Fisheries-induced evolution of the circadian system and collective personality traits

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ABSTRACT

The circadian system is phylogenetically conserved across a wide range of taxa. It orchestrates organismal biological processes and is linked to life history and risk-taking behavior. Fisheries-induced evolution affects life history and may also alter behavioral traits in exploited populations. Thus, intensive and selective harvesting may lead to the evolution of the circadian system and thereby affect temporal organization of physiological processes. We present experimental results based on zebrafish (*Danio rerio*) selection lines exposed to either large or small size-selective mortality relative to a control, simulating evolutionary responses to intensive fishing. We show that size-selective harvest leads to evolution of life-history, risk-taking and shoaling behaviors, and importantly, also shifts the molecular circadian clockwork. Evolutionary effects of size-selective harvesting on risk-taking and shoaling were linked to daily activity rhythms in the small, but not in the large, size-selective mortality scenario. Both small and large size-selective mortality induced an evolutionary shift of the molecular circadian clockwork in the same direction in the brain and liver. Interestingly, the shifts in the molecular circadian clockwork disappeared in the clock output pathway, resulting in similar transcription profile among size-selective scenarios. We reveal that size-selective harvesting not only alters life-histories, but also leaves an evolutionary legacy in the behavioral and physiological machinery of exploited fish populations. These changes can affect catchability, yield, recovery and the way exploited fish

population use space and time and maybe difficult to be reversed even when harvesting is halted.

KEYWORDS: boldness, shoaling, collective, self-feeding, life history, gene expression

Significance statement:

Harvesting constitutes a strong driver of life history evolution in wild populations, but there is limited knowledge whether harvesting also leads to adaptive changes in the underlying physiology and behavior of exploited populations. We use a multi-generation zebrafish (*Danio rerio*) harvesting experiment simulating two different fisheries scenarios and we show that size-selective mortality affect the circadian system and collective fish personality traits. Such adaptations were present eight generations after size-selective harvesting was stopped, indicating induced evolution of the circadian system and behavioural traits in response to size-selective harvesting.

INTRODUCTION

Harvesting drives the evolution of animal populations on contemporary time scales by creating selection pressures that substantially depart from natural conditions (1-3). Unselective, yet elevated harvesting-induced mortality alone triggers the evolution of a fast life history characterized by elevated reproductive investment, reduced age and size at maturation, reduced post maturation growth and longevity (4, 5). The evolution of a fast life history is reinforced by harvesting mortality being size-selective (6). Life history evolution can alter correlated traits, such as physiological and behavioral traits (6-9). However, physiological and behavioral adaptations in response to harvesting are much less understood compared to harvesting-induced changes to life-histories (2, 8). Our study fills this gap using an experimental system simulating fisheries-induced evolution in response to size-selective harvest.

Fisheries-induced evolution can affect physiological and behavioral traits through at least two key mechanisms. The first relates to correlated selection responses of adaptive changes in life-histories (10). For example, the pace-of-life syndrome proposes that life-history traits, such as growth rate or maturation size, are systematically and genetically related to behavioural and physiological traits (11). Accordingly, life-history adaptations caused by fisheries can also be expected to indirectly affect the physiology and behavior of exploited fishes (5, 6, 9). Secondly, fisheries can also directly select on physiological and behavioural traits, e.g., because bold, aggressive, social or stress-resilient fishes are preferentially harvested with certain gears (12-16). Theory (6, 9) and experimental harvesting with model fish species (17-19) have indeed shown that both elevated and size-selective harvesting typical of most real fisheries foster evolutionary adaptation of behavioral traits. What is largely unknown is the underpinning mechanisms linking life history, physiology and behavior in response to harvesting (8, 20) and how such mechanisms contribute to shape the evolutionary trajectories in a multivariate selection environment (21).

One candidate pathway linking life-history, behavior and physiology is the circadian system. The circadian system is widely conserved phylogenetically (22) and controls daily rhythms of physiology and behavior (23). Thereby, the circadian clock fulfills an adaptive function helping the organism to anticipate and maintain synchronization to external 24-h environmental cycles (23-26). Functional circadian

83 clocks are essential for precise timing of activity, thereby helping the organism to avoid
84 or minimize risk of predation while maximizing foraging (27). Recent studies reported
85 an insofar unknown relationship of animal personality, particularly an animal's
86 tendency to take risks, and the circadian system (28, 29) suggesting genetic
87 correlations among traits. Zebrafish (*Danio rerio*) of different coping styles, commonly
88 referred to as proactive/reactive behavioural phenotypes (30), were found to be
89 systematically linked to different properties of circadian rhythms (29). Moreover, life
90 history changes correlate with the circadian clock (e.g., 31, 32). Accordingly,
91 evolutionary adaptations of the circadian system in exploited populations may occur in
92 response to both behavioral or life history adaptations. Here, we report a study
93 documenting evidence for evolutionary shifts in the circadian system in response to
94 intensive size-selective harvesting using a model system based on zebrafish size-
95 selected lines.

96 The proximate mechanisms governing fisheries-induced evolution are difficult
97 to disentangle in the wild because phenotypic changes are also affected by plasticity
98 (2). A powerful means to single out the cause-and-effect of selective harvesting is
99 conducting selection experiments with model fish species in the laboratory (33). We
100 use a long-term harvesting experimental system based on zebrafish selection lines
101 exposed to strong directional harvest selection (a 75% per-generation harvest rate)
102 acting on either large or small fish, relative to a control line harvested randomly with
103 respect to body size (17). Zebrafish were selected in a social environment over five
104 generations. Afterwards, selection was halted for eight further generations to remove
105 all "maternal" effects (i.e. a summary term for all non-genetic ecological effects on the
106 phenotype of the offspring), allowing clean comparisons among the lines at F_{13} in
107 terms of long-term outcomes of harvesting-induced evolution on behavioural traits
108 and the circadian system. The experimental zebrafish lines that we used in the present
109 study maintained key life-history adaptations until F_{13} that were already induced at F_9
110 (17; see also Fig. S1), indicating that harvesting-induced evolution of life-history did not
111 recover to pre-harvesting levels.

112 Previous results using the zebrafish model system have shown that the
113 selection lines differ evolutionarily in reproductive allocation, adult body size, risk-
114 taking behavior, trait variability, allele composition and transcriptomic profiles (17, 19,

115 34, 35). Moreover, size-selection affected single nucleotide polymorphisms occurring
116 within regulatory areas of genes related to the circadian system (17), in particular
117 *guca1c* that is involved in the light reception cascade in the retina and pineal gland (36)
118 and *tph2* that is involved in serotonin synthesis (37). These data collectively provided
119 strong evidence that size-selection over just five generations affected both genes and
120 phenotypes, suggesting that size-selection could also have left a molecular legacy in
121 the circadian system. In the present work, we test the hypothesis that risk taking
122 behavior and the circadian system share common pathways (29) and we predicted that
123 harvesting-induced evolution of risk-taking behavior has altered the circadian system
124 in the zebrafish selection lines. We also expected that possible changes in shoaling
125 behavior (i.e., sociability; 19) would affect the social synchronization of individual
126 circadian clocks (Bloch et al., 2013), with consequent changes in the collective
127 circadian output at the group level. Finally, we expected that changes of daily
128 behavioral rhythms would be mirrored by the underlying circadian molecular
129 clockwork in the central (i.e., brain; possibly associated with the control of swimming
130 activity) and peripheral (i.e., liver; possibly associated with the control of feeding
131 activity) oscillators, and by neuroendocrinological molecular markers related to energy
132 balance, appetite and growth.
133
134 **RESULTS**
135 ***Shoaling and risk-taking behavior***
136 We first studied zebrafish shoaling behavior in a round experimental arena (see
137 *Methods* for details). We found significant ($p < 0.001$) repeatability of inter-individual
138 distance among shoal members (R = 0.70 [confidence interval: 0.49 – 0.85]) over time
139 (i.e., 150 and 190 days post fertilization, dpf; Fig. 1A). Moreover, we found that the
140 small-harvested line formed significantly ($t_{71} = -3.52$; $p < 0.001$) more cohesive (i.e.,
141 smaller inter-individual distance) shoals than controls, yet no significant ($t_{71} = 0.90$; $p =$
142 0.373) differences existed among the large-harvested line and the controls (Fig. 1B).
143 Next, we studied zebrafish risk-taking behavior using a diving test in groups (see
144 *Methods* for details). We found significant ($p < 0.001$) repeatability for the time spent
145 by the shoals feeding at the surface (R = 0.62 [confidence interval: 0.39 – 0.79]) over
146 time (i.e., 230 and 240 dpf; Fig. 1C). The small-harvested line spent significantly ($p <$

147 0.01) more time feeding at the surface (i.e., a risky environment) than the control,
148 while the large-harvested line spent significantly ($p < 0.05$) less time than the control at
149 the risky surface (Fig. 1D). Hence, the large-harvested line was found to be significantly
150 shyer, while the small-harvested line was bolder than the control.
151
152 ***Daily and circadian activity rhythms***
153 We recorded robust daily activity rhythms of swimming (Fig. 2A, B) and self-feeding
154 (Fig. 2C, D) behaviour in a separate group of zebrafish between 170 and 190 dpf (see
155 *Methods* for details; Fig. S2, S3). In all selection lines, the behavioral rhythms revealed
156 significant 24-hour periodicities during both light-dark (LD) trials (first LD trial, days 1-
157 15; second LD trial, days 22-36) and significant free-running rhythmicity under
158 constant dim light conditions, with periods close to 24-h (Fig. S3, Table 1, see
159 *Supplement* for details).
160 All the three zebrafish lines displayed the typical diurnal swimming activity
161 rhythms (Fig. 2A, B). The total swimming activity during the photophase was
162 significantly ($p < 0.001$) repeatable over the two LD trials (R = 0.78 [confidence interval:
163 0.53 – 0.92]; Fig. 3A), with the small-harvested line being significantly ($t_{30} = 3.52$; $p <$
164 0.01) more active than controls (Fig. 3A). The selection lines were also significantly ($p <$
165 0.05) repeatable in their early peaks of swimming activity (R = 0.58 [confidence
166 interval: 0.19 – 0.84]). In particular, the small-harvested line concentrated significantly
167 ($t_{30} = 2.96$; $p < 0.01$) more of their daily activity during the first four hours of the
168 photophase relative to controls (Fig. 3B). By contrast, the late peaks of swimming
169 activity were not significantly ($p = 0.123$) repeatable traits (Fig. 3C), therefore this
170 behaviour was not considered as a stable behavioral trait and was thus not compared
171 statistically among the selection lines.
172 All lines displayed self-feeding activity rhythms during the last hours of the
173 scotophase, immediately prior light-on (Fig. 2C, D). The self-feeding activity was
174 significantly ($p < 0.05$) repeatable (R ranged from 0.45 to 0.52) over the two LD trials in
175 all the four consecutive hourly sections that were analyzed (from three hours before to
176 one hour after light on, Fig. 4). At the onset of the self-feeding events (06:00 - 08:00 h;
177 darkness) both size-selected lines were significantly ($p < 0.01$) more active than
178 controls (Fig. 4A, B). Subsequently, during the hour before light-on (08:00 - 09:00 h),

