

Morphological variations of wild populations of Nile tilapia (*Oreochromis niloticus*) living in extreme environmental conditions in the Kenyan Rift-Valley

Titus Chemandwa Ndiwa · Dorothy Wanja Nyingi ·
Julien Claude · Jean-François Agnèse

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Abstract In this study, we use geometric morphometric method to establish morphological differences between natural populations of Nile tilapia from two extreme environmental conditions (high temperature and salinity) in Kenya, and compare them to two populations from regions experiencing less extreme conditions. To determine genetic influence on morphology, we correlated genetic data with morphological data. The study observed significant morphological differences between all studied populations, including three closely related hot spring populations (Bogoria, Chelaba and Turtle Springs), and two populations with similar genetic background inhabiting saline environments in Lake Turkana basin (Turkana and Crocodile Lake populations). In addition, allometric growth patterns of the seven

populations differed significantly, demonstrating that developmental changes (plastic or adaptive) operated between these populations. Positive correlation between morphometric and genetic data confirmed the influence of genetic factors on morphology. All observed differences were attributed either to genetic and/or environmental factors, which seemed to play a major role in influencing morphology of wild Nile tilapia populations. We recommend further studies to be carried out under controlled conditions to confirm the role of temperature, pH and salinity in morphological diversification of Nile tilapia.

Keywords Morphometrics · Salinity · Temperature · Nile tilapia · Rift-Valley · Kenya

T. C. Ndiwa (✉) · D. W. Nyingi
Department of Clinical Studies, College of Agriculture and
Veterinary Sciences, University of Nairobi, P. O Box
29053-00625, Nairobi, France
e-mail: ndiwatitus@yahoo.com

T. C. Ndiwa · D. W. Nyingi
Kenya Wetlands Biodiversity Research Team, National Museums
of Kenya, P.O. Box 40658-00100, Nairobi, Kenya

J. Claude
Département Forme, UMR IRD 226 CNRS 5554, Institut des
Science de l'Evolution, Université de Montpellier 2, Montpellier,
France

J.-F. Agnèse
Département Conservation et Domestication, UMR IRD 226
CNRS 5554, Institut des Science de l'Evolution, Université de
Montpellier 2, Montpellier, France

Introduction

Cichlids are tropical freshwater fishes well known for their high rates of speciation often resulting in rapid radiation. They exhibit remarkably high levels of genetic and morphological diversity, which affect their morphology, ecology, behaviour and genomes (Nelson 1994; Barlow 2000; Chakrabarty 2005). A large extent of cichlid diversity is observed in three main East African Lakes (Victoria, Tanganyika and Malawi) where 1800 to 2000 species (60 % of all cichlid species worldwide) are present in species flocks (Greenwood 1984; Turner et al. 2001). These cichlid species flocks are primarily monophyletic groups of closely related species inhabiting the same ecosystem, and evolved

from a single ancestral species (Greenwood 1974). Within these three East African Lakes, some cichlid species went through rapid morphological diversification and speciation, leading to formation of many new species within a few thousand generations (Sage et al. 1984; Meyer et al. 1990; Owen et al. 1990). In Lake Victoria for instance, the radiation of monophyletic haplochromine species occurred within a geologically short time of about 750,000 years and resulted to the emergence of approximately 500 cichlid species (Greenwood 1984; Abila et al. 2004; Kerschbaumer and Sturmbauer 2011). Additionally, evolutionary older cichlid assemblages of Lake Tanganyika have shown the highest degree of morphological differentiations (Salzburger et al. 2002).

Under the ecological theory of diversification (Schluter 2000), a strong relationship exists between speciation events and colonization of new environments, or niche diversification, which may in turn induce changes in organism's behaviour, morphology and physiology (Price et al. 2003). Generally, selection pressures that exist in new environments favour divergence of populations from their ancestral forms. A classic example is provided by the case of the three spined sticklebacks (*Gasterosteus aculeatus*) in Canada, where two sympatric forms (limnetic and benthic) emerged as a consequence of ecological divergence (Schluter and McPhail 1993). This example among others (Meyer 1987; Day et al. 1994; Imre et al. 2002) has provided evidence of morphological diversifications as a result of environmental variations. However, Gagnaire et al. (2013) proposed that ecological divergence may actually involve multiple facets of species divergence on which divergent selections may operate, hence the need for an integrative approach to study the process of speciation. Some of the factors proposed to influence divergence include variations in quantitative phenotypic traits, regulation in gene expression and differential transmission of particular allelic combinations (Gagnaire et al. 2013).

