Aquaculture 279 (2008) 120–125

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Fluctuating asymmetry, abnormalities and parasitism as indicators of environmental stress in cultured stocks of goldfish and carp

D. Almeida [a], A. Almodóvar [a], G.G. Nicola [b], B. Elvira [a,*]

[a] Department of Zoology and Physical Anthropology, Faculty of Biology, Complutense University of Madrid, E-28040 Madrid, Spain
[b] Department of Environmental Sciences, University of Castilla-La Mancha, E-45071 Toledo, Spain

ARTICLE INFO

Article history:
Received 24 October 2007
Received in revised form 31 March 2008
Accepted 1 April 2008

Keywords:
Animal welfare
Developmental instability
Fluctuating asymmetry
Stress
Carassius auratus
Cyprinus carpio

ABSTRACT

Knowledge about the effects of captivity and environmental stress on fish populations is basic to culture them for ornamental, conservation or exploitation purposes. The aim of this study was to test how captivity affect morphological development of bilateral characters and whether levels of fluctuating asymmetry, morphological abnormalities and ectoparasites reflect developmental stress caused by adverse environmental conditions in two species of cyprinids, goldfish *Carassius auratus* and two varieties of carp *Cyprinus carpio*, scale and mirror carp. Samples from captivity were caught in an artificial pond (Madrid City, Spain), which presents poor environmental conditions; while samples from nature were captured in the Ebro River basin (NE Spain). Six meristic and four morphometric bilateral characters were measured and compared between the two samples. Additionally, the incidence of morphological abnormalities and ectoparasites was also recorded. Captive goldfish presented significant lower number of scales of the lateral line, scale rows above lateral line and length of pectoral and ventral fins. Captive carps showed significant shorter first barbels and longer ventral fins. Ornamental goldfish showed significant higher levels of fluctuating asymmetry than wild samples for number of branched rays of pectoral fin, lateral line, scale rows below lateral line and length of pectoral and ventral fins. Ornamental carps presented a similar trend for the same characters, except for branched rays and length of pectoral fins, together with the length of second barbels. In the artificial pond, the incidence of abnormalities was higher in carp, whereas goldfish showed a higher occurrence of ectoparasites. These facts support the hypothesis of a different species response to environmental stress. The overall results indicate that fish from the artificial pond are subjected to environmental stress caused by adverse conditions such as overcrowding and lower water quality. Moreover, estimated parameters in this study mean a useful tool to detect developmental instability in confined fish.

1. Introduction

Captivity can produce several changes on cultured fish for ornamental, conservation or exploitation purposes. Thus, artificial environmental conditions can affect morphology, physiology and behavior of fishes (Kihslinger and Nevitt, 2006). This is due to both the restrictions to their ecological requirements (Barber, 2007) and the lack of natural physical, chemical or biological stimuli, which would trigger mechanisms of their life history strategies (Patterson et al., 2004).

Environmental stress can give rise to decreased developmental stability of individuals, which may result in reduced performance of fitness components (Clarke, 1995; Møller and Swaddle, 1997). Developmental instability of individuals and populations is most often estimated by their level of fluctuating asymmetry (FA), which occurs when otherwise bilateral traits show small random variations in the size of the two sides of the character around a bilateral symmetry axis (Palmer and Strobeck, 1986). The degree of FA reflects the inability of individuals to maintain homeostasis during development when faced with stress; either genetic or environmental (see Møller and Swaddle, 1997; Dongen, 2006 for a review).

In fish, individual and population levels of bilateral asymmetry have been shown to relate positively to a wide range of abiotic, biotic and genetic stresses. Abiotic factors such as acidification, toxic chemicals or heavy metals are common stressors which produce elevated levels of FA (Allenbach et al., 1999; Franco et al., 2002; Estes et al., 2006). Furthermore, FA could be sensitive to different levels of individual density in captive conditions (Leary et al., 1991). Finally, FA seems to increase under genetic stresses such as hybridization, inbreeding and loss of genetic variation (Mazzi et al., 2002), particularly in reared fishes (Wilkins et al., 1995; Young et al., 1995; Palma et al., 2001; Fessehaye et al., 2007). Several studies have

* Corresponding author. Tel.: +34 91 3944946; fax: +34 91 3944947.
E-mail address: belvira@bio.ucm.es (B. Elvira).

0044-8486/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2008.04.003

also showed the direct relationship between environmental stress and the increase of abnormalities (Taylor, 2001; Lemly, 2002) and parasites in fishes (Reimchen, 1997; Landsberg et al., 1998; Schwaiger, 2001). Development in poor environmental conditions reduces the efficiency of the immune system and individuals may be more susceptible to parasitism (Valtonen et al., 2003; Binuramesh et al., 2005).

Goldfish *Carassius auratus* (L.) and carp *Cyprinus carpio* L. are two species of cyprinids native to temperate Europe and Asia, where mostly inhabit backwaters and feed on aquatic vegetation, invertebrates and detritus (Barus et al., 2002; Szczerbowski, 2002). These species have been cultured for centuries in many countries as ornamentals, adding several strains (e.g. fantail or lionhead goldfish, and mirror, leather or koi carp). Carp is also exploited as sport fish and for human feeding (Balon, 2004). Moreover, they have been introduced in many open waters all around the world and have been well acclimatized due to their generalist character (Hickley et al., 2004; Zambrano et al., 2006). In Spain, both species of cyprinids were introduced in the 17th century as ornamentals (Elvira and Almodovar, 2001). Some varieties of carp, such as mirror or leather carp are found in nature, although they appear with low frequency due to the simple heredity of that character (absence of scales), which is only determined by two *loci*. From the other hand, varieties of goldfish (see above) have been a product of intense artificial selection and they do not appear in nature.