179 and the first hour after light-on (09:00 - 10:00 h), the small-harvested line, but not the
180 large-harvested line, was more active in demanding food than controls (Fig. 4C, D). To
181 obtain a more quantitative measure of feeding behavior, we measured the amount of
182 food demanded every two days normalized by the biomass of each fish group during
183 the first days of the first LD trial. Significant ($F_{2,33} = 5.161$, $p < 0.05$) differences among
184 lines during the first two days were found. Specifically, the large-harvested fish
185 demanded more food per fish mass (0.80 ± 0.58 g/g) than controls (0.18 ± 0.36 g/g),
186 but these differences disappeared over time (Fig. S4).
187
188 ***Core clock genes***
189 At the end of the behavioral activity trials we sacrificed the fish, under constant-
190 lighting conditions, to measure circadian transcript abundance with RT-qPCR (see
191 *Methods* for details). Core clock genes expression revealed significant differences in
192 their circadian oscillations among the zebrafish lines (Table 2). In the brain, the control
193 line showed a strong trend ($p = 0.052$) in the circadian rhythmicity of *arntl1a* and a
194 clear circadian oscillation of *clock1a* with a peak at the same Circadian Time (CT) of
195 *arntl1a* (CT0 for both genes; Table 2). By contrast, the small-harvested line lost
196 circadian rhythmicity of *arntl1a* and *clock1a* with respect to the control, while the
197 large-harvested line shifted its peaks of circadian expression for both genes (CT16
198 instead of CT0, Table 2 and Fig. 5). Results of *per1b* indicated significant circadian
199 patterns in all lines with main differences in peaks in both size-selected lines that
200 shifted from CT8 to CT12 relative to the control (Fig. 5, Table 2). In the liver, the small-
201 harvested fish showed similar circadian rhythmicity to control fish in terms of *arntl1a*,
202 while the large-harvested line revealed only a trend ($p = 0.086$) in the circadian
203 oscillation (Table 2). The control line did not show any circadian rhythmicity in terms of
204 *clock1a* and *per1b*, however, the size-selected lines revealed clear circadian
205 rhythmicity for these two genes with slightly different peaks (Table 2 and Fig. 5).
206
207 ***Light-inducible genes***
208 In the brain, the control line showed no significant rhythmicity of *per2*, while the size-
209 selected lines oscillated with peaks at different circadian times (CT12 and CT8 for large-
210 and small-harvested lines, respectively; Table 2, Fig. 5). Results of *cry1a* indicated

211 significant circadian rhythmicity with different peaks in the size-selected lines relative
212 to the control: the large-harvested line shifted the peak from CT8 to CT12, while the
213 small-harvested line shifted from CT8 to CT16 (Table 2 and Fig. 5). In the liver, both
214 size-selected lines lost circadian rhythmicity of *per2* with respect to the control. The
215 small-harvested line lost rhythmicity for *cry1a*, while the large-harvested line showed
216 the same peak compared to the control (Table 2 and Fig. 5).
217
218 ***Clock output genes***
219 The circadian expression of clock output genes showed less peak shifts compared to
220 the other two groups of genes that we investigated (Table 2). In the brain, all lines had
221 a circadian rhythmicity of *tef1* and *dbpa* with peaks at the same circadian time.
222 However, the large-harvested line showed a trend (p = 0.078) in the circadian
223 rhythmicity of *dbpa* (Table 2 and Fig. 5). In the liver, both size-selected lines shifted the
224 peak of the circadian oscillation of *tef1* from CT12 to CT8 compared to the control
225 (Table 2 and Fig. 5). By contrast, *dbpa* did not show differences among the lines. The
226 control line only showed a trend (p = 0.095) for the circadian rhythmicity of *dbpa*
227 (Table 2 and Fig. 5).
228
229 ***Clock controlled genes related to growth and energy balance***
230 The circadian expression of genes related to physiological processes, such as energy
231 balance (*lepa*) and growth (*igf1*), also revealed significant differences between the
232 size-selected lines and controls. The circadian oscillation of *lepa* expression in the brain
233 was only significant in the size-selected lines, but not in the control, with peaks at the
234 same circadian time (CT8). By contrast, we did not find significant circadian oscillation
235 of *lepa* in the liver (Table 3, Fig. 6). Finally, the *igf1* circadian oscillation in the liver was
236 significant only in the control line (Table 3, Fig. 6).
237
238 **DISCUSSION**
239 Using an experimental system simulating fisheries-induced evolution following size-
240 selection, we reveal the evolutionary legacy left by five generations of size-selective
241 harvesting on shoaling and risk-taking behaviors as well as on the circadian molecular
242 and behavioral outputs. In particular, we demonstrate that individuals of the small-

243 harvested line (which is conceivable in recreational fisheries managed with a maximum
244 size limit or in commercial gears having an upper bounds on mesh sizes, e.g. gill nets)
245 took more risk during feeding at the surface, were more cohesive, were more active at
246 the beginning of the photophase and extended self-feeding activity during photophase
247 relative to the control. We also found the large-harvested line (which can be expected
248 in recreational fisheries managed with a minimum-length limit or in commercial
249 fisheries operating with a trawl) to be shyer and to initially demand more food per
250 biomass than controls, and there was also a trend for this line to be less cohesive than
251 control. The evolutionary effects of size-selective harvesting on risk taking, shoaling
252 and foraging seem to be linked to daily activity rhythms, particularly in the small-
253 harvested line. From a molecular perspective, we also found shifts of the clock genes
254 circadian expression in the brain and liver in the same direction for both size-selected
255 lines. Importantly, the molecular shifts of the clock genes disappeared in the clock
256 output pathway, suggesting the occurrence of a switch leading to overall similar daily
257 rhythms at the behavioral level in all three selection lines. This suggests that being
258 overall active during the day and look for food around sunrise has strong fitness value
259 for zebrafish, no matter what the molecular clock is signaling.
260
261 ***Collective personality traits and daily activity rhythms***
262 The behavioural traits we studied represented stable and repeatable traits and thus
263 were indicative of zebrafish collective personality traits (38, 39). Importantly, we
264 demonstrated that shoaling and risk-taking behaviors were repeatable behavioral
265 traits over ontogenetic time. We also revealed daily swimming and self-feeding activity
266 rhythms (except for late peaks of swimming activity) to be repeatable across
267 experimental stages.
268 We documented behavioral adaptations in all the studied collective personality
269 traits, indicating that the evolutionary response to the disruptive size-selective
270 harvesting treatments allowed the small-harvested fish to elevate boldness to increase
271 their foraging efficiency, while the opposite was found in the large-harvested
272 treatment which showed increased shyness. These results agreed with predictions
273 from theory (6, 12). Feeding during the selection experiment occurred with clumped
274 food at the surface of the water during daylight (17), which can be considered a risky

275 environment for zebrafish (i.e. avian predation; 40). Therefore, bold individuals
276 spending more time at the surface, as the small-harvested line, were likely more
277 efficient at foraging. Our interpretation is additionally supported by the fact that the
278 small-harvested line formed more cohesive shoals relative to controls. Social fish
279 species, such as zebrafish, can forage more effectively in groups than solitary
280 individuals (e.g., 41). Therefore, being more cohesive could provide additional foraging
281 advantages in the small-, but not in the large-harvested line. By contrast, the large-
282 harvested line evolved increased shyness, most likely a response to the evolution of a
283 fast life-history (6). Thus, our findings are fully consistent with the idea that elevated
284 and positively size-selective harvesting selected for increased timidity/shyness (6, 12).

285 As regarding daily activity rhythms, the small-harvested line was found to be
286 more active during the photophase and concentrated swimming activity at the
287 beginning of the photophase. These findings again suggested the evolution of a
288 foraging-optimizing timing of activity, likely linked to boldness. Our finding are
289 supported by a recent study demonstrating that proactive zebrafish (i.e., risk-taking
290 individuals) are more active in the first hours of photophase compared to reactive ones
291 (29). Relatedly, the self-feeding activity events of the small-harvested line extended to
292 the hours before and after light-on. The timing of feeding is a key aspect for survival
293 and is traded-off against predation risk and food availability (24). The ecological
294 significance of zebrafish feeding during the last hours of darkness may be related to
295 avoidance of visual predators (e.g. birds; 40) and/or to storing energy to be prepared
296 for spawning that occurs at the beginning of photophase (42). However, the food
297 demand sensor was located close to the water surface (42; see also Fig. S2) and an
298 increase of self-feeding events towards light-on by the small-harvested line could
299 indicate an additional increase of foraging efficiency and safeguarding energy intake in
300 risky contexts. A further support for a link between boldness and daily activity rhythms
301 has been reported in a recent study that revealed artificial light at night affects risk-
302 taking behavior in the Trinidad guppy, *Poecilia reticulata* (43).

303 Overall, our results indicated an asymmetrical adaptation among behavior and
304 daily activity rhythms in the size-selective treatments as previously reported for life
305 history traits in other selection experiments (20, 21, 44). In fact, we demonstrated an
306 adaptive behavioral link between boldness and daily activity rhythms in the small-, but

307 not in the large-, harvested line. Fisheries-induced evolution of behavior likely has a
308 complex and multivariate nature (9, 21), thereby complicating generalizations related
309 to evolutionary correlations among traits (45-47). The somewhat asymmetrical
310 adaptation of behavior in the two size-selection lines did not extend to all traits that
311 we measured, which could be related to a functional limitation of the range of
312 evolution of our model species (21, 48).
313
314 ***Brain clock gene shift***
315 Purely size-selective harvesting modified the circadian clock transcript oscillations in
316 the same direction in both size-selected lines relative to controls. This result was a
317 surprise. In fact, despite the differences documented in the timing of daily swimming
318 and self-feeding activity rhythms among size-selection lines, we did not expect shifts in
319 the underlying molecular clockwork because all lines revealed an overall diurnal
320 swimming activity, while the self-feeding activity was mainly performed within a range
321 of 4-5 hours (Fig. 2). Conversely to expectations, we found that peaks of clock gene
322 expressions in the brain were shifted up to eight hours in both size-selection lines with
323 respect to controls. Yet, the peak of the oscillations of the clock output genes (*tef1* and
324 *dbpa*) showed a strong consistency among the three lines. Both *tef1* and *dbpa* belong
325 to a group of clock-controlled genes that feedback onto the core clock (49), and *tef1* is
326 also involved in the molecular switching mechanism controlling for photoperiodic
327 responsiveness in mammals (50), suggesting that the shifts documented in the core
328 clock and light-inducible genes can be switched before the clock output pathway. The
329 occurrence of a switch in the circadian clock was also reinforced by the fact that the
330 size-selected lines at F_9 showed genetic changes in areas of genes associated with
331 serotonin synthesis (17). Serotonin is a key element in the synthesis of melatonin that
332 is rhythmically secreted by the pineal gland (51). Melatonin represents a major
333 rhythmic output of the fish circadian system controlling photoperiodic-dependent
334 functions and synchronization of biological processes (51, 52). Thus, the serotonergic
335 system could have played a role in the switch mechanism we document in response to
336 size-selective harvest (17).
337 Our results did not indicate a link between harvesting-induced evolution of risk-
338 taking or other behaviors and the circadian molecular machinery in the brain because

