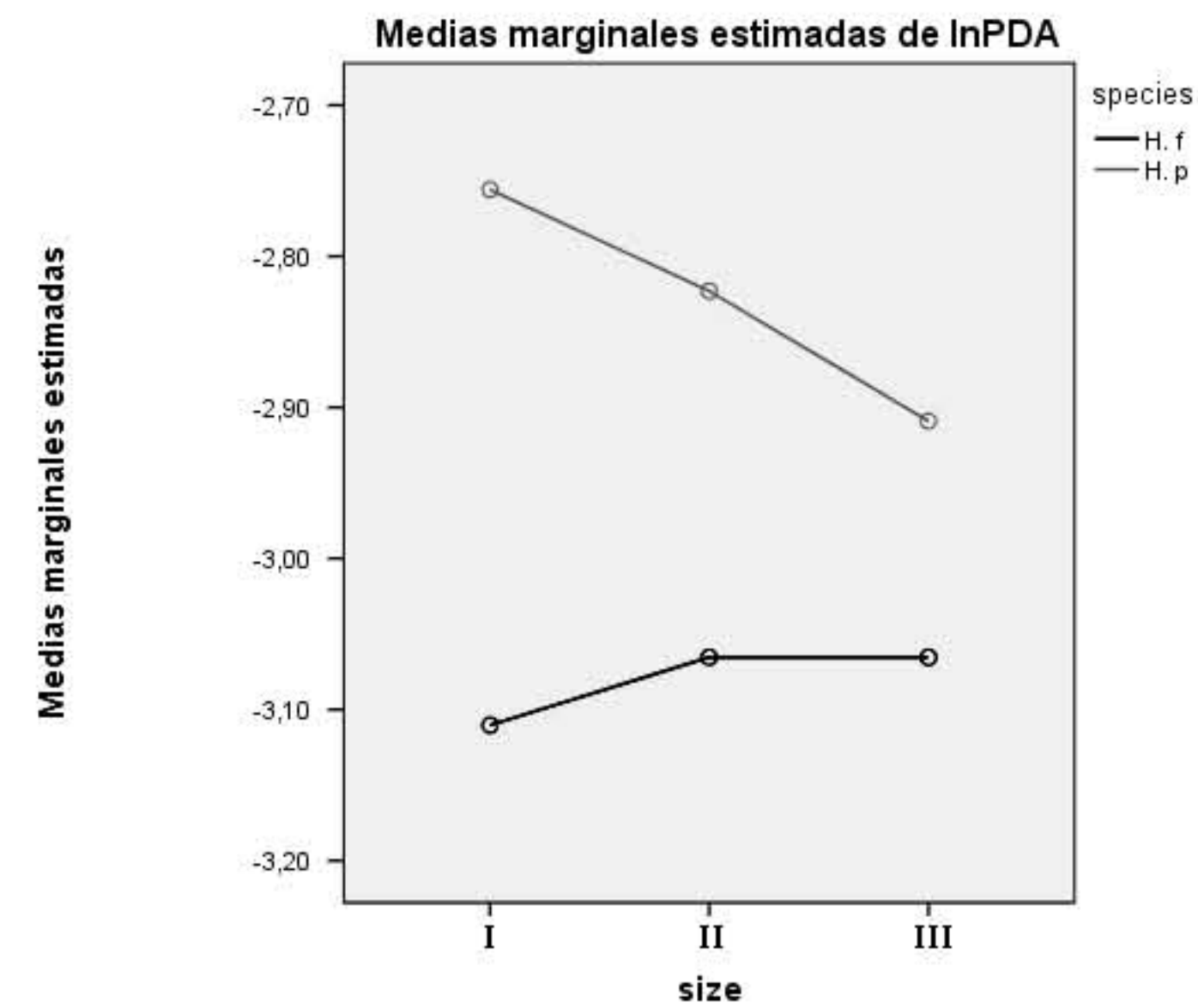
Las covariables que aparecen en el modelo se evalúan en los siguiente valores: lnCS = 1,9705401918732380



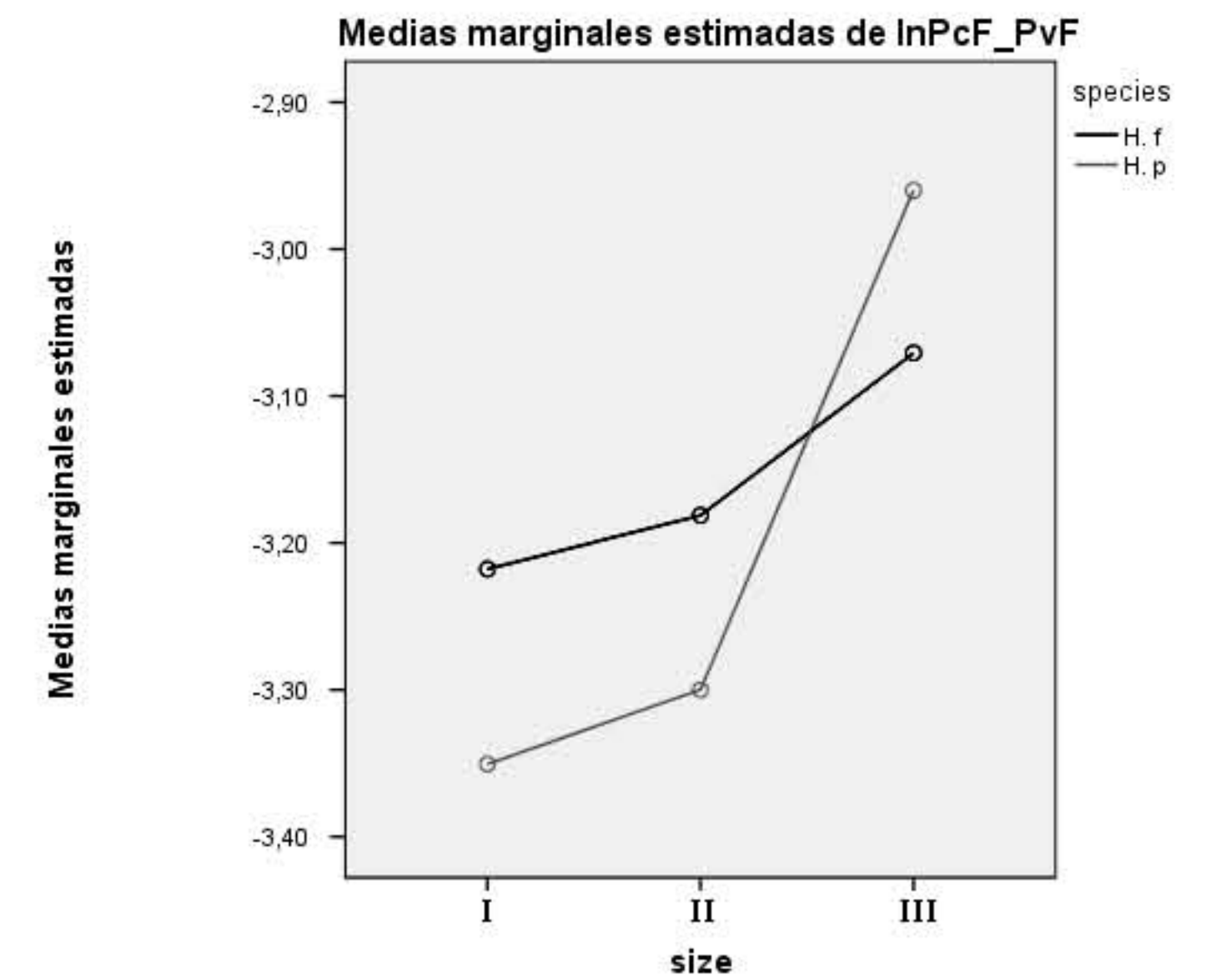
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