The aim of this study was to test whether levels of fluctuating asymmetry, morphological abnormalities and ectoparasites reflect developmental stress caused by adverse environmental conditions due to captivity in goldfish *C. auratus*, and two varieties of carp *C. carpio*, scale and mirror carp.

2. Materials and methods

2.1. Study areas and fish sampling

The study was conducted in an artificial pond located in Retiro Park (Madrid, central Spain). The pond has a 35565 m^2 area and 58265 m^3 water volume, and the fish fauna mainly consists of goldfish and carp, as well as of largemouth bass *Micropterus salmoides* (Lacépède), pumpkinseed *Lepomis gibbosus* (L.) and mosquitofish *Gambusia holbrooki* Girard. Fishes were stocked in 1982 and they have been able to breed in that artificial environment. Retiros's pond presents a high eutrophication level according to the standards of OECD (1982) (Table 1), and a poor developed plant and benthic community probably due to the lack of suitable substrate. Furthermore, fishes can feed on organic wastes, which are thrown by people into the water, what can give rise to nutritional disturbances. Goldfish (pond goldfish, standard length range 182 to 265 mm, n=39) and two varieties of carp *C. carpio*, scale (pond scale carp, standard length range 223 to 417 mm, n=29) and mirror carp (pond mirror carp, standard length range 258 to 421 mm, n=43) were collected in Retiro's pond in October of 2001, after the pond was completely emptied. All specimens were identified, counted and weighed (to within 1 g) in order to calculate fish densities and biomasses.

In order to assess whether captivity conditions in Retiro Park affect developmental stability, captive fish were compared with wild fish from nine localities from the Ebro River basin (Navarra, northern Spain). This area belongs to the “cyprinids zone” and is clearly influenced by Mediterranean climate. Goldfish (wild goldfish, standard length range 48 to 161 mm, n=45) and scale carp (wild scale carp, standard length range 94 to 356 mm, n=45) were collected in this basin by electrofishing during summer of 2003. Fish specimens were identified, counted and weighed (to within 1 g) in order to estimate densities and biomasses, by means of removal sampling without replacement or Zippin's method (1956).

Carps from Retiro Park and the Ebro River basin belong to the subspecies *Cyprinus carpio carpio*, according to the morphological characters described by Barus et al. (2002) and the historical data of stocking in Europe (Balon, 1995a,b). Likewise, goldfish corresponds to the subspecies *Carassius auratus auratus*, after Szczerbowski (2002). Moreover, both species have been highly translocated between Iberian localities and there is no morphological or genetic evidence supporting any difference in stocks coming from river basins or artificial ponds.

Captive and wild samples were collected taking into account a similar age rank. Fish were distributed in the following age year classes: from 2+ to 6+ for pond goldfish; from 2+ to 6+ for pond scale carp; from 2+ to 6+ for pond mirror carp; from 1+ to 4+ for wild goldfish; from 1+ to 4+ for wild scale carp. Confined and wild fish showed different proportions for every age class probably due to the difficulties to breed in Retiro's pond and the scarce number of old individuals captured in open waters.

Fish were killed by lethal dose of anaesthetic in accordance to the recommended ethical guidelines, fixed in 70% alcohol and deposited at the Museum of Comparative Morphology and Anatomy of Vertebrates, Complutense University of Madrid.

Table 1
Physicochemical features of water in the study areas

Water quality parameters (mg/L)	Retiros's pond[a]	Ebro River basin[b]
Dissolved oxygen (DO)	6.57±0.25	8.75±0.25
Total suspended solids (TSS)	9.65±1.82	8.73±0.51
Biological oxygen demand (BOD_5)	6.36±0.75	3.35±0.21
Chemical oxygen demand (COD)	7.44±2.05	4.84±0.82
Total nitrogen (TN)	11.51±0.58	3.02±0.42
Total phosphorus (TP)	0.19±0.04	0.07±0.02

Water quality monitoring were monthly carried out for 3-day periods in 2001. Results are means±SE. [a]Source: Autonomous Community of Madrid (Spain); [b]Source: Hydrographic Confederation of the Ebro River. Ministry of Environment (Spain).

2.2. Morphological measurements

A total of 10 bilateral characters were studied, following Holcík et al. (1989). Six meristic traits were taken: number of branched rays of pectoral and ventral fins, number of scales of the lateral line, number of scale rows above and below lateral line, as well as the number of gill rakers. Four morphometric traits were also considered: length of first and second barbels, length of pectoral and ventral fin. These ten variables were chosen due to their functional importance as sensory (lateral line and barbels), structural (scale rows), locomotive (fins) and feeding (gill rakers) traits. Character size was calculated as the average between both sides ($(R+L)/2$)). Body size was measured as standard length (mm). All measurements were taken by the same person (D. A.). Morphometric bilateral characters were measured to the nearest 0.01 mm, using an electronic digital calliper. Measurement error was estimated following Yezerinac et al. (1992) from three repeated measures of each variable. The measurements were taken at 1-week intervals with no reference to previous values.

2.3. Data analysis

2.3.1. General analyses

Assumptions of normality of distributions and homogeneity of variances were verified through Kolmogorov–Smirnov and Levene's tests, respectively. All statistical analyses were performed with STATISTICA 6.0. The significance level for all statistical tests was set at α=0.05.