339 the size-selection lines should have shown opposing adaptations in the circadian
340 system mirroring the adaptations in collective personality traits. Instead, the circadian
341 molecular shifts were pointing into the same direction in the two size-selection lines
342 relative to controls. Recent studies on zebrafish demonstrated that proactive and
343 reactive phenotypes in zebrafish explain a large fraction of the variation of the brain
344 transcriptome including expression of genes related to the circadian system (28).
345 Moreover, proactive phenotypes were found to have more robust molecular (i.e., clock
346 genes daily expression), humoral (i.e., daily secretion of melatonin) and behavioral
347 (i.e., locomotor activity) oscillations of the circadian system (29). However, both of the
348 cited studies measured transcripts abundance under LD conditions; therefore the truly
349 endogenous nature of the circadian molecular clockwork could have been masked by
350 the LD cycle. In our study, we measured transcript oscillations under constant
351 conditions, thereby providing a truly endogenous effect of size-selective harvesting on
352 the molecular circadian clockwork, and in our work both size-selection lines that
353 differed strongly in boldness responded similarly in the underlying molecular circadian
354 clockwork.
355 A possible explanation for our results could be related to a link between
356 harvesting induced evolution of life-history and the circadian system. In particular,
357 both size selected lines were previously reported to show evolution of a reduced age
358 and size at maturation compared to controls (17). Indeed, clock genes associate with
359 quantitative trait loci related to life history traits in fish, such as growth rate and length
360 in *Oncorhynchus kisutch* (53) and developmental rate and spawning time in
361 *Oncorhynchus mykiss* (32, 54). In particular, the core clock gene *clock* (i.e., *clock1a*, one
362 of the genes used here to characterize the core clock molecular machinery) is mainly
363 mapped in regions related to life history traits in salmonid fishes (32, 55). Therefore,
364 the possibility of a common molecular pathway or a pleiotropic effect of the brain core
365 clock machinery exist that is associated with the maturation schedule that evolved in
366 parallel directions in both size-selection lines according to previous studies (17).
367
368 ***Liver clock gene shift***
369 The size-selected lines showed a shift in the peak of oscillations of some clock genes in
370 the liver, following a similar pattern to that observed in the brain. Moreover, the size-

371 selected lines did not reveal circadian rhythmicity of the light-inducible genes. Only the
372 large-harvested line indicated a circadian oscillation of *cry1a*. However, the clock
373 output genes had consistent peaks of circadian oscillations. The mechanisms governing
374 the synchronization of the liver's peripheral oscillator in fishes are not fully
375 understood, both LD and feeding play an important role in maintaining fitness (26, 56).
376 This could be the reason why we were not able to detect a clear effect of size-selective
377 harvesting in the liver circadian system as sampling was performed under constant dim
378 light to avoid a masking effect of the light-darkness cycle (57). The serotonergic system
379 also plays an important role in feeding processes and other behaviors in fish (57-59),
380 reinforcing the idea proposed above of its role as a mediator in the molecular switch of
381 the circadian clockwork.

382 We observed differences in the genes related to growth and energy balance
383 among the size-selection lines. The *lepa* expression in the brain displayed significant
384 circadian variations in both size-selected lines compared with the absence of
385 rhythmicity in the control group. Moreover, both size-selected lines showed a
386 disruption of the *igf1* circadian rhythm in the liver. Results on both genes indicated a
387 modification of growth and reproductive allocation processes, lipid and protein
388 metabolism and energy balance (59), as previously reported for both size selected lines
389 at the phenotypical level at F_9 (17). Modifications of the insulin growth factors
390 pathways in the zebrafish's skeleton muscle have also been documented in previous
391 size-selection experiments with zebrafish in other labs (20), supporting the idea that
392 this pathway could have a major role in mediating growth processes in response to
393 size-selection. Phenotypically, the large-harvested line also demanded more food
394 compared to the control at the beginning of the experimental phase, and earlier work
395 has revealed changes in genes related to growth-related processes and energy release
396 in the liver at F_{11} (35). Therefore, it is plausible that size-selective harvest has
397 evolutionarily altered the allocation of energy as well as the behavioral mechanisms of
398 energy acquisition.

399 Our interpretation of altered energy allocation caused by harvesting is also
400 supported by the results of the Lester biphasic growth model (60) that describes life-
401 time growth patterns and energy allocation to soma vs. reproduction in the zebrafish
402 selection lines (Fig. S1). The growth trajectories indicate harvest-induced changes in

403 energy allocation patterns at F_9 and F_{13} (Fig. S1), with the large-harvested line
404 investing early and intensively into reproduction, and the small-harvested one
405 investing early but at low intensity into gonad production and more intensively in
406 somatic growth (17). Note the terminal size attained by the large-harvested line was
407 previously found to be smaller than the other two lines (17; see also Fig. S1), while the
408 terminal size of the small-harvested and control lines was found to be similar. The
409 same pattern was revealed at F_{13}, suggesting evolutionary fixation and lack of
410 phenotypic rebound eight generations after selection was halted. The phenotypic data
411 suggests the evolution of a mechanism unrelated to energy allocation through which
412 the small-harvested line is able to attain similar terminal size to the control fish. This
413 mechanism, as elaborated above, could be mediated by a correlated response of
414 foraging efficiency in risky contexts.

415 Collectively, our results indicate the occurrence of a switch downstream the
416 circadian clock both in central (brain) and peripheral (liver) oscillators, in response to
417 five generations of size-selection in zebrafish. This is the first documented evidence
418 that classical harvesting patterns of fisheries can leave a legacy in the molecular
419 circadian system of evolving fish populations. There are three mechanisms used to
420 explain circadian system switches (61): *i)* altered properties of the circadian clock
421 leading to different activity patterns; *ii)* identical properties of the circadian clock
422 switching in the output pathway, leading to different activity patterns; and *iii)* identical
423 properties of the circadian clock subjected to masking that ultimately determine the
424 activity patterns. Our results suggest the presence of a further mechanism where
425 altered properties of the circadian clock are switched in the molecular output
426 pathway, leading to overall similar daily activity patterns. The role of the clock output
427 in determining diurnal and nocturnal phenotypes is also supported by a recent study
428 with the fruit fly *Drosophila melanogaster* (62), where ten generations of artificial
429 selection for diurnal and nocturnal phenotypes were sufficient to obtain highly
430 divergent strains where most differentially expressed genes were associated with the
431 clock output (62). The switching mechanism we describe here could be confounded by
432 the effect of size-selective harvesting on social interactions. In particular a possible
433 alteration of shoaling behavior could alter the social synchronization of circadian clocks
434 of the shoal members (63). Our results indicate that shoaling behavior (measured as

435 inter-individual distance among shoal members) was significantly altered in the small-
436 harvested line and also showed a trend to be different, in a different direction, in the
437 large-harvested line, suggesting that a social-mediated mechanism is not likely the
438 cause of the switching described for both size-selected lines in the present study.
439

440 **Conclusions**

441 The main impacts of human activities on biological rhythms of wildlife are related to
442 diurnal disturbance as documented in an increase of nocturnality in mammalian
443 species in response to human presence (64). Another important human-induced
444 change of activity rhythms is represented by artificial light at night that has been
445 demonstrated to have a profound impact on circadian rhythms in a wide range of taxa
446 (65). Using a selection experiment simulating fisheries-induced evolution, our work
447 adds to this literature by showing that size-selective harvesting as is typical in many
448 fisheries or under specific harvest regulations can substantially shift the circadian
449 molecular clockwork, yet without strong changes in the daily behavioral activity
450 rhythms of our model species. Moreover, the changes of risk-taking and shoaling
451 behaviors provide empirical evidence for size-selection to leave a strong behavioural
452 legacy, supporting recent theory (6). Our work shows that the evolutionary impact of
453 fisheries far extends from life-history traits to behavioral traits and to the circadian
454 system, providing advancements in the understanding of the molecular architecture
455 controlling the evolutionary trajectories of populations exposed to a multivariate
456 anthropogenic selection. Evolutionary changes as the ones documented here can have
457 important consequences for organism performance, social groups, population
458 dynamics, recovery, and ultimately fisheries quality (12, 13, 66).
459

460 **MATERIAL AND METHODS**

461 ***Selection lines***

462 Our study system consists of wild-collected zebrafish (*Danio rerio*) from West Bengal in
463 India (for more details see 67). The parental wild-collected population was
464 experimentally harvested by exposing them to strong directional selection (a 75% per-
465 generation harvest rate) for either large body size (large-harvested) or small body size
466 (small-harvested). A third line was harvested randomly with respect to size and used as

467 control (17). Each selection line was replicated twice for a total of six selection lines.
468 Size-selective harvesting treatments were applied during the first five generations (17)
469 and then harvesting halted for eight generations until F_{13}. At F_9, the large-harvested
470 line evolved a smaller adult length and weight and higher relative fecundity compared
471 to controls (17). By contrast, the small-harvested line showed reduced reproductive
472 investment and no change in adult length compared to the control line (17). Both size
473 selected line evolved different maturation schedules by maturing smaller and at
474 younger age than the control line (for more details see 17) and such differences are
475 still detected at F_{13} (the generation used here) as indicated by results of a Lester
476 biphasic growth model and associated life-history traits (See Fig. S1). These changes
477 indicated a harvest-induced change in energy allocation patterns and no recovery to
478 pre-harvesting levels until F_{13}, with the large-harvested line investing early and
479 intensively into reproduction, and the small-harvested line investing early but at low
480 intensity into gonad production and more intensively in somatic growth. Moreover,
481 small-harvested juveniles at F_{10} were significantly more explorative and bolder than
482 controls, suggesting evolution of personality (17). In summary, the genetically diverged
483 selection lines differed significantly in their reproductive investment, adult body size,
484 juvenile behavior, trait variability, allele composition and gene expression (17, 34, 35).
485 Zebrafish selection lines for the present experiment where from F_{13}. They were
486 reared in groups and under *ad libitum* feeding and maintained under the following
487 conditions: water temperature at 26 ± 0.5 °C; photoperiod at 12:12 h LD cycle (lights
488 on/off at 07:00 and 19:00, respectively); fed three times per day with dry food
489 (TetraMin, Tetra) mainly in the first part of the light hours (at 09:00; 11:00 and 13:00).
490 Fish were reared and manipulated following the guidelines of the European Union
491 (2010/63/EU) and the Spanish legislation (RD 1201/2005 and Law 32/2007). The
492 experimental protocols were approved by the Spanish National Committee and the
493 Committee of the University of Murcia on Ethics and Animal Welfare.
494
495 ***Shoaling and risk-taking behavior experiments***
496 Zebrafish is a social species that forms shoals (40), and both risk-taking behavior and
497 circadian rhythms are sensitive to social modulation (68, 69). Therefore, we used