The Nile tilapia (*Oreochromis niloticus*), the subject of our study, is a teleost fish of the family Cichlidae, has its native range in Africa and is widely known for its importance in aquaculture. This species has been recognized for its ability to tolerate a wide range of salinity, dissolved oxygen and temperature (Farmer and Beamish 1969; Avella et al. 1993), high fecundity and rapid growth rate (Welcomme 1967) and an omnivorous mode of feeding (El-Sayed 1999). Due to these

attributes, the species has the ability to adapt rapidly to new environments, showing a wide range of biological responses to different environmental conditions both in culture and in nature (Schofield et al. 2011; Grammer et al. 2012). As a result of this plasticity (or of the high degree of physiological tolerance found in the Nile Tilapia), the species has been able to successfully establish itself in extreme environmental conditions such as temperate winter conditions (Peterson et al. 2005; Grammer et al. 2012), hot springs (Trewavas 1983; Nyingi et al. 2009; Ndiwa et al. 2014) and saline waters (Schofield et al. 2011). Studies by Collyer et al. (2005) in pupfish (*Cyprinodon tularosa*) noted that the geographical spread of species across a broad range of ecological conditions is accompanied by equally diverse morphological variations, which are strongly correlated to the environmental conditions.

Within the Kenyan Rift Valley, Nile tilapia has colonized numerous newly formed habitats within a system characterised by a number of drainages comprising a series of rivers, springs and lakes. This complex system is a host to four out of the seven described sub-species of *Oreochromis niloticus* by Trewavas (1983): *O. n. baringoensis* from Lake Baringo; *O. n. sugutae* from River Suguta; *O. n. vulcani* from Lake Turkana. Recently Nyingi et al. (2009) discovered a natural population from Lake Bogoria Hotel genetically differentiated from but nevertheless close to the Lake Baringo population. The purpose of the current study is to investigate whether populations of Nile Tilapia have undergone significant morphological diversifications, and whether these differences can be related to environmental factors (either as the result of phenotypic plasticity or adaptive evolution).

According to Collyer et al. (2005, 2007), variations in salinity have the potential to affect fish body shape. These authors reported a general decrease in body circumference to overall body size at high salinity on pupfish (*Cyprinodon Tularosa*). Similarly, high water temperatures can influence both the growth rate and development rate of an organism (Atkinson 1995). In Zebra fish (*Danio rerio*) for instances, raising of juveniles under four different developmental temperatures resulted to highly variable phenotypes (Sfakianakis et al. 2011). A similar study by Georgakopoulou et al. (2007) on European seabass (*Dicentrarchus labrax*, L) showed that samples reared under lower temperatures (15 °C) were more slender than those reared under higher temperature (20 °C). Even though no studies have been

carried out to establish the effect of water pH on fish shape, extreme changes in water pH have been reported to cause fish deaths by altering other aspects of water chemistry, for instance increase in pH increases toxicity of ammonia (Schofield 1976; Wurts and Durborow 1992).

Taking into consideration the local genetic variations observed in three recently discovered populations of *O. niloticus* within the hot springs (Chelaba, Lake Bogoria Hotel and Turtle) of Loboï Swamp (Nyngi et al. 2009; Ndiwa et al. 2014), we hypothesized that these fish populations may also differ in shape due to the influence of high salinity and high temperature. In addition to the Loboï Swamp fish, another population of *O. niloticus* found in the northern part of the Kenyan Rift-Valley also experiences extreme environmental conditions. The Crocodile Lake, one of the three Lakes located on the Central Island of Lake Turkana is saline, with electrical conductivity as high as 10,590 $\mu\text{S}/\text{cm}$ and alkaline with a pH of around 10.0 (Avery 2010). Depending on the level of Lake Turkana, Crocodile Lake can either be submerged or emerged. During high water level the lake has been known to disappear (Hay 1976; Cerling 1979) while in low water levels, it remains isolated. The last time the two lakes were reported to have connected was in 1902 (Hopson 1982). So far only three species of fish have been reported to survive in Crocodile Lake namely: *Clarias gariepinus*, *Haplochromis* sp., and *O. niloticus*.