2.3.2. Morphological analysis

All variables were $\log_{10}$-transformed and correlations between means of both meristic (Spearman rank correlation) and morphometric

Table 2
Densities and biomasses of fishes in the study areas

	Retiros's pond	Ebro River basin (mean ± SE)
	Goldfish	
Density (ind./ha)	63.83	122.67 ± 20.90
Biomass (kg/ha)	23.20	4.91 ± 1.48
	Carp	
Density (ind./ha)	4440.60	279.87 ± 99.80
Biomass (kg/ha)	4910.67	60.30 ± 16.98
	Largemouth bass	
Density (ind./ha)	4.78	61.13 ± 26.15
Biomass (kg/ha)	1.94	17.78 ± 8.71
	Barbel	
Density (ind./ha)	–	2490.52 ± 437.64
Biomass (kg/ha)	–	147.55 ± 60.23
	Other fishes	
Density (ind./ha)	9.84	4881.83 ± 1498.11
Biomass (kg/ha)	0.09	78.76 ± 29.34
	Total fishes	
Density (ind./ha)	4519.05	7836.02 ± 1446.31
Biomass (kg/ha)	4935.89	309.41 ± 57.25

(Pearson r correlation) characters and standard length were performed. To compare mean values of morphological traits between captive and wild samples, General Linear Models (GLM) were used: analyses of variance (ANOVA) and covariance (ANCOVA) with standard length as the covariate. Subsequent post-hoc contrasts (Tukey–Kramer honestly significant difference HSD test) were employed for comparison of means from more than two groups.

2.3.3. Body condition

Body condition was estimated for every group in each fish using the Fulton's condition factor (K):

$$K = 100W/SL^3,$$

where W is the weight of the fish in g and SL is the standard length in cm.

ANOVA and Tukey tests were performed to compare different fish groups.

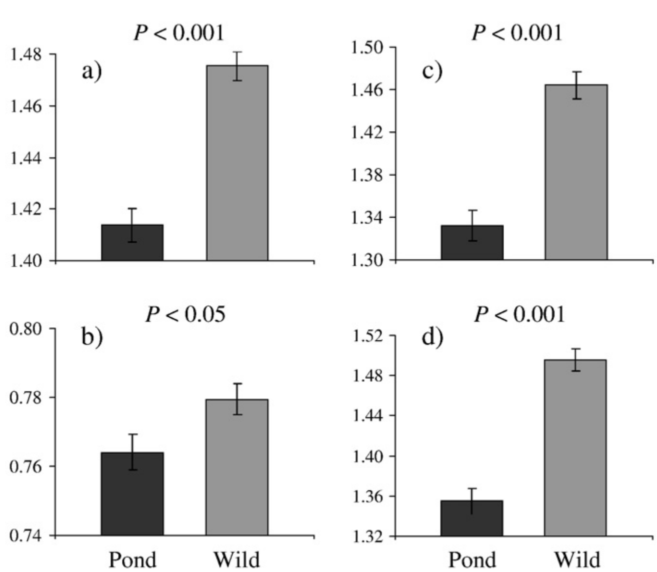

Fig. 1. Variation in several bilateral characters (character size; log mean ± SE) of different goldfish groups. (a) Number of scales of the lateral line. (b) Number of scale rows above lateral line. (c) Length of pectoral fin. (d) Length of ventral fin.

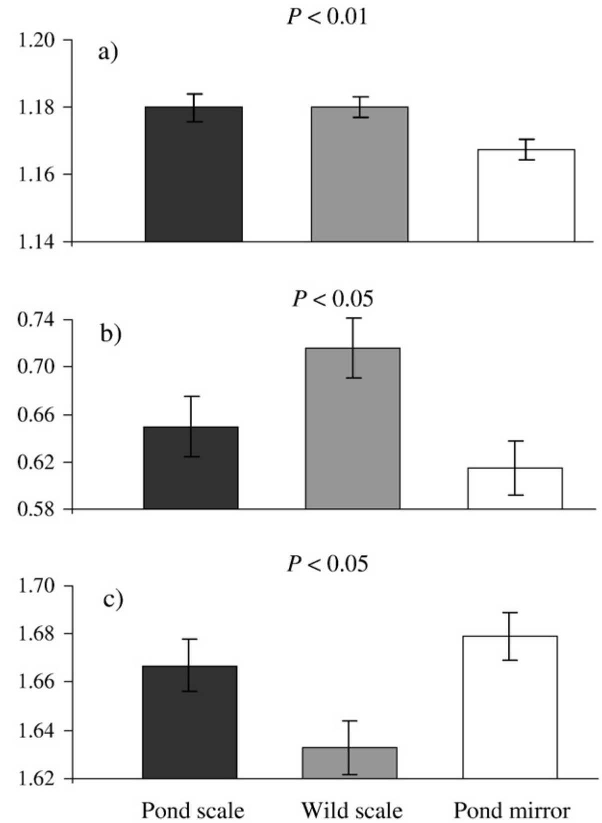

Fig. 2. Variation in several bilateral characters (character size; log mean ± SE) of different carps groups. (a) Number of branched rays of pectoral fin; pond mirror carp vs. pond and wild scale carps. (b) Length of first barbel; wild vs. pond. (c) Length of ventral fin; wild vs. pond.