498 zebrafish shoals for testing our hypothesis because it is the appropiate reflection of the
499 phenotypes in the original environment that the selection experiment operated on.
500 Shoaling behavior was recorded two consecutive times when zebrafish were
501 150 and 190 dpf (at about the same age used for the circadian experiment, see below).
502 We randomly selected 6 groups of 8 fishes for each of the six selection line (12 groups
503 for each treatment: large-harvested, small-harvested and control line). The groups
504 were transferred to 3-L rearing boxes at 30 dpf and maintained under the conditions
505 explained above. The standard length of the experimental fish groups at 170 dpf was
506 within the following range: large-harvested (1.9 – 2.7 cm); control (2.1 – 2.8 cm); small-
507 harvested (2.1 – 2.7 cm). Shoals behavior was measured in a white round arena
508 (diameter of 60 cm) with 10 cm of water as reported in other studies that have tested
509 shoaling behavior in zebrafish (70). The arena was placed on a table behind a white
510 curtain to avoid any kind of disturbance to the fish during the experimental trials. On
511 top of the arena (at about 1 m) we placed a webcam (Logitech C920; resolution: 1920 x
512 1080 pixels; frame rate: 30 frames per second) that was connected to a computer
513 were video recordings were stored. Behavior was measured between 09:00 and 13:00
514 h. We started the experimental trials two hours after lights on to avoid measuring
515 behavior when mating usually occurs (first hours after lights on; see 40). The fish group
516 was introduced in the experimental arena by gently pouring them together with the
517 water from the rearing boxes and left undisturbed for 25 min. Then, inter-individual
518 distance was measured for five min as reported in other studies (70).
519 We used the same groups of zebrafish to perform an additionally behavioral
520 test on the time spent feeding at the surface of the water. We repeated trials two
521 times (i.e., at 230 and 240 dpf). The groups of eight zebrafish were moved from the 3-
522 liter rearing box in a new experimental tank (width x length x height = 10 x 30 x 25 cm)
523 with 22 cm of water. The experimental tank was placed on a table behind a white
524 curtain. On the side of the experimental tank (at about 50 cm) we placed a webcam (as
525 described above). Zebrafish were introduced in the experimental tank and left
526 undisturbed for 3 min before starting the experimental trial. That time was sufficient
527 to remove the diving response usually seen in these methodological approach (71).
528 Then, a small quantity of food was added at the surface and at the same time we
529 started video recording for 30 s (sufficient time for zebrafish to consume almost all the

530 food) to quantify the cumulative time each of the eight fish spent feeding at the
531 surface of the experimental tank (one third of the water column = at about 7 cm). All
532 video recordings were analyzed with automated behavioral tracking (EthoVision XT 9,
533 Noldus Information Technologies Inc.; www.noldus.com).
534
535 ***Circadian experimental set up and design***
536 Activity rhythms were recorded in a parallel experiment with a new set of fish when
537 they were at about 170 dpf. We randomly selected 3 groups of 15 fishes for each of
538 the six selection line (6 groups and 90 fishes for each treatment: large-harvested,
539 small-harvested and control line). The mass of the experimental fish groups was within
540 the following range: large-harvested (3.6 – 5.7 g); control (3.3 – 6.5 g); small-harvested
541 (4.6 – 6.1 g). Each 15-fish group was then placed in a 8-L experimental aquarium
542 (30×13×23 cm; see Fig. S2) equipped with aeration and mechanical and biological
543 filters. Light was provided by white LEDs (LED Flex-strip 1043-W, Solbright, RAYTE,
544 Murcia, Spain), with a light intensity of 435 lux at the water surface. The photoperiod
545 was set at a 12:12 h LD cycle, and a heater (75 W Magictherm; Prodac, China) kept the
546 water temperature at 26 ± 0.5 °C. The lights were set to turn on and off at 09:00 h and
547 21:00 h, respectively.
548 The experimental set up we used was previously designed to record at the same
549 time swimming (as a proxy of locomotor activity) and self-feeding activity by a group of
550 zebrafish (Fig. S2; see also 42). An infrared photocell (E3S-AD62 Omron, Kyoto, Japan)
551 was installed in the middle of the longer side of each aquarium, 15 cm below the water
552 surface. The sensitivity of this photocell was adjusted to detect fish at any distance
553 from the sensor to cover the full length of the aquarium (30 cm) and measure the
554 swimming activity. The self-feeding system consisted of another photocell located in
555 the upper corner of the aquarium, 2.5 cm below the water surface and 1.5 cm from
556 the side. The sensitivity of the photocell was adjusted to detect fish approaching within
557 a range of 1 cm independently of the size range used in the experiment. The photocell
558 was connected to a commercial feeder (Eheim, Deizisau, Germany) and when zebrafish
559 interrupted the infrared light beam, the feeder turned on and at about 0.2% g
560 flakes/biomass dropped into the aquarium. The feeder was placed in the same area, 10
561 cm from the sensor (for more details see 42). Swimming and self-feeding activities

562 were recorded and stored in a computer every 10 min. Before starting experimental
563 trials, fishes were acclimated to laboratory conditions and to the use of the self-feeder
564 for 15 days.

565 After the above mentioned acclimation period we recorded the swimming and
566 self-feeding activity for 38 days (from at about 170 to 210 dpf). The experimental trial
567 was subdivided in four consecutive phases: (first LD trial: 15 days) 12:12 h LD cycle to
568 investigate daily swimming and self-feeding rhythms; (constant conditions: 7 days)
569 constant dim light (3.5 lux at the water surface) to investigate the endogenous free-
570 running rhythms of swimming and self-feeding activity; (second LD trial: 15 days) 12:12
571 h LD cycle to investigate their resynchronization; (final constant conditions) constant
572 dim light to ascertain the endogenous expression patterns of clock genes. Moreover,
573 during the first 6 days of the first LD trial we measured the daily amount of food
574 demanded normalized by the mass of the fish group to have more quantitative
575 information on the self-feeding behavior of the fishes.

576

577 ***Gene expression analysis***

578 The first group of genes was composed by genes related to the core transcriptional-
579 translational feedback loop driving circadian oscillation in vertebrates (*per1b, clock1a,*
580 *arntl1a*). The second group involved light-inducible genes (*per2, cry1a*). The third
581 group were genes related to the circadian clock output (*dbpa, tef1*), and the fourth
582 group was composed of circadian clock controlled genes (*lepa*, and *igf1*) related to
583 growth and energy balance. *lepa* (*lepa*) was measured both in brain and liver and *igf1*
584 only in liver. The gene *lepa* acts as satiation signal in teleosts and has implications in
585 energy balance and glucose homeostasis, it is mainly secreted in the liver with a daily
586 rhythm of expression (57, 72). The gene *lepa* is also expressed in the teleost brain
587 where expression increases after a meal (73). The gene *igf1* is involved in growth
588 processes and signals lipid metabolism and is secreted with a daily rhythm of
589 expression (57).

590 Gene expression was measured at the end of the activity rhythms experiment.
591 Samples were collected throughout a 24-hour cycle at six different time points
592 (circadian time, CT: 0, 4, 8, 12, 16 and 20). Fish were exposed for 24 h to the second
593 constant dim light phase. We sampled a total of five fishes for each time point and

594 each treatment. Fishes were killed on ice and then the whole brain and liver were
595 dissected and put in RNAlater at -80°C. Total RNA was isolated using a trizol reagent
596 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The amount,
597 quality and composition of isolated RNA were analyzed by Nanodrop ND-1000
598 (Thermo Fisher Scientific Inc., Wilmington, USA). DNase-treated RNA was used to
599 perform cDNA synthesis in a final volume of 20 µl using the iScript cDNA synthesis kit
600 (Biorad, USA). The reaction was performed at 46°C for 20 min, followed by a 1-min
601 inactivation step at 95°C.

602 cDNA was PCR-amplified with the StepOnePlus Real- Time PCR System (Applied
603 Biosystems, Foster City, CA, USA) using SsoAdvanced™ Universal SYBR® Green
604 Supermix (Biorad, USA). The thermal cycling conditions were as follows: 30 s of
605 denaturation at 95°C, followed by 40 cycles of a 15-s denaturation step at 95°C, and
606 then by an annealing-elongation step for 30 s at 60°C. After amplification, a melting
607 curve analysis was performed to confirm amplicon specificity. All the samples were run
608 in triplicate.

609 Gene-specific primers are indicated in Table S1. The relative expression levels of
610 each sample were calculated by the $2^{-\Delta\Delta CT}$ method (74), using geometric mean of three
611 housekeeping genes (*βactin*, *GADPH* and *EF1α*) in both tissues.

612

613 ***Statistical analysis***

614 Inter-individual distance and time spent feeding at the surface of the water were
615 transformed by finding the exponent (lambda), which made the values of the response
616 variable as normally distributed as possible with a simple power transformation. Next,
617 we calculated the adjusted repeatability (selection lines as fixed effect; 75) across age
618 for inter-individual distance among group members (150 and 190 dpf) and for time
619 spent feeding at the surface (230 and 240 dpf). Finally, we modelled the behavioral
620 measures using linear mixed-effects models (76) with selection lines as fixed effect and
621 shoal nested within trials as random intercepts. Model fitting was assessed by checking
622 the plot of the residuals vs. the fitted values.

623 Time series of swimming and self-feeding activity were visually inspected by
624 plotting actograms and then using a Chi-square periodogram (77) to scan for the
625 presence of significant ($p < 0.05$) periodicity in the range 8-28 h and percentage of

626 variance (%V) explained by each period, reported as a measure of rhythms' robustness
627 (78). Waveform analysis (24-h based) was carried out in both LD trials, and raw time
628 series values (i.e. number of infrared light beam interruptions) were transformed in %
629 of maximum to standardize the data. The Midline Estimating Statistic Of Rhythm
630 (MESOR) was computed and represented as a horizontal threshold in waveform plots
631 (78).