Both the Loboï and Crater Lake populations have adapted to extreme conditions, high temperature and high salinity levels respectively, while other closely related populations (Lakes Baringo, Turkana and Victoria) do not experience such conditions. These populations thus present an opportunity to observe how extreme conditions affect the morphology of the fish. To achieve this goal, we compared individuals from seven populations, four with extreme conditions (hot springs; Lake Bogoria Hotel Spring, Chelaba Spring, Turtle Spring and saline; Crocodile Lake populations), and three that experience more favourable environmental conditions (Lakes Baringo, Turkana and Victoria). Six of the studied Nile tilapia populations occur in their natural habitats except the Lake Victoria population, which was introduced into Lake Victoria from Lake Albert in Uganda (Fryer and Isles 1972; Trewavas 1983).

The study provides an insight into morphological responses of these fish populations to extreme

temperature and salinity. Occurrence of phenotypic differences between the populations studied will confirm that the environment or/and the genetic background play an important role in evolution of cichlids, while lack of phenotypic differences will imply that the high degree of tolerance in Nile Tilapia is related to its propensity to invade new environment, and in keeping a very canalized phenotype.

Materials and methods

Specimens from three hot spring populations of Loboï swamp drainage system (Chelaba, Lake Bogoria Hotel and Turtle) and four other lakes (Baringo, Turkana, Crocodile and Victoria) were studied (Table 1; Fig. 1). The three hot springs are characterised by elevated water temperatures (approximately 36 °C), low dissolved oxygen concentration (3.5–3.9 mg/l^{-1}) and slight acidity pH (6.4–6.9) (Ashley et al. 2004). The springs drain into Loboï swamp of about 1.5 km^2 , which is covered by dense vegetation of *Typha domingensis* and *Cyperus papyrus*. As the water exits the swamp, it is poorly aerated (Dissolved Oxygen (DO) = 0.2–1.1 mg/l^{-1}) and has a higher pH (7.4–8.3) (Ashley et al. 2004). Comparatively, Crocodile Lake is a crater formed island lake within the Lake Turkana. It is characterised by relatively higher water pH (10) and almost three times higher salinity (conductivity, 10,590 $\mu\text{S}/\text{cm}$) than Lake Turkana, which has pH and a conductivity of 9.3 and 3420 $\mu\text{S}/\text{cm}$ respectively (Avery 2010). Lake Baringo, a fresh water Lake located about 52 km from the Loboï swamp has DO concentration ranging from 5.89–6.7 mg/L , temperature range of 22.1–31.8 °C, pH ~ 8.5 and conductivity of about 580 $\mu\text{S}/\text{cm}$ (Omondi et al. 2014). Lake Victoria, also fresh water body is characterized by water temperatures ranging from 23.8 to 31.5 °C, pH 6.2–9.2, and conductivity of approximately 170 $\mu\text{S}/\text{cm}$ (Ochumba 1990; Crul 1995).

Fish specimens were collected from the hot springs (Chelaba, Lake Bogoria Hotel and Turtle) and Lakes Baringo, Victoria, Turkana and Crocodile using seine nets and scoop nets. Fish were anaesthetised and killed using an overdose of MS-222 before fixing in 4 % formalin solution for seven days. The formalin was washed and voucher specimens preserved in 70 % ethanol, and are curated as reference material at the National Museums of Kenya. The standard lengths

Table 1 Names of the seven populations of *O. niloticus* studied, their respective sample sizes (N), coordinates of study sites, habitat characteristics, surface water temperature range, conductivity (salinity) and size of the water body