2.3.4. FA analysis

Paired measurements of bilateral characters were collected from each individual and transformed into signed asymmetry values according to the formula right−left ($R-L$). Presence of directional asymmetry (normal distribution with a mean different from zero) was checked by testing whether the means of signed right minus left values were significantly different from zero using t-tests and the subsequent sequential Bonferroni corrections (Palmer and Strobeck, 1986; Møller and Swaddle, 1997). Presence of antisymmetry (a bimodal or platykurtic distribution with a mean of zero) was checked by examining the kurtosis (Zar, 1996) of the $R-L$ distributions. The magnitude of absolute FA was assessed as the unsigned difference between the measurements of the right and left sides ($|R-L|$). Spearman rank and Pearson correlations were performed between absolute values of FA for meristic and morphometric characters, respectively and character size. To test for differences in levels of FA between samples, GLM were used (Øxnevad et al., 2002; Fessehaye et al., 2007): ANOVA or ANCOVA when FA was correlated with the character size; both analyses were followed by Tukey tests for comparing more than two groups.

2.3.5. Ectoparasitism and abnormalities

The occurrence of morphological abnormalities (hypertrophy of mouth, nodules and spinal deformities) and ectoparasites (*Lernaea* spp., Crustacea, Copepoda) were also recorded in each individual. Carps are mainly parasited by *L. cyprinacea* L. and goldfish by *L. carassi* Tidd, although these two and other *Lernaea* species can parasite both cyprinids, above all under high density conditions. These species of parasites were chosen due to the easiness to be counted on the skin and moreover, they are ectoparasites which can present a high prevalence on cyprinids under captive and natural conditions. A χ^2 test (with Yates' correction) was performed to test the differences between wild and captive samples.

3. Results

Table 1 shows some physicochemical features of water in the study areas. Artificial pond presented a lower water quality with reference to the natural system. Fish densities and biomasses of the study areas are summarized in Table 2. Estimated fish density in Ebro River basin was higher than Retiro's pond, whereas fish biomass was much higher in the artificial environment. Goldfish density was double in wild environment, although biomass was much higher in the artificial environment. Carp showed both density and, above all, biomass higher in the ornamental pond. Carp was the most abundant fish in Retiro Park and its biomass (4910.67 kg/ha) clearly shows the high overcrowding occurring in the pond.

Spearman and Pearson correlations between means of bilateral characters and body size were highly significant ($P<0.001$) for the meristic variables number of scale rows below lateral line in both species, branched rays of pectoral fin only in goldfish and for all morphometric characters. Spearman correlations were also significant for the number of scales of the lateral line in goldfish ($P<0.05$) and branched rays of ventral fin in carps ($P<0.01$).

Significant differences were found between captive and wild goldfish for the characters number of scales of the lateral line (ANCOVA, $F_{1,80}=28.92$, $P<0.001$) and scale rows above lateral line (ANOVA, $F_{1,81}=4.99$, $P<0.05$), as well as for the length of pectoral (ANCOVA, $F_{1,81}=21.24$, $P<0.001$) and ventral fins (ANCOVA, $F_{1,81}=45.02$, $P<0.001$). Captive goldfish always showed lower means of these traits (Fig. 1). Carp groups also showed significant differences between captive and wild samples for branched rays of pectoral fin (ANOVA, $F_{2,114}=5.39$, $P<0.01$), the lengths of first barbel (ANCOVA, $F_{2,110}=3.94$, $P<0.05$) and ventral fin (ANCOVA, $F_{2,113}=3.28$, $P<0.05$). Both groups of pond carps showed shorter first barbels and longer ventral fins than wild counterparts (Tukey test, $P<0.05$) (Fig. 2). The number of branched rays of pectoral fin was similar between captive and wild scale carps (Tukey test, $P>0.05$).

Mean body condition of goldfish from Retiro's pond was significantly lower ($K=2.92$, SE=0.11) than that of fish from Ebro River basin ($K=3.10$, SE=0.03) (ANOVA, $F_{1,82}=197.38$, $P<0.001$).

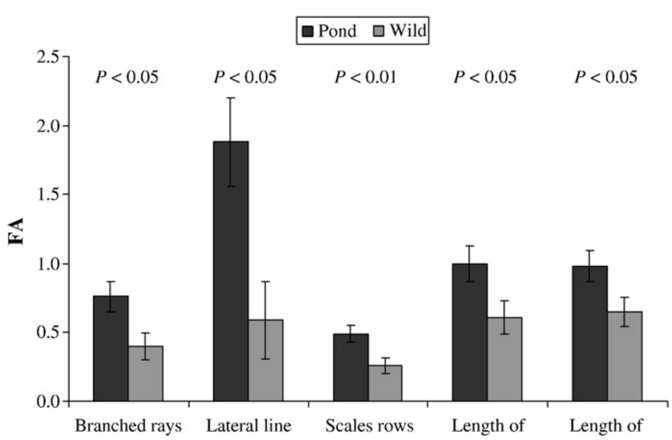

Fig. 3. Variation in fluctuating asymmetry (FA; means±SE) for several bilateral characters of goldfish groups.

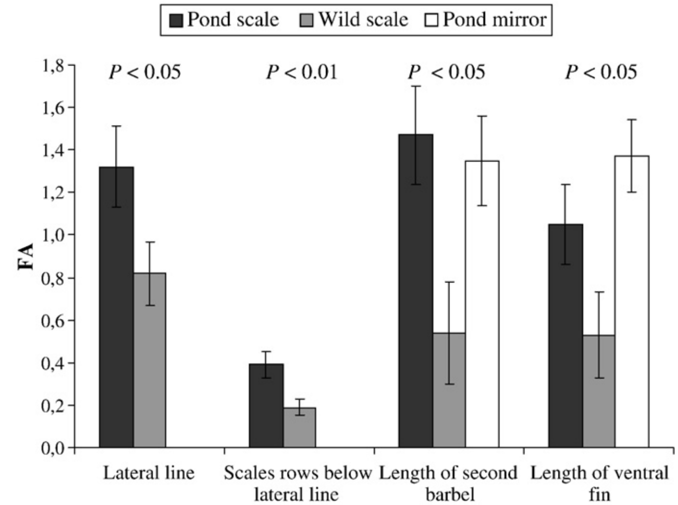

Fig. 4. Variation in fluctuating asymmetry (FA; means±SE) for several bilateral characters of carps groups; wild vs. pond for length of second barbel and ventral fin.