632 As regarding swimming activity, we estimated total activity for each group by
633 calculating the area under the waveform curve during both LD trials (78). Next, early
634 and late peaks of activity were calculated as percentage of activity during the first
635 (early) and last (late) four hours of photophase. As regarding feeding activity, we
636 estimated feeding events for each group by calculating the area under the waveform
637 curve during four different hourly sections: *i*) from 06:00 to 07:00; *ii*) from 07:00 to
638 08:00; *iii*) from 08:00 to 09:00 (i.e., the hour before lights on); *iv*) from 09:00 to 10:00
639 (i.e., the hour after lights on). We calculated the adjusted repeatability (selection lines
640 as fixed effect) of these behavioral traits over the two LD trials as described above. We
641 lambda transformed them as above and modelled the data using a linear mixed-effects
642 models (76) with selection lines as fixed effect and shoals nested within LD trials as
643 random intercepts. Finally, we modelled the amount of food demanded by using a
644 linear model with selection lines and days of experiment (1-2, 3-4, 5-6) as fixed effects.
645 Model fitting was assessed by checking the plot of the residuals vs. the fitted values.
646 Periodicity in gene expression was checked using RAIN, a robust non-parametric
647 method for the detection of rhythms in biological data that can detect arbitrary
648 waveforms and has been widely applied in the measurement of circadian transcripts
649 abundance (79).

650 Periodogram and waveform analysis were performed using the software
651 Eltemps (www.el-temps.com). The rest of analyses were implemented using R 3.3.1
652 (https://www.R-project.org/) and the following additional packages: "rain" (79);
653 "rcompanion" (https://CRAN.R-project.org/package=rcompanion), "rptR" (80), and
654 "nlme" (81). In all cases, we used a 95% confidence interval.

655

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Author contributions

V.S., J.F.L., C.B. and R.A. designed research; V.S., J.F.L. and E.F. performed research; V.S. analyzed data; V.S., J.F.L., E.F., C.B., and R.A. interpreted results; V.S. and R.A wrote the paper with contributions from all the co-authors.

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874 **Table 1 -** The output of the periodogram analysis for swimming and self-feeding
875 activity rhythms during the three steps of the experiment (first light-darkness trial:
876 LD1; constant dim light: LL; and second light-darkness trial: LD2) according to the three
877 selection lines (large-harvested: LH, small-harvested: SH and control: RH). Periods (T)
878 or free running periods (*tau*) are averaged according to the number of groups (N; the
879 proportion indicates the number of groups with significant rhythms) and the
880 robustness of periodicities are expressed as percentage of variance explained (%V)
881 together with the midline estimating statistic of rhythm (MESOR). The standard errors
882 are reported between parentheses.

Phenotype	Experimental Phase	Line	N	T or *tau* (se)	%V (se)	MESOR (se)
Swimming	LD1	LH	6/6	24.00 (0.00)	47.41 (4.54)	0.070 (0.006)
		RH	6/6	24.00 (0.02)	29.74 (10.38)	0.103 (0.011)
		SH	5/6	24.02 (0.02)	52.48 (10.13)	0.116 (0.012)
	LL1	LH	4/6	20.88 (1.70)	29.43 (11.23)	-
		RH	2/6	26.33 (0.00)	30.29 (8.59)	-
		SH	2/6	23.42 (0.33)	23.54 (1.89)	-
	LD2	LH	6/6	24.03 (0.02)	52.45 (8.54)	0.081 (0.017)
		RH	6/6	24.00 (0.03)	51.01 (14.30)	0.066 (0.006)
		SH	5/6	24.00 (0.00)	56.96 (20.82)	0.108 (0.018)
Self-feeding	LD1	LH	6/6	24.00 (0.00)	46.44 (7.45)	0.018 (0.003)
		RH	6/6	23.97 (0.02)	38.89 (18.18)	0.016 (0.009)
		SH	6/6	24.01 (0.03)	43.21 (6.35)	0.026 (0.005)
	LL1	LH	4/6	23.56 (0.52)	28.59 (6.73)	-
		RH	3/6	22.56 (1.31)	18.86 (3.43)	-
		SH	1/6	16.83 (-)	16.03 (-)	-
	LD2	LH	6/6	24.03 (0.02)	46.07 (11.67)	0.013 (0.003)
		RH	6/6	24.01 (0.01)	32.29 (7.92)	0.008 (0.003)
		SH	6/6	24.00 (0.02)	37.87 (10.17)	0.016 (0.003)

883

884

885 **Table 2 -** The output of the analysis with RAIN (a robust non-parametric method for
886 the detection of rhythms in biological data) regarding the circadian oscillation of
887 transcript abundance of the genes related to the circadian system. Gene expression
888 was measured at the end of the experiment in constant dim light. Transcripts are
889 subdivided according to their role in the circadian system for each selection lines
890 (large-harvested: LH, small-harvested: SH and control: RH) and in each tissue (brain
891 and liver). The timing of the peak of the circadian oscillation (CT0, CT4, CT8, CT12, CT16
892 or CT20) is reported together with the *p* value (N is between 4 and 5). Significant
893 circadian rhythmicity is reported in bold.
894

895

Clock mechanism	Gene	Line	Brain		Liver	
			Peak	*p* value	Peak	*p* value
Core clock	*arntl1a*	LH	**16**	**< 0.01**	16	0.086
		RH	0	0.052	**0**	**< 0.01**
		SH	16	0.682	**0**	**< 0.01**
	clock1a	LH	**16**	**< 0.01**	**0**	**< 0.001**
		RH	**0**	**< 0.05**	0	0.472
		SH	20	0.279	**20**	**< 0.001**
	per1b	LH	**12**	**< 0.001**	**16**	**< 0.01**
		RH	**8**	**< 0.01**	8	0.878
		SH	**12**	**< 0.001**	**12**	**< 0.05**
Light-inducible	*per2*	LH	**12**	**< 0.001**	0	0.112
		RH	16	0.117	**16**	**< 0.05**
		SH	**8**	**< 0.05**	20	0.136
	cry1a	LH	**12**	**< 0.001**	**16**	**< 0.01**
		RH	**8**	**< 0.001**	**16**	**< 0.01**
		SH	**16**	**< 0.001**	20	0.120
Clock output	*tef1*	LH	**8**	**< 0.05**	**8**	**< 0.01**
		RH	**8**	**< 0.001**	**12**	**< 0.001**
		SH	**8**	**< 0.05**	**8**	**< 0.05**
	dbpa	LH	8	0.078	**8**	**< 0.05**
		RH	**8**	**< 0.05**	8	0.095
		SH	**8**	**< 0.001**	**8**	**< 0.05**

909

910

Table 3 - The output of the analysis with RAIN (a robust non-parametric method for the detection of rhythms in biological data) regarding the circadian oscillation of transcript abundance of the genes related to neuroendocrinological aspects of energy balance, appetite and growth. Gene expression was measured at the end of the experiment in constant dim light. Transcripts are subdivided according to their function, for each selection lines (large-harvested: LH, small-harvested: SH and control: RH) and in the corresponding tissue (brain and liver). The timing of the peak of the circadian oscillation (CT0, CT4, CT8, CT12, CT16 or CT20) is reported together with the *p* value (N is between 4 and 5). Significant circadian rhythmicity is reported in bold.

Gene	Function	Tissue	Line	Peak	*p* value
lepa	Appetite	Brain	LH	**8**	**<0.05**
			RH	8	0.456
			SH	**8**	**<0.01**
	Food intake	Liver	LH	20	0.580
			RH	12	0.422
			SH	12	0.876
igf1	Growth	Liver	LH	4	0.227
			RH	**4**	**<0.01**
			SH	4	0.134

930 **FIGURE CAPTIONS**

931

932 **Figure 1 –** Group behavioral traits of Zebrafish shoals composed of eight members
933 measured from 190 to 240 dpf during five-minute video recordings. Inter individual
934 distance (A) was measured at 190 and 210 dpf, while time spent feeding at the surface
935 (B) was measured at 230 and 240 dpf. Trial values are reported on the left with
936 repeatability score and confidence intervals, while boxplots are reported on the right.
937 The different colors represent the selection lines: red for large-harvested (LH; N = 12
938 for each trial) grey for control (RH; N = 11) and blue for small-harvested (SH; N = 12).
939 Boxplots represent the median (bold centerline), the 25th (top of the box) and 75th
940 percentile (bottom of the box). Significant differences are indicated by horizontal lines
941 (*: *p* value < 0.05; **: *p* value < 0.01; ***: *p* value < 0.001).

942

943 **Figure 2 -** Mean waveforms (time scale 24 h) for swimming (A, B) and self-feeding (C,
944 D) activity of Zebrafish shoals are expressed as percentage of the maximum during the
945 first (LD1: days 1-15) and second (LD2: days 22-36) light-darkness trial. Each point
946 represents the 10-min binned mean across all the experimental days in LD1 and LD2
947 for all the groups. The different colors represent the selection lines: red for large-
948 harvested (LH; N = 6) grey for control (RH; N = 6) and blue for small-harvested (SH; N =
949 6). The horizontal lines represent the midline estimating statistic of rhythm as reported
950 in Table 1. The vertical lines represent the standard error (*N* between 75 and 90). Grey
951 shadowed areas represent the darkness hours (lights on is at zeitgeber time = 12;
952 experimental time = 09:00).

953

954 **Figure 3 –** Total swimming activity during photophase (area under the waveform
955 curve, AUC; A) together with early (B) and late (C) peaks of activity (percentage of
956 activity during the first and last four hours of photophase, respectively) measured
957 during the first (LD1: days 1-15) and second (LD2: days 22:36) light-darkness trial. LD1
958 and LD2 values are reported on the left side together with repeatability score and
959 confidence intervals (n.s. = repeatability is not significant). Boxplots are reported on
960 the right side. The different colors represent the selection lines: red for large-
961 harvested (LH; N = 6) grey for control (RH; N = 6) and blue for small-harvested (SH; N =

962 5). Boxplots represent the median (bold centerline), the 25th (top of the box) and 75th
963 percentile (bottom of the box). Significant differences are indicated by black horizontal
964 lines (**: *p* value < 0.01).
965
966 **Figure 4 –** Self-feeding activity (area under the waveform curve, AUC) during the last
967 three hours of scotophase (grey shadow: 06:00 - 07:00; 07:00 - 08:00; 08:00 - 09:00)
968 and the first hour of photophase (09:00 - 10:00) measured during the first (LD1: days
969 1-15) and second (LD2: days 22-36) light-darkness trial. LD1 and LD2 values are
970 reported on the left side together with repeatability score and confidence intervals.
971 Boxplots are reported on the right side. The different colors represent the selection
972 lines: red for large-harvested (LH; N = 6) grey for control (RH; N = 6) and blue for small-
973 harvested (SH; N = 6). Boxplots represent the median (bold centerline), the 25th (top of
974 the box) and 75th percentile (bottom of the box). Significant differences are indicated
975 by black horizontal lines (**: *p* value < 0.01; ***: *p* value < 0.001).
976
977 **Figure 5 -** Mean transcript abundance of the genes related to the circadian system in
978 the brain (left) and liver (right) at different circadian time for the three selection lines
979 (large-harvested: LH, small-harvested: SH and control: RH; N between 4 and 5).
980
981 **Figure 6 -** Transcript abundance of the circadian clock controlled genes related to
982 growth and energy balance. Lines were used to better indicate the circadian oscillation
983 of the mean transcript abundance for the three selections lines (large-harvested, LH:
984 red; small-harvested, SH: blue; and control, RH: grey; N between 4 and 5). Boxplots
985 represent the median (bold centerline), the 25th (top of the box) and 75th percentile
986 (bottom of the box).
987
988