Population	Number	Co-ordinates	Habitat	Temperature	Conductivity	Surface area
Bogoria Hotel Spring	41	0°21'44"N, 36°03'04"E	Hotspring	32–36 °C	<100 $\mu\text{S/cm}$	1.5 Km^2
Chelaba Spring	30	0°21'30"N, 36°02'58"E	Hotspring	32–36 °C	<100 $\mu\text{S/cm}$	1.5 Km^2
Turtle Spring	24	0°21'44"N, 36°02'41"E	Hotspring	32–36 °C	<100 $\mu\text{S/cm}$	1.5 Km^2
Crocodile Lake	28	3°29'34"N, 36°01'54"E	Saline	25–30 °C	10,590 $\mu\text{S/cm}$	<1 Km^2
Lake Turkana	38	3°30'46"N, 35°54'53"E	Saline	26–27 °C	3420 $\mu\text{S/cm}$	6405 Km^2
Lake Baringo	43	0°36'43"N, 36°01'31"E	Normal	22–32 °C	580 $\mu\text{S/cm}$	130 Km^2
Lake Victoria	33	0°38'17"N, 33°58'35"E	Normal	23–32 °C	170 $\mu\text{S/cm}$	68,800 Km^2

(distance from the tip of the snout to the base of caudal fin) of the fish were measured using a digital Vernier calliper in order to estimate their sizes.

Body shape of the samples was quantified using landmark based geometric morphometric method (Rohlf and Marcus 1993). Images of preserved samples