Significant differences were also found between carp groups from the pond and from the nature (ANOVA, $F_{2,114}=102.89$, $P<0.001$). Both varieties of captive carps showed Fulton's factors significantly lower ($K=2.77$, SE=0.02) than that of fish from natural conditions ($K=2.84$, SE=0.05) (Tukey test, $P<0.001$).

The analysis of asymmetry distributions showed that means did not differ significantly from zero after sequential Bonferroni corrections. Asymmetry distributions for all variables were either mesokurtic (−2<Standardized kurtosis value<2) or leptokurtic (Standardized kurtosis value>2). Tests hence showed that the asymmetry distributions were not directionally distributed or antisymmetric, so all characters were considered to show fluctuating asymmetry.

Absolute asymmetry values were significantly correlated with character size for the number of scales of the lateral line and above lateral line in goldfish (Spearman correlation, $P<0.05$) and highly correlated for all morphometric characters of carp (Pearson correlation, $P<0.001$).

There was a significant difference between captive and wild goldfish in the mean level of FA for branched rays of pectoral fin (ANOVA, $F_{1,80}=5.81$, $P<0.05$), scales of the lateral line (ANCOVA, $F_{1,80}=6.24$, $P<0.05$) and scale rows below lateral line (ANOVA, $F_{1,81}=8.46$, $P<0.01$), as well as for two morphometric characters, pectoral (ANOVA, $F_{1,81}=4.68$, $P<0.05$) and ventral fins (ANOVA, $F_{1,82}=4.70$, $P<0.05$). Captive individuals showed higher levels of FA for all traits (Fig. 3). The mean level of FA was also significantly different between captive and wild carp for scales of the lateral line (ANOVA, $F_{1,71}=4.45$, $P<0.05$), scale rows below lateral line (ANOVA, $F_{1,71}=8.06$, $P<0.01$), second barbel (ANCOVA, $F_{2,110}=4.07$, $P<0.05$) and ventral fin (ANCOVA, $F_{2,113}=3.18$, $P<0.05$). Captive carps exhibited higher mean values of FA for all those characters (Fig. 4).

Table 3 lists the proportion of abnormal morphologies and individuals with parasites for each group of cyprinids, as well as χ^2 test (with Yates' correction) results for comparisons with wild cyprinids. The frequency of morphological abnormalities was significantly higher in captive carps (scale 31.8%; mirror 26.5%) compared with their wild counterparts (2.3%). Hypertrophy of mouth was the abnormality which contributed more to the statistical differences in carps. Likewise, the frequency of ectoparasites increased significantly in captive individuals of goldfish (50%), compared with wild fish (9.8%). Frequencies of abnormalities in captive goldfish (11.4%) and parasitism in captive carps (scale 11.5%;

Table 3
Comparison by χ^2 test (with Yates' correction) of the incidence of abnormalities (a) and ectoparasites (b) between wild and captive cyprinids

(a)			
Group	Number of specimens without hypertrophy of mouth	Number of specimens with hypertrophy of mouth	*P*
Pond goldfish	39	0	–
Wild goldfish	45	0	
Pond scale carp	23	6	<0.01
Wild scale carp	45	0	
Pond mirror carp	37	6	<0.05
Wild scale carp	45	0	
Group	Number of specimens without nodules	Number of specimens with nodules	*P*
Pond goldfish	35	4	>0.05
Wild goldfish	45	0	
Pond scale carp	28	1	>0.05
Wild scale carp	44	1	
Pond mirror carp	42	1	>0.05
Wild scale carp	44	1	
Group	Number of specimens without spinal deformities	Number of specimens with spinal deformities	*P*
Pond goldfish	39	0	–
Wild goldfish	45	0	
Pond scale carp	29	0	–
Wild scale carp	45	0	
Pond mirror carp	41	2	>0.05
Wild scale carp	45	0	
Group	Number of specimens without total abnormalities	Number of specimens with total abnormalities	*P*
Pond goldfish	35	4	>0.05
Wild goldfish	45	0	
Pond scale carp	22	7	<0.01
Wild scale carp	44	1	
Pond mirror carp	34	9	<0.05
Wild scale carp	44	1	
(b)			
Group	Number of specimens without ectoparasites	Number of specimens with ectoparasites	*P*
Pond goldfish	26	13	<0.05
Wild goldfish	41	4	
Pond scale carp	26	3	>0.05
Wild scale carp	43	2	
Pond mirror carp	38	5	>0.05
Wild scale carp	43	2	

mirror 13.2%) were also higher than wild fish (goldfish, absent; carp 4.7%, respectively), although significant differences were not found.

4. Discussion

The degree of developmental instability observed in captive goldfish and carp for some morphological characters may be related to its functional importance. Captive goldfish showed a significant reduced development of sensory (lateral line), structural (number of scales rows above lateral line) and locomotive (fins length) characters. In carp, differences between captive and wild fish were also found for sensory (first barbels), and locomotive (ventral fin length) characters. Asymmetry in functionally important traits for fish such as fins probably makes locomotion less efficient, as has been observed for some domesticated animals (Møller and Swaddle, 1997; Knierim et al., 2007). The increased asymmetry in locomotive and sensory traits observed in captive goldfish and carp is perhaps a reflection of the decreased need for efficient swimming in an artificial environment without water current and capacity to perceive changes in the surrounding water pressure (Chagnaud et al., 2006). The lower development of a chemosensory organ like barbels could be promoted by the poor physicochemical water conditions. A recent review of Balon (2004) about the principal domesticated fishes in history indicated that goldfish and carp quickly undergo physiological and morphological changes under captive conditions, probably due to nutritional and habitat differences.