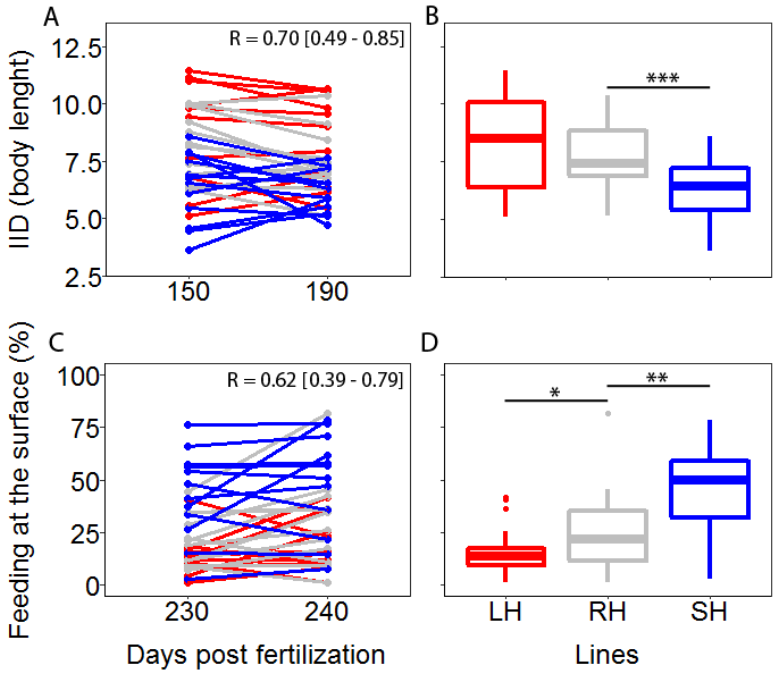

A
B
C
D
R = 0.70 [0.49 - 0.85]
R = 0.62 [0.39 - 0.79]
IID (body lenght)
Feeding at the surface (%)
12.5
10.0
7.5
5.0
2.5
100
75
50
25
0
150
190
230
240
***
*
**
LH
RH
SH
Days post fertilization
Lines

Fig. 1

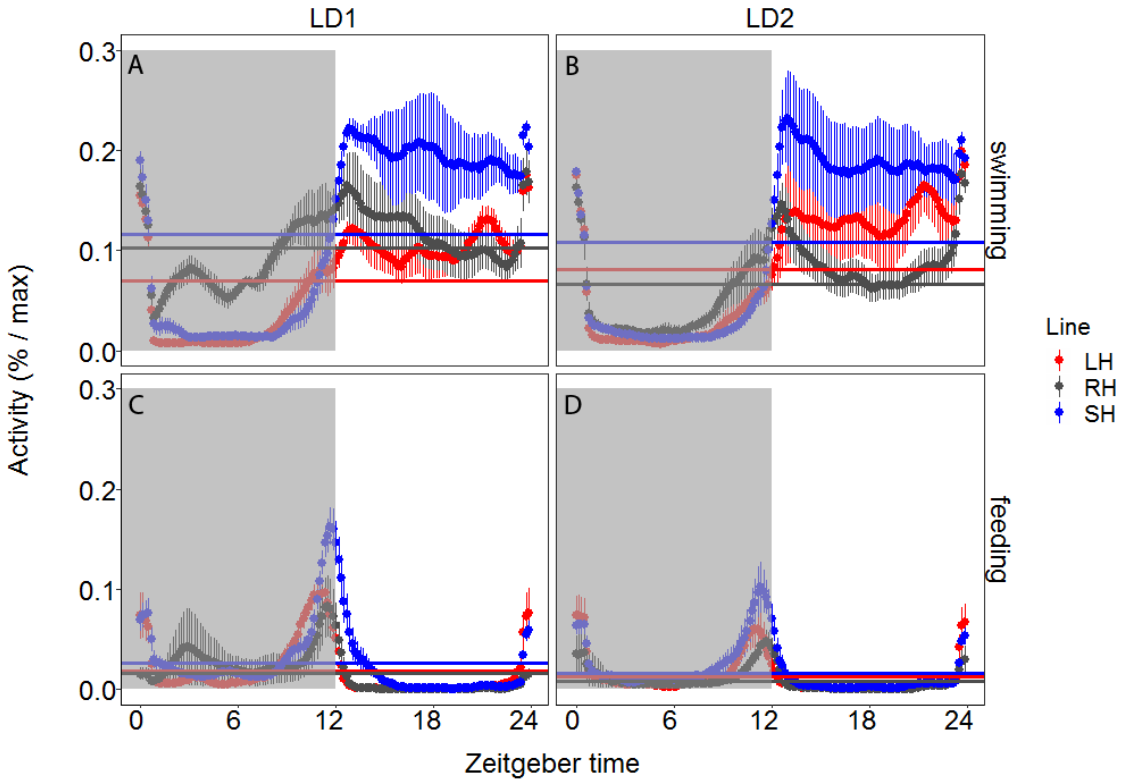

LD1
LD2
A
B
C
D
swimming
feeding
Activity (% / max)
0.3
0.2
0.1
0.0
0
6
12
18
24
Zeitgeber time
Line
LH
RH
SH

Fig. 2

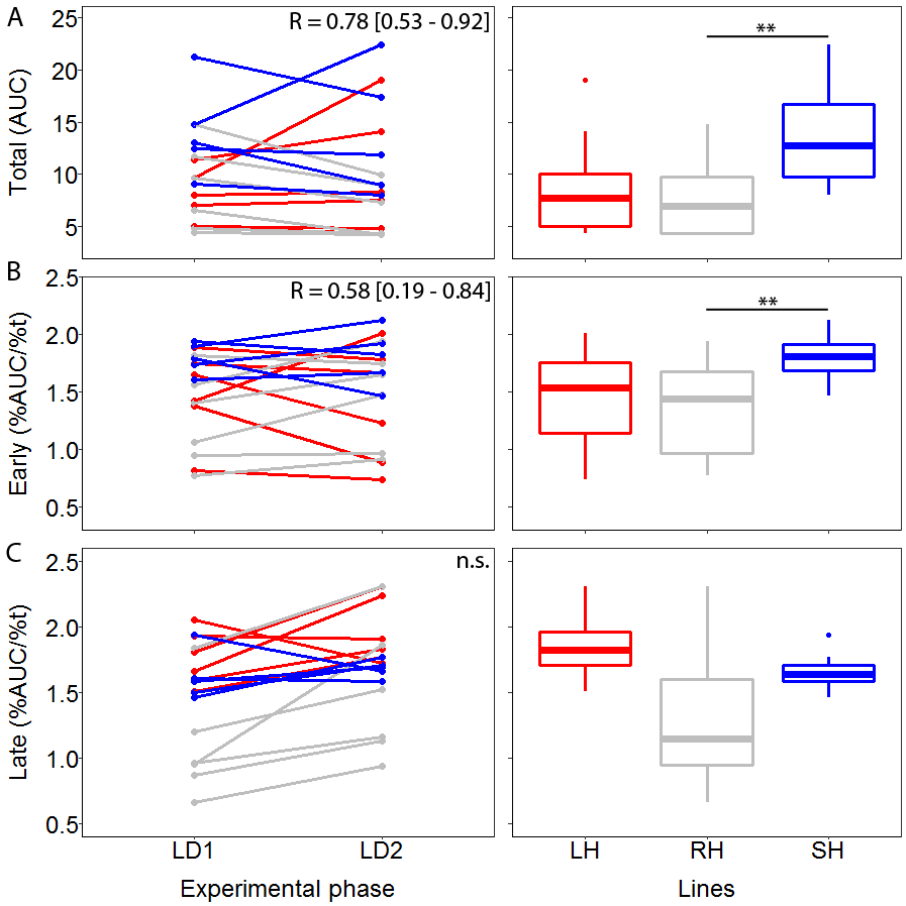

A
B
C
Total (AUC)
Early (%AUC/%t)
Late (%AUC/%t)
R = 0.78 [0.53 - 0.92]
R = 0.58 [0.19 - 0.84]
n.s.
**
**
LD1
LD2
Experimental phase
LH
RH
SH
Lines

Fig. 3

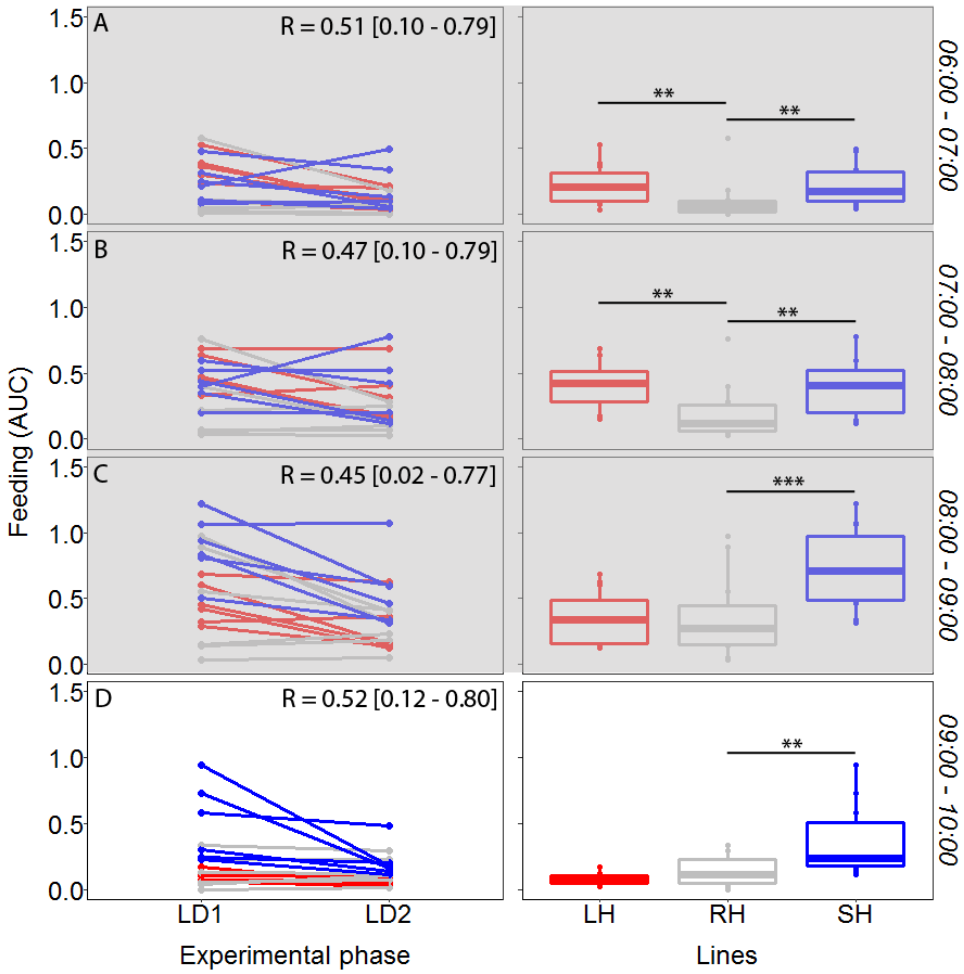

A
R = 0.51 [0.10 - 0.79]
B
R = 0.47 [0.10 - 0.79]
C
R = 0.45 [0.02 - 0.77]
D
R = 0.52 [0.12 - 0.80]
Feeding (AUC)
1.5
1.0
0.5
0.0
**
***
06:00 - 07:00
07:00 - 08:00
08:00 - 09:00
09:00 - 10:00
LD1
LD2
Experimental phase
LH
RH
SH
Lines