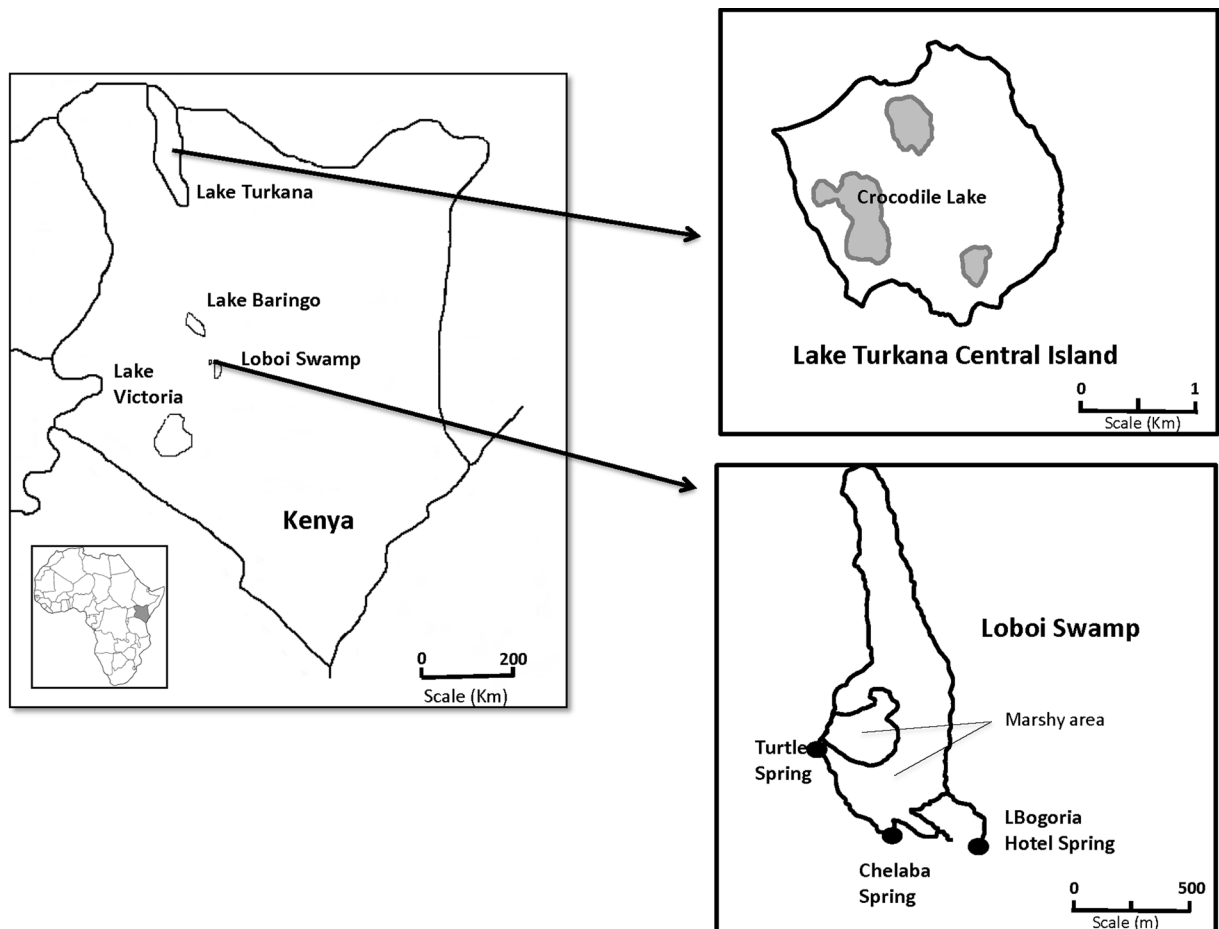


Fig. 1 Map showing the location of all the six natural populations and one introduced population of *O. niloticus*: Lake Turkana at Lake Turkana drainage system, Crocodile Lake in the Central Island of Lake Turkana, Lake Baringo at Lake Baringo drainage

system, Turtle Spring, Chelaba Spring and Bogoria Hotel Spring in the Lobi Swamp, and Lake Victoria (introduced) at *L. Victoria* drainage system

of fish were obtained by using a flatbed scanner (Herler et al. 2007) and imported into the program tpsUtil version 1.53 (Rohlf 2012). A total of 14 landmarks (Fig. 2) were chosen based on their capacity to capture overall body shape, and their coordinates (x,y) digitized on the left hand side of each sample using the program tpsDig version 2.16 (Rohlf 2010). The coordinates were saved in a tps format data file and used for further morphometric analysis. A total of 237 adult fish specimens from seven wild population of Nile tilapia were analysed. Maturity of the specimens was ascertained by observing their gonads. Their standard length sizes ranged from 40 to 207 mm.

Data analysis

The data file was imported into R software program and analysed using Momocs (Bonhomme et al. 2014), Geomorph (Adams and Otarola-Castillo 2013; Dryden 2013) and Ape packages (Padis et al. 2004; Padis 2012). Coordinates were scaled to unit centroid size, translated, rotated and optimally superimposed through the generalized partial Procrustes Analysis. Procrustes coordinates were projected into Euclidian tangent shape space for further analysis (Dryden and Mardia 1998; Claude 2008). In order to establish morphological variations between the different populations of fish, we used principal component analysis (PCA) of the superimposed coordinates of the landmarks. Variations were visualised by plotting multivariate ordination of specimens on the

first two principal component (PC) axes (PC1 and PC2). Contribution of original variables on corresponding eigenveectors or PC loadings were analysed to establish shape features that had the greatest influence on each PC.

Nested MANCOVA models were conducted on the principal components (PC) of shape data in order to assess the influence of locality, and environmental factors. Habitat and locality were considered as explanatory variables and centroid size as a covariate in the model. Size was added as a covariate to understand the effect of habitat and locality on allometric growth patterns.

$$Response \sim Size + Habitat + Habitat:Size + Habitat:locality + Habitat:Locality:Size$$

To represent and appraise morphological differences between the localities, a linear discriminant analysis was performed on the dataset of the Procrustes coordinates.

The Fst values for 5 populations of Nile tilapia (Chelaba, Bogoria, Turtle, Turkana, Baringo and Victoria) used in the current study were obtained from our previous studies (Ndiwa et al. 2014). Alternatively, the Fst values for Crocodile Lake and Lake Victoria populations were obtained from unpublished data. All our Fst values were calculated from microsatellite molecular data using Genepop software (Rousset 2008). A mantel test analysis (Mantel 1967) was performed through permutation to correlate the Fst Values and