Results of the present study are consistent with the hypothesis that environmental stress under captivity increases the level of fluctuating asymmetry in fish. Thus, fluctuating asymmetry appears to be a useful indicator for stress-induced developmental instability in goldfish and carp. These findings agree with previous studies focused on the effects of exposure to different water contaminants on developmental instability of fish. For example, Øxnevad et al. (2002) found an increase in FA for gill rakers of Eurasian Perch *Perca fluviatilis* L. with lake acidification. Likewise, Estes et al. (2006) observed an increased level of FA in mosquitofish *Gambusia holbrooki* subjected to paper mill effluents, and Green and Lochmann (2006) found significant differences in FA among groups of channel catfish *Ictalurus punctatus* (Rafinesque) exposed to different sublethal concentrations of isopropyl methylphosphonic acid.

The comparative analysis indicated that goldfish and carp showed a subtle different response to environmental stress under captivity conditions. Goldfish presented a similar trend for the same characters as carp, except for the number of branched rays and the length of pectoral fin, which is related to manoeuvrability and propulsion. Interspecific differences were more marked for the incidence of external abnormalities and ectoparasites. Thus, within the artificial lake, the frequency of phenodeviants was higher in carp, whereas goldfish showed a higher occurrence of ectoparasites. The increase of phenodeviants under adverse environmental conditions has also been observed by Graham et al. (1993), who found a higher frequency of lower jaw deformities in populations of goldfish that occupied polluted lakes compared with unpolluted ones, where none of the fish had abnormal growth patterns. Moreover, Sato (2006) found that the occurrence of deformities in small, isolated populations of Kirikuchi charr *Salvelinus leucomaenis japonicus* Oshima was an important ecological indicator which reflected the decline in fitness. In that study the proportion of deformed individuals were much lower than figures observed in Retiro's pond.

The higher incidence of abnormalities under artificial conditions could be partly explained by loss of genetic variability and inbreeding (Wang and Ryman, 2001; Shikano et al., 2005; Kocour et al., 2006). Poor environmental conditions are known to reduce the efficiency of the immune system (Gershwin et al., 1985), which explains the higher incidence of parasitism in the artificial lake. Further, the direct relationship of developmental instability and parasitism has been observed in more than thirty studies across some plant and animal taxa (see Møller, 1996, 2006 for a review). However, the reduction of selection forces under captive conditions may also contribute to the intensity of parasite infection. The overall results indicate that fish from the artificial lake are subjected to a high environmental stress caused by adverse conditions such as overcrowding, nutritional disturbances, unsuitable habitat, eutrophication, poor water quality and even the close presence of predatory fishes.

Swain (1987) has suggested that meristic characters are not as accurate as indicators of developmental stability as metric traits, because they only become asymmetric once stress reaches a threshold level. The higher levels of asymmetry found in some meristic characters in both species in Retiro's pond reinforces the study findings. Furthermore, traits that are functionally important, such as fins, seem to exhibit extremely stable developmental trajectories and hence very small levels of fluctuating asymmetry (Møller and Höglund, 1991), which was not the case in the present study. Evidence from a range of taxa indicates that increasing population density results in decreased developmental stability (Møller and Swaddle, 1997). Overcrowding could impair inmune response by a neuroendocrine pathway (Binuramesh et al., 2005; Davis, 2006), increasing the proportion of parasite individuals in case of goldfish.

5. Conclusion

In conclusion, adverse conditions found in confined environments such as the artificial lake of Retiro Park affect morphological development of some traits of functional importance for fish. Further, fluctuating asymmetry can be used as an indicator of poor body condition, caused by nutritional stress or poor habitat quality. Improving environmental conditions of cultured fish would enhance individual performance (lower levels of FA) and reduce the effects of parasitism. Also, morphological abnormalities which hurts the marketing image and downgrade the commercial value of the fish probably would reduce.

Acknowledgements

Financial support was provided by the Department of Environment of the Municipality of Madrid. D. Almeida held a doctoral fellowship (FPI 2004-2008) from Complutense University of Madrid.

References

Allenbach, D.M., Sullivan, K.B., Lydy, M.J., 1999. Higher fluctuating asymmetry as a measure of susceptibility to pesticides in fishes. Environ. Toxicol. Chem. 18, 899–905.

Balon, E.K., 1995a. Origin and domestication of the wild carp, *Cyprinus carpio*: from Roman gourmets to the swimming flowers. Aquaculture 129, 3–48.

Balon, E.K., 1995b. The common carp, *Cyprinus carpio*: its wild origin, domestication in aquaculture, and selection as colored nishikigoi. Guelph Ichthyol. Rev. 3, 1–55.

Balon, E.K., 2004. About the oldest domesticates among fishes. J. Fish Biol. 65 (Supp. A), 1–27, doi:10.1111/j.1095-8649.2004.00563.x.

Barber, I., 2007. Parasites, behaviour and welfare in fish. Appl. Anim. Behav. Sci. 104 (3–4), 251–264, doi:10.1016/j.applanim.2006.09.005.