Fig. 4

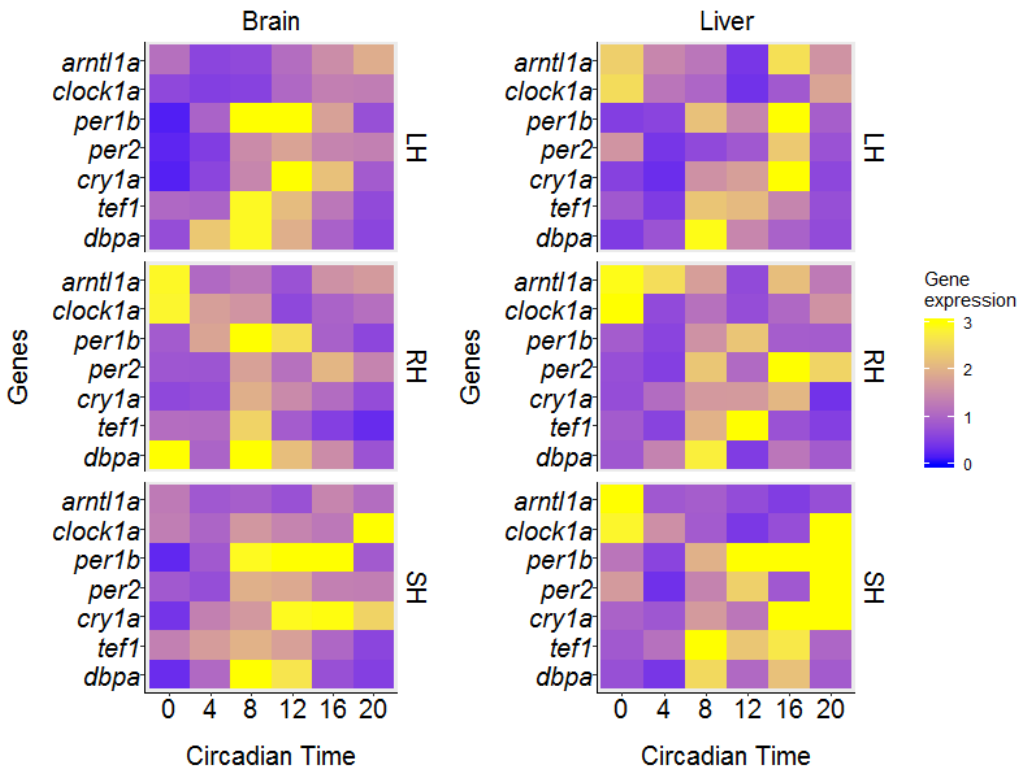
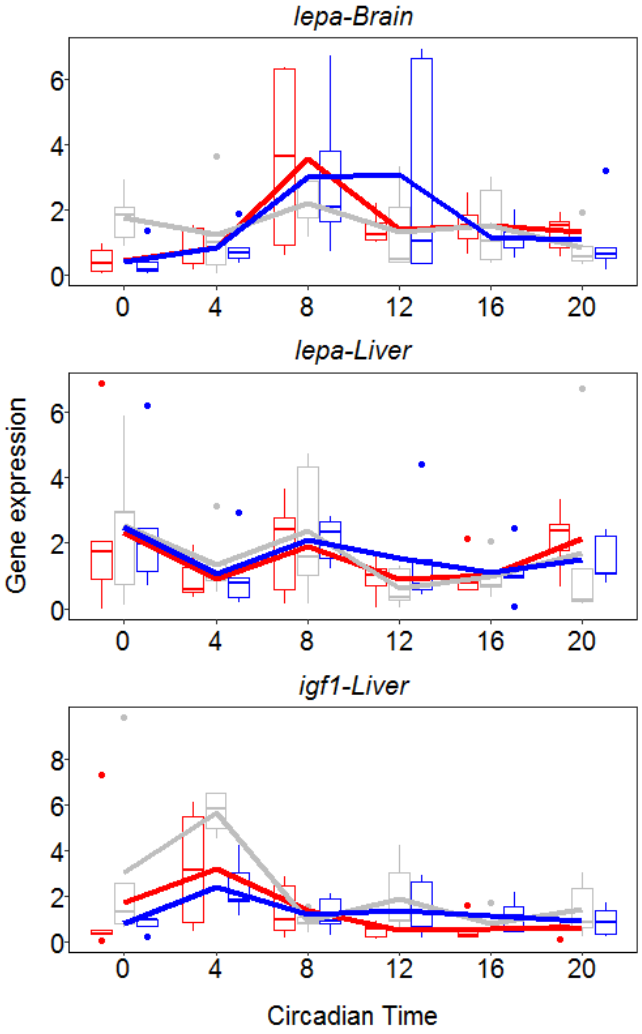

Brain
Liver
arntl1a
clock1a
per1b
per2
cry1a
tef1
dbpa
LH
RH
SH
Genes
0 4 8 12 16 20
Circadian Time
Gene expression
3
2
1
0

Fig. 5



1010

1011

1012

1013 Fig. 6

1014

1015 **SUPPLEMENTARY INFORMATION**

1016

1017 **SUPPLEMENTARY TEXT**

1018 **Swimming and feeding activity rhythms**

1019 Visual inspection of actograms evidenced diurnal swimming activity (with some
1020 evident bimodal peaks at lights on/off) while feeding activity indicated a main peak in
1021 the last part of the darkness hours (Fig. S2).

1022 The Chi-square periodogram analysis identified a significant 24-hours
1023 periodicity in swimming activity during both light-dark (LD) trials. Interestingly during
1024 the first LD trial the robustness of rhythms of the control line is lower (29.74±10.38
1025 %V; Table 1) than the other two lines (large-harvested: 47.41±4.54 %V; small-
1026 harvested: 52.48±10.13 %V, Table 1) in fact the control line showed a higher swimming
1027 activity during darkness (Fig. 2). Such differences were no more evident during the
1028 second LD trial when all lines have similar robust rhythms (large-harvested: 52.45±8.54
1029 %V; control: 51.01±14.30 %V; small-harvested: 56.96±20.82 %V; Table 1) and the
1030 control line did not show higher swimming activity during darkness (Fig. 2). Finally
1031 swimming activity showed variable significant free running rhythmicity under constant
1032 dim light (Table 1). The Chi-square periodogram also detected bimodality (peaks of
1033 significant 12-hours periodicity) in swimming activity in both LD trials.

1034 Self-feeding activity rhythms had a significant 24-hours periodicity during both
1035 LD trials. Robustness was similar in all the lines during the first (large-harvested:
1036 46.44±7.45 %V; control: 38.89±18.18 %V; small-harvested: 43.21±6.35 %V, Table 1)
1037 and second (large-harvested: 46.07±11.67 %V; control: 32.29±7.92 %V; small-
1038 harvested: 37.87±10.17 %V) LD trial. Finally under constant dim light the self-feeding
1039 activity indicated variable free running rhythmicity (Table 1). The Chi-square
1040 periodogram did not detect bimodality in self-feeding activity rhythms.

1041 During the first LD trial bimodality was detected in 3 out of 6 groups in the
1042 large-harvested line (robustness: 25.17±12.20 %V), in three out of six groups in the
1043 control line (robustness: 8.50±1.73 %V) and in one out of five groups in small-
1044 harvested line (robustness: 23.18 %V). During the second LD trial bimodality was still
1045 present in two out of six groups in large-harvested line (robustness: 25.97±7.98 %V), in

1046 four out of six groups in the control line (robustness: 21.79±3.82), and in two out of
1047 five groups in small-harvested line (robustness: 9.17±0.97 %V).

1048 **SUPPLEMENTARY TABLE**

1049

1050 **Table S1 –** Primers used for RT-qPCR together with the references used to design them. The function of each gene is reported together with the
1051 tissue (Brain: B; Liver: L) and the forward and reverse primers.

Function	Tissue	Genes	Forward	Reverse	Reference
Circadian clock: *Core clock*	B / L	*per1b*	CCGTCAGTTTCGCTTTTCTC	ATGTGCAGGCTGTAGATCCC	(1)
		clock1a	CTGGAGGATCAGCTGGGTAG	CACACACAGGCACAGACACA	(1)
		arntl1a	TAGAGCGCTGTTTGCTGATG	GACCCGTGGACTTCAGTGAC	(2)
Circadian clock: *Light-inducible*	B / L	*per2*	ATGTCGATGGCTTTAGGCAG	CGAGACATCCAGAAGGTGCT	(1)
		cry1a	TCCGCTGTGTGTACATCCTC	CAAACACTGCAGCAAAAACC	(1)
Circadian clock: *Clock output*	B / L	*dbpa*	GTCCAGGCCAATTTCTCAAA	CACAGCAGTCCTTCCCTCTC	(3)
		tef1	AAAGCTCTGCTTGAGTACCCTTT	ACTCCTCCAGATCCATGTACTCC	(3)
Growth	L	*igf1*	TCCGTCTCCTGTTCGCTAAATC	TTTGGGTCTCCAGCAAAAGC	(4)
Appetite	L/B	*lepa*	AACTGCAGGCAAAGACCATC	GCGGGAATCTCTGGATAATG	(4)
Housekeeping	B / L	*β-actin*	GCCTGACGGACAGGTCAT	ACCGCAAGATTCCATACCC	(1)
		gadph	GTGGAGTCTACTGGTGTCTTC	GTGCAGGAGGCATTGCTTACA	(5)
		ef1a	CCTTCGTCCCAATTTCAGG	CCTTGAACCAGCCCATGT	(6)

1052

1053 **REFERENCES USED IN THE SUPPLEMENTARY TABLE S1**

1054 1. Cavallari N*, et al.* (2011) A blind circadian clock in cavefish reveals that opsins mediate peripheral clock photoreception. *PLoS Biol.*
1055 9(9):e1001142.

1056 2. Weger M*, et al.* (2013) Real-time in vivo monitoring of circadian E-box enhancer activity: a robust and sensitive zebrafish reporter line
1057 for developmental, chemical and neural biology of the circadian clock. *Dev. Biol.* 380(2):259-273.