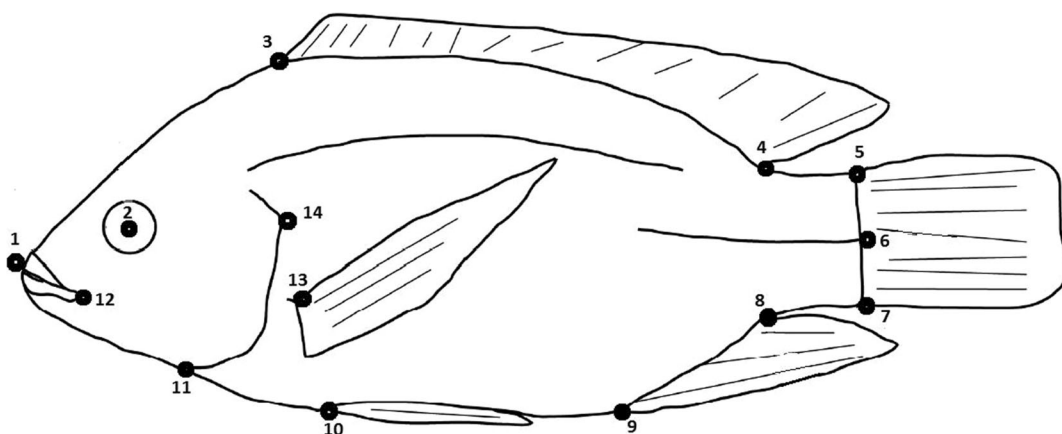


Fig. 2 Position of 14 different landmarks used in the study: (1) anterior tip of the snout with mouth closed, (2) centre of the eye, (3 & 4) anterior and posterior insertions of the dorsal fin, (5) dorsal origin of caudal fin, (6) point halfway between dorsal and ventral origin of caudal fin, (7) ventral origin of caudal fin, (8) posterior

insertion of anal fin, (9) anterior origin of anal fin, (10) anterior origin of pelvic fin, (11) juncture of the ventral edge of operculum with the ventral outline, (12) most posterior end of the mouth, (13) anterior origin of pectoral fin and (14) most posterior end of operculum

Mahalanobis distance. This analysis aimed at confirming the influence of genetic factors on the morphology. Lastly, minimum spanning network based on Mahalanobis distance was constructed on R based on Ape package (Padis et al. 2004; Padis 2012) in order to visualize phenotypic relationship between the seven wild populations of Nile tilapia.

Results

Comparison of body sizes of the fish samples from the seven populations of Nile tilapia (Fig. 3) indicated minimal variations between specimens from Lake Baringo, Chelaba Spring, Crocodile Lake and Turtle Spring. However, greater body size differences were observed between the latter populations and populations from Bogoria Spring, Lake Turkana and Lake Victoria. Body size comparison among individuals of same population showed least variations within Crocodile Lake (Fig. 3).

The first shape PC accounted for 23 % of the observed variations. This PC was mostly related with the variation of the head, caudal peduncle and anal fin (Fig. 4). The PC2 on the other hand accounted for 14.5 % of variation mostly within the depth of the body and caudal peduncle (Fig. 4a).

Even though there were overlaps between most of the populations under study, significant morphological differences were observed between all populations. A strong separation was observed between the hot spring populations and both Crocodile Lake, and Lake Turkana populations based on PC1. However, PC2 showed greater differentiation between Lakes Turkana and Crocodile populations than the latter (Fig. 4a).

Surprisingly, we observed that populations of Lakes Turkana and Victoria were similar morphologically. These results were unexpected since the two systems have different ecological characteristics.

Differences between populations and habitats

Both differences in habitats and localities significantly explained the shape variations between the seven populations of Nile tilapia studied (Table 2). Linear discriminant analysis based on the sampling locations correctly assigned 84.8 % of the individuals to their respective localities. All samples from Lake Victoria were assigned to their real group (Table 3). However, most of individuals from the hot springs populations that were not correctly assigned to their respective localities were either assigned to another hot spring population(s), or to L. Baringo (Table 3). Assignment of individuals from other localities is shown on Table 3.