Barus, V., Penaz, M., Kohlmann, K., 2002. *Cyprinus carpio* (Linnaeus, 1758). In: Banarescu, P., Paepke, H.J. (Eds.), The Freshwater Fishes of Europe. Cyprinidae 2/III and Gasterosteidae, vol. 5. AULA-Verlag, Wiesbaden, pp. 85–179 (III).

Binuramesh, C., Prabakaran, M., Steinhagen, D., Dinakaran, M., 2005. Effect of chronic confinement stress on the immune responses in different sex ratio groups of *Oreochromis mossambicus* (Peters). Aquaculture 250, 47–59, doi:10.1016/j.aquaculture.2005.03.043.

Chagnaud, B.P., Bleckmann, H., Engelmann, J., 2006. Neural responses of goldfish lateral line afferents to vortex motions. J. Exp. Biol. 209, 327–342, doi:10.1242/jeb.01982.

Clarke, G.M., 1995. Relationships between developmental stability and fitness: application for conservation biology. Conserv. Biol. 9 (1), 18–24, doi:10.1046/j.1523-1739.1995.09010018.x.

Davis, K.B., 2006. Management of physiological stress in finfish aquaculture. N. Am. J. Aquac. 68, 116–121, doi:10.1577/A05-007.1.

Dongen, S.V., 2006. Fluctuating asymmetry and developmental instability in evolutionary biology: past, present and future. J. Evol. Biol. 19 (6), 1727–1743, doi:10.1111/j.1420-9101.2006.01175.x.

Elvira, B., Almodovar, A., 2001. Freshwater fish introductions in Spain: facts and figures at the beginning of the 21st century. J. Fish. Biol. 59 (A), 323–331, doi:10.1006/jfbi.2001.1753.

Estes, E.C.J., Katholi, C.R., Angus, R.A., 2006. Elevated fluctuating asymmetry in eastern mosquitofish (*Gambusia holbrooki*) from a river receiving paper mill effluent. Environ. Toxicol. Chem. 25, 1026–1033.

Fessehaye, Y., Komen, H., Kezk, M.A., van Arendonk, J.A.M., Bovenhuis, H., 2007. Effects pf inbreeding on survival, body weight and fluctuating asymmetry (FA) in Nile tilapia, *Oreochromis niloticus*. Aquaculture 264, 27–35, doi:10.1016/j.aquaculture.2006.12.038.

Franco, A., Malavasi, S., Pranovi, F., Nasci, C., Torricelli, P., 2002. Ethoxyresorufin *O*-deethylase (EROD) activity and fluctuating asymmetry (FA) in *Zosterisessor ophiocephalus* (Teleostei, Gobiidae) as indicators of environmental stress in the Venice lagoon. J. Aquat. Ecosyst. Stress Recov. 9 (4), 239–247, doi:10.1023/A:1024010813669.

Gershwin, M.E., Beach, R.S., Hurley, L.S., 1985. Nutrition and immunity. Academic Press, Orlando. 417 pp.

Graham, J.H., Freeman, D.C., Emlen, J.M., 1993. Developmental stability: a sensitive indicator of populations under stress. In: Landis, W.G., Hughes, J.S., Lewis, M.A. (Eds.), Environmental Toxicology and Risk Assessment. ASTM STP, vol. 1179. Society for Testing and Materials, Philadelphia, pp. 136–158.

Green, C.C., Lochman, S.E., 2006. Fluctuating asymmetry and condition in golden shiner (*Notemigonus crysoleucas*) and channel catfish (*Ictalurus punctatus*) reared in sublethal concentrations of isopropyl methylphosphonic acid. Environ. Toxicol. Chem. 25, 58–64.

Hickley, P., Muchiri, S.M., Britton, J.R., Boar, R.R., 2004. Discovery of carp, *Cyprinus carpio*, in the already stressed fishery of Lake Naivasha, Kenya. Fish. Manage. Ecol. 11 (2), 139–142, doi:10.1046/j.1365-2400.2003.00379.x.

Holcík, J., Banarescu, P., Evans, D., 1989. A general introduction to fishes. In: Holcík, J. (Ed.), The Freshwater Fishes of Europe. General Introduction to Fishes, Acipenseriformes, vol. 1. AULA-Verlag, Wiesbaden, pp. 18–147 (II).

Kihslinger, R.L., Nevitt, G.A., 2006. Early rearing environment impacts cerebellar growth in juvenile salmon. J. Exper. Biol. 209, 504–509, doi:10.1242/jeb.02019.

Knierim, U., Van Dongen, S., Forkman, B., Tuyttens, F.A.M., Špinka, M., Campo, J.L., Weissengruber, G.E., 2007. Fluctuating asymmetry as an animal welfare indicator — a review of methodology and validity. Physiol. Behav. 92 (3), 398–421, doi:10.1016/j.physbeh.2007.02.014.

Kocour, M., Linhart, O., Vandeputte, M., 2006. Mouth and fin deformities in common carp: is there a genetic basis? Aquac. Res. 37 (4), 419–422, doi:10.1111/j.1365-2109.2005.01425.x.

Landsberg, J.H., Blakesley, B.A., Reese, R.O., Mcrae, G., Forstchen, P.R., 1998. Parasites of fish as indicators of environmental stress. Environ. Monit. Assess. 51 (1–2), 211–232, doi:10.1023/A:10005991420265.

Leary, R.F., Allendorf, F.W., Knudsen, K.L., 1991. Effects of rearing density on meristics and developmental stability of rainbow trout. Copeia 1, 44–49.