1058 3. Mracek P*, et al.* (2012) Regulation of *per* and *cry* genes reveals a central role for the D-box enhancer in light-dependent gene
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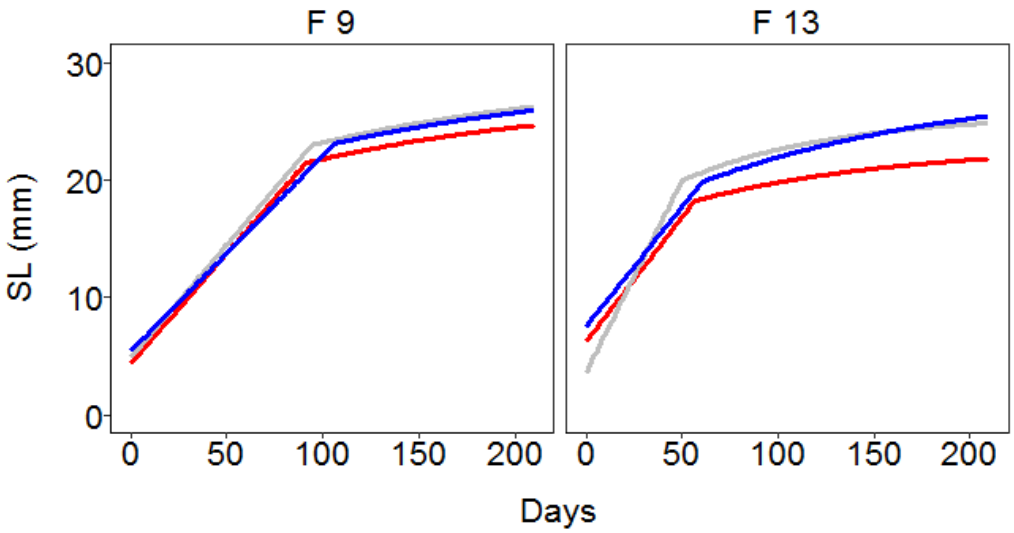
1063 type, developmental stage and chemical treatment. *BMC Mol. Biol.* 9(1):102.
1064 6. Weger BD*, et al.* (2011) The light responsive transcriptome of the zebrafish: function and regulation. *PloS one* 6(2):e17080.

1065

1066 **SUPPLEMENTARY FIGURES**



1067

1068 **Figure S1 -** Fits of the Lester biphasic growth model at generations F_9 and F_{13}. Colors
1069 represent the different selection lines: large-harvested (red), control (grey) and small-
1070 harvested (blue). Adapted from Uusi-Heikkilä et al. (2015) and Sbragaglia et al. (2019).

1071

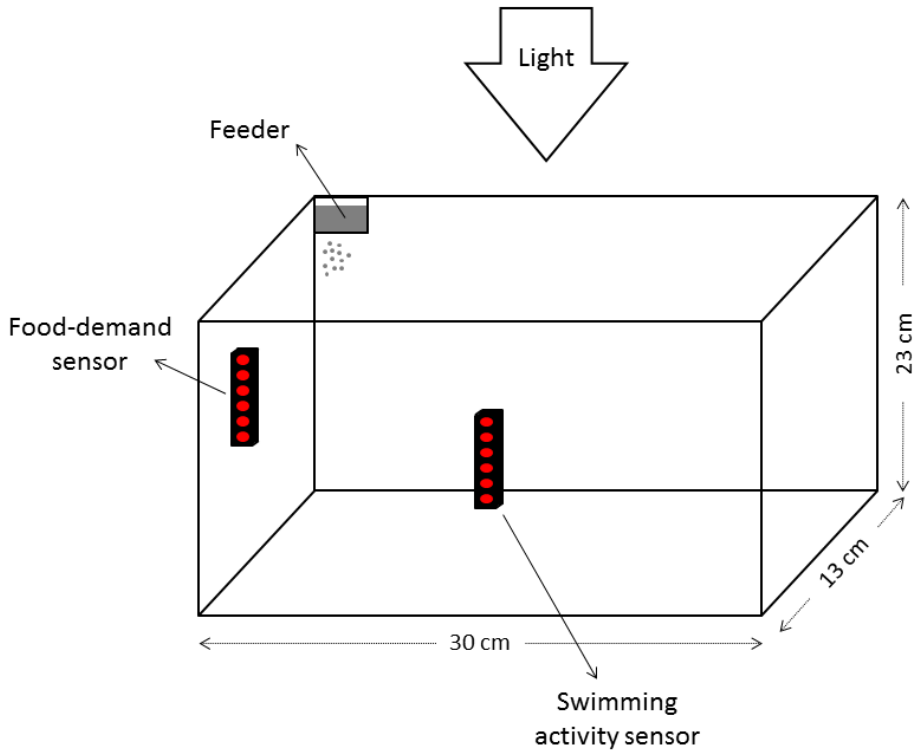


Figure S2 – A schematic representation of the experimental aquarium used in this study to measure swimming and self-feeding activity rhythms. Light illumination was provided from above. An infrared sensor was placed in the central part of the aquarium to measure swimming activity, while another infrared sensor was places to one of the corners and connected to the nearby feeder (for more details see Del Pozo et al., 2011).

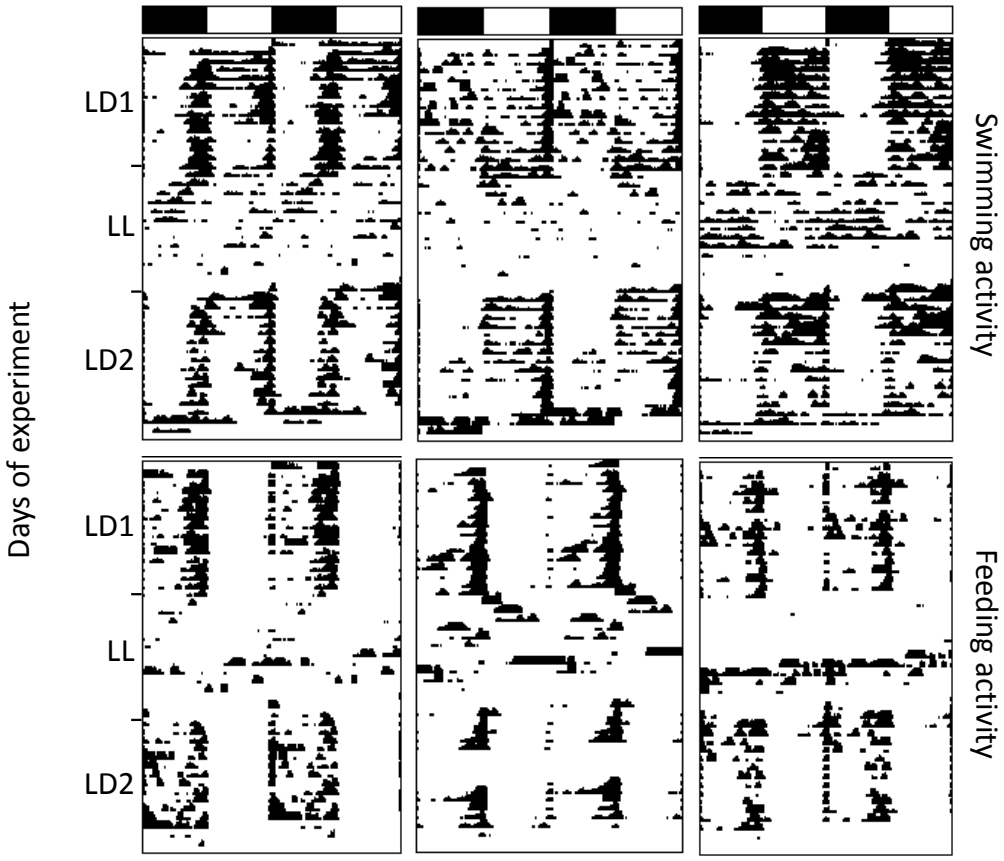


Figure S3 - Six representative double plotted (time scale 48 h) actograms (one for each selection line) representing locomotor and self-feeding activity over 38 days of experiment (left: large-harvested line; middle: control line, right: small-harvested line). Swimming and self-feeding activity of zebrafish are indicated by the height of black areas that represent the number of infrared light beam interruptions/10 min. Open/filled bars at top represent a 12-h light/12-h darkness photoperiod for the first (LD1) and second (LD2) light-darkness trial, while during LL zebrafish shoals were exposed to constant dim light.

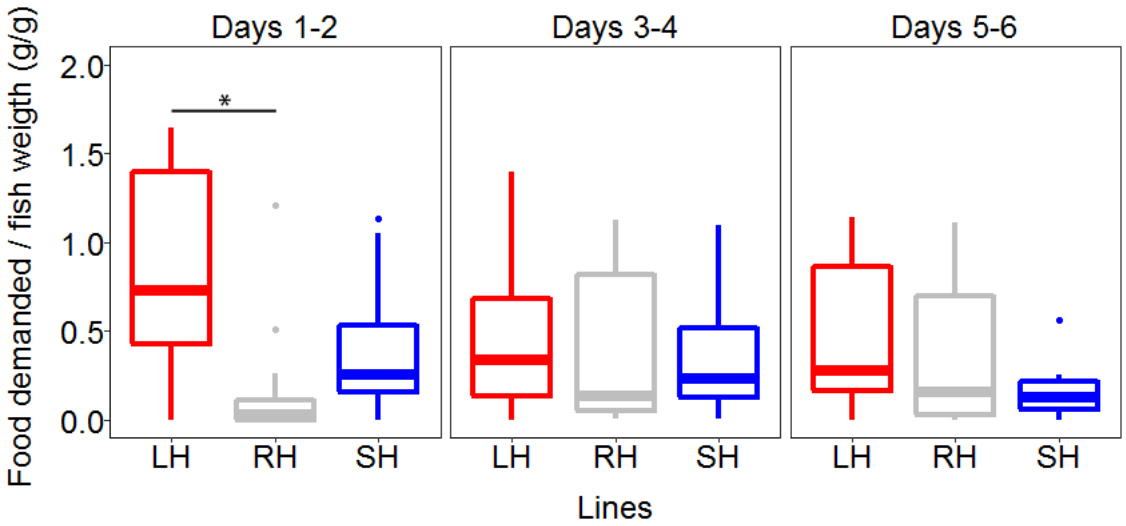


Figure S4 – Boxplots of the amount of food demanded (g) normalized on the weight of the fish group (g) during the first 6 days in the first light-darkness trial (LD1: days 1-6). The different colors represent the selection lines: red for large-harvested (LH; N = 6) grey for control (RH; N = 6) and blue for small-harvested (SH; N = 6). Boxplots represent the median (bold centerline), the 25th (top of the box) and 75th percentile (bottom of the box). Significant differences are indicated by black horizontal lines (*: *p* value < 0.05).