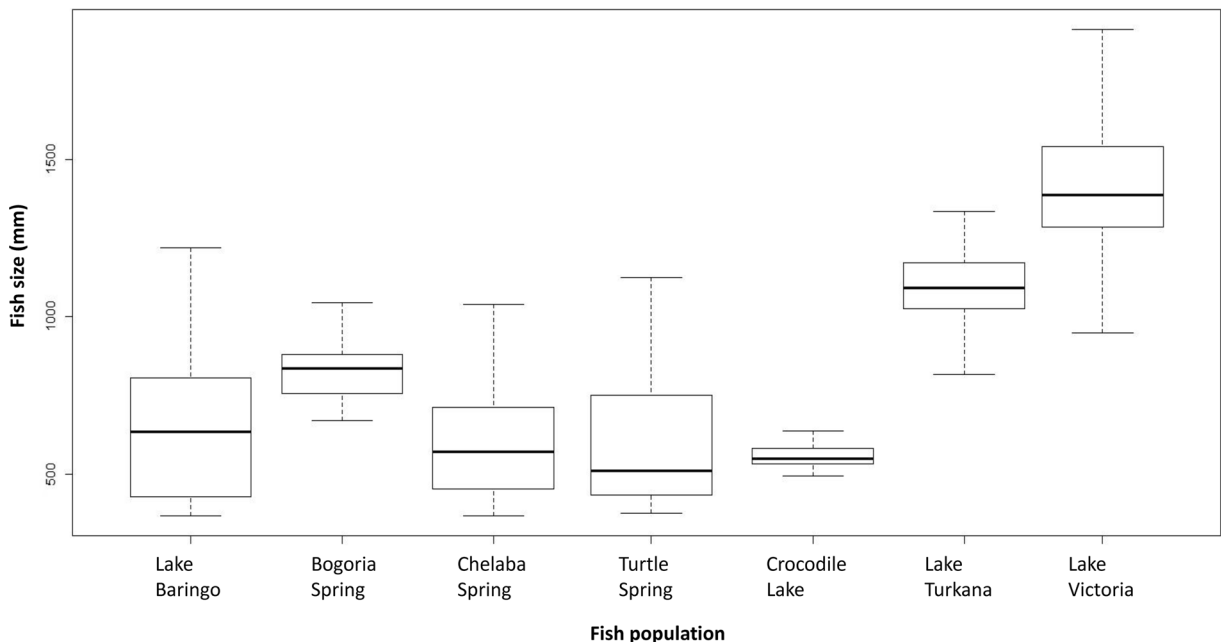


Fig. 3 Size distribution of the fish samples collected from the seven localities

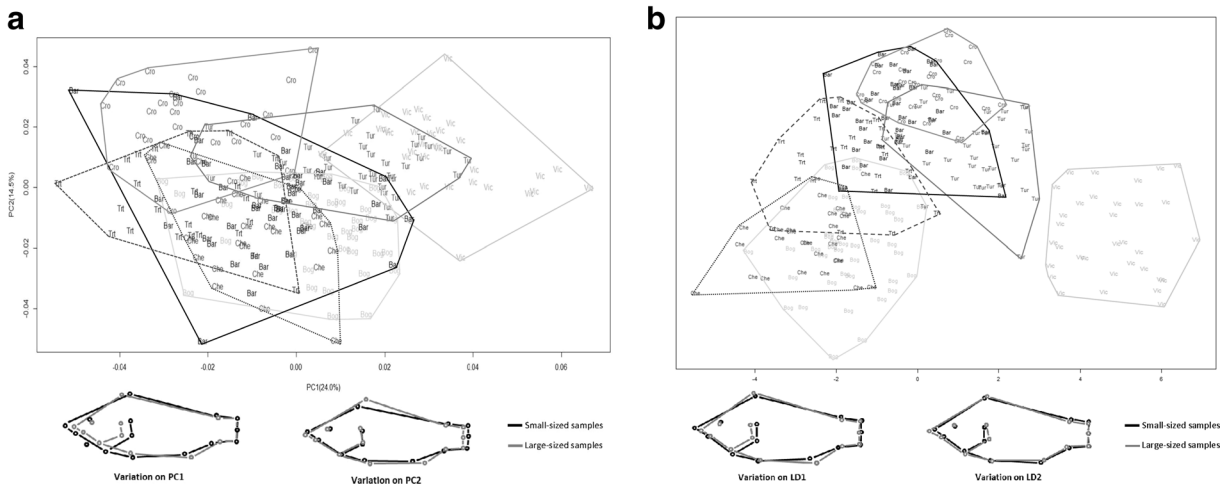


Fig. 4 **a** Differentiation of the seven populations of Nile tilapia based on Principal Component Analysis (PCA). On fish shape images (PC 1, 2), Grey = mature (large samples); Black = small (young) samples. In the scatterplot, cro = crocodile; Tur = Turkana; Che = Chelaba; Bog = Bogoria; Trt = Turtle; Bar = Baringo; Vic = Victoria **b** Differentiation of the seven

populations of Nile tilapia using Linear Discriminant Analysis (LDA). On fish shape images (LDA 1, 2), Grey = mature (large samples); Black = small (young) samples. In the scatterplot, cro = crocodile; Tur = Turkana; Che = Chelaba; Bog = Bogoria; Trt = Turtle; Bar = Baringo; Vic = Victoria

Linear discriminant analysis (LDA) clustered the seven populations of *O. niloticus* into two distinct groups. The first cluster consisted entirely of individuals from Lake Victoria, while the second cluster comprised the other six populations (Fig. 4b).

However, partial separations were observed among the hot spring populations and also among Lakes Turkana and Crocodile populations (Fig. 4b). Shape variations related with both LDA1 and LDA2 were attributed to differences within the head and the caudal region.

Because we observed a significant interaction of locality with size, we compared small sized individuals to large sized individuals from each locality in order to establish their differences and similarity in growth. Generally, we observed a decrease in head size and length of the mouth with increase in size in all the six

populations except Crocodile Lake, where no marked differences were observed between the heads of the small and large sized samples. We also observed differences in body depth between the small and large sized samples (Fig. 5).

The closely related hot spring populations displayed different patterns of growth irrespective of their close spatial relationship. In Bogoria spring, small sized individuals were characterized by more elongated mouths compared to the other two spring populations. On the other hand, the heads of small sized individuals from Chelaba Spring showed slight elongation with greater dorsal displacement of their mouths. We also observed posterior displacement of the anal fin as individuals increase in size for Bogoria and Chelaba populations. Finally, large sized samples from Chelaba and Turtle Springs had elongated caudal peduncle length compared

Table 2 Results of multi-variate analysis of covariance (MANCOVA) performed on body shape considering all the samples

Factor	df	Pillai	approx. F	num. df	den. df	P-value
Size	1	0.8282	40.167	24	200	<0.0001
Habitat	2	1.2667	14.464	48	402	<0.0001
Habitat:Size	2	0.3991	2.088	48	402	<0.0001
Habitat:Locality	4	2.0469	8.864	96	812	<0.0001
Habitat:Locality:Size	4	0.5402	1.321	96	812	0.0268

Table 3 Linear discriminant analysis (LDA) assignment of individuals to different localities and groupings according to habitat types, percentages of assignment are in brackets

Origin of the fish	False assignment	Habitat grouping
Lake Bogoria Hotel Spring (82.5 %)	Chelaba Spring (10 %) Lake Baringo (5 %) Turtle Spring (2.5 %)	Hotspring - Hotspring Hotspring - Normal Hotspring - Hotspring
Chelaba Spring (78.13 %)	Bogoria Spring (12.5 %) Turtle Spring (9.37 %)	Hotspring - Hotspring Hotspring - Hotspring
Turtle Spring (76.19 %)	Chelaba Spring (4.76 %) Crocodile Lake (4.76 %) Lake Baringo (14.29 %)	Hotspring - Hotspring Hotspring - Saline Hotspring - Normal
Lake Baringo (91.43 %)	Bogoria Spring (8.57 %)	Normal - Hotspring
Lake Turkana (94.59 %)	Crocodile Lake (5.41 %)	Saline - Saline
Crocodile Lake (83.87 %)	Turtle Spring (3.23 %) Lake Turkana (3.23 %) Lake Baringo (9.67 %)	Saline - Hotspring Saline -Saline Saline - Normal
Lake Victoria (97.06 %)	Lake Baringo (2.94 %)	Normal - Normal