Lemly, A.D., 2002. Symptoms and implications of selenium toxicity in fish: the Belews Lake case example. Aquat. Toxicol. 57, 39–49 doi:0166-445X/02/$.

Mazzi, D., Largiader, C.R., Bakker, T.C.M., 2002. Inbreeding and developmental stability in three-spined sticklebacks (*Gasterosteus aculeatus* L.). Heredity 89, 293–299, doi:10.1038/sj.hdy.6800138.

Møller, A.P., 1996. Parasitism and developmental instability of hosts: a review. Oikos 77, 189–196.

Møller, A.P., 2006. A review of developmental instability, parasitism and disease: infection, genetics and evolution. Infect. Genet. Evol. 6, 133–140, doi:10.1016/j.meegid.2005.03.005.

Møller, A.P., Höglund, J., 1991. Patterns of fluctuating asymmetry in avian feather ornaments: implications for models for sexual selection. Proc. Roy. Soc. London S. B. 245, 1–5.

Møller, A.P., Swaddle, J.P., 1997. Asymmetry, developmental stability, and evolution. Oxford Series in Ecology and Evolution. Oxford University Press, New York. 291 pp.

OECD, 1982. Eutrophication of water: monitoring, assessment and control. Organization of Economic Co-operation and Development, Paris. 154 pp.

Øxnevad, S.A., Heibo, E., Vøllestad, L.A., 2002. Is there a relationship between fluctuating asymmetry and reproductive investment in perch (*Perca fluvialtilis*)? Can. J. Zool. 80, 120–125, doi:10.1139/Z01-215.

Palma, J., Alarcón, J.A., Álvarez, C., Zouros, E., Magoulas, A., Andrade, J.P., 2001. Developmental stability and genetic heterozigosity in wild and cultured stocks of gilthead sea bream (*Sparus aurata*). J. Mar. Biol. Assoc. UK 81, 283–288.

Palmer, A.R., Strobeck, C., 1986. Fluctuating asymmetry: measurement, analysis, patterns. Annu. Rev. Ecol. Syst. 17, 391–421.

Patterson, D.A., MacDonald, J.S., Hinch, S.G., Healey, M.C., Farrell, P., 2004. The effect of exercise and captivity on energy partitioning, reproductive maturation and fertilization success in adult sockeye salmon. J. Fish Biol. 64, 1039–1059, doi:10.1111/j.1095-8649.2004.00370.x.

Reimchen, T.E., 1997. Parasitism of asymmetrical pelvic phenotypes in stickleback. Can. J. Zool. 75, 2084–2094.

Sato, T., 2006. Occurrence of deformed fish and their fitness-related traits in Kirikuchi charr, *Salvelinus leucomaenis japonicus*, the southernmost population of the genus *Salvelinus*. Zool. Sci. 23, 593–599.

Schwaiger, J., 2001. Histopathological alterations and parasite infection in fish: indicators of multiple stress factors. J. Aquat. Ecosyst. Stress Recov. 8 (3–4), 231–240, doi:10.1023/A:1012954608541.

Shikano, T., Ando, D., Taniguchi, N., 2005. Relationships of vertebral deformity with genetic variation and heterosis in the guppy *Poecilia reticulata*. Aquaculture 246, 133–138, doi:10.1016/j.aquaculture.2005.01.010.

Swain, D.P., 1987. A problem with the use of meristic characters to estimate developmental stability. Amer. Nat. 129, 761–768.

Szczerbowski, J.A., 2002. *Carassius auratus* (Linnaeus, 1758). In: Banarescu, P., Paepke, H. J. (Eds.), The Freshwater Fishes of Europe. Cyprinidae 2/III and Gasterosteidae, vol. 5. AULA-Verlag, Wiesbaden, pp. 5–41 (III).

Taylor, D.S., 2001. Physical variability and fluctuating asymmetry in hererozigous and homozygous populations of *Rivulus marmoratus*. Can. J. Zool. 79, 766–778, doi:10.1139/cjz-79-5-766.

Valtonen, E.T., Holmes, J.C., Aronen, J., Rautalahti, I., 2003. Parasite communities as indicators of recovery from pollution: parasites of roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*) in Central Finland. Parasitology 126, S43–S52, doi:10.1017/S0031182003003494.

Wang, J., Ryman, N., 2001. Genetic effects of multiple generations of supportive breeding. Conserv. Biol. 15 (6), 1619–1631, doi:10.1046/j.1523-1739.2001.00173.x.

Wilkins, N.P., Gosling, E., Curatolo, A., Linnane, A., Jordan, C., Courtney, H.P., 1995. Fluctuating asymmetry in Atlantic salmon, European trout and their hybrids, including triploids. Aquaculture 137, 77–85.

Yezerinac, S.M., Lougheed, S.C., Handford, P., 1992. Morphological variability and enzyme heterozygosity: individual and population level correlations. Evolution 46 (6), 1959–1964.

Young, W.P., Wheeler, P.A., Thorgaard, G.H., 1995. Asymmetry and variability of meristic characters and spotting in isogenic lines of rainbow trout. Aquaculture 137, 67–76.

Zambrano, L., Martinez-Meyer, E., Menezes, N., Peterson, A.T., 2006. Invasive potential of common carp (*Cyprinus carpio*) and Nile tilapia (*Oreochromis niloticus*) in American freshwater systems. Can. J. Fish. Aquat. Sci. 63, 1903–1910, doi:10.1139/F06-088.

Zar, J.H., 1996. Biostatistical analysis, 3rd edn. Prentice-Hall, New Jersey, USA.

Zippin, C., 1956. An evaluation of the removal method of estimating animal population. Biometrics 12, 163–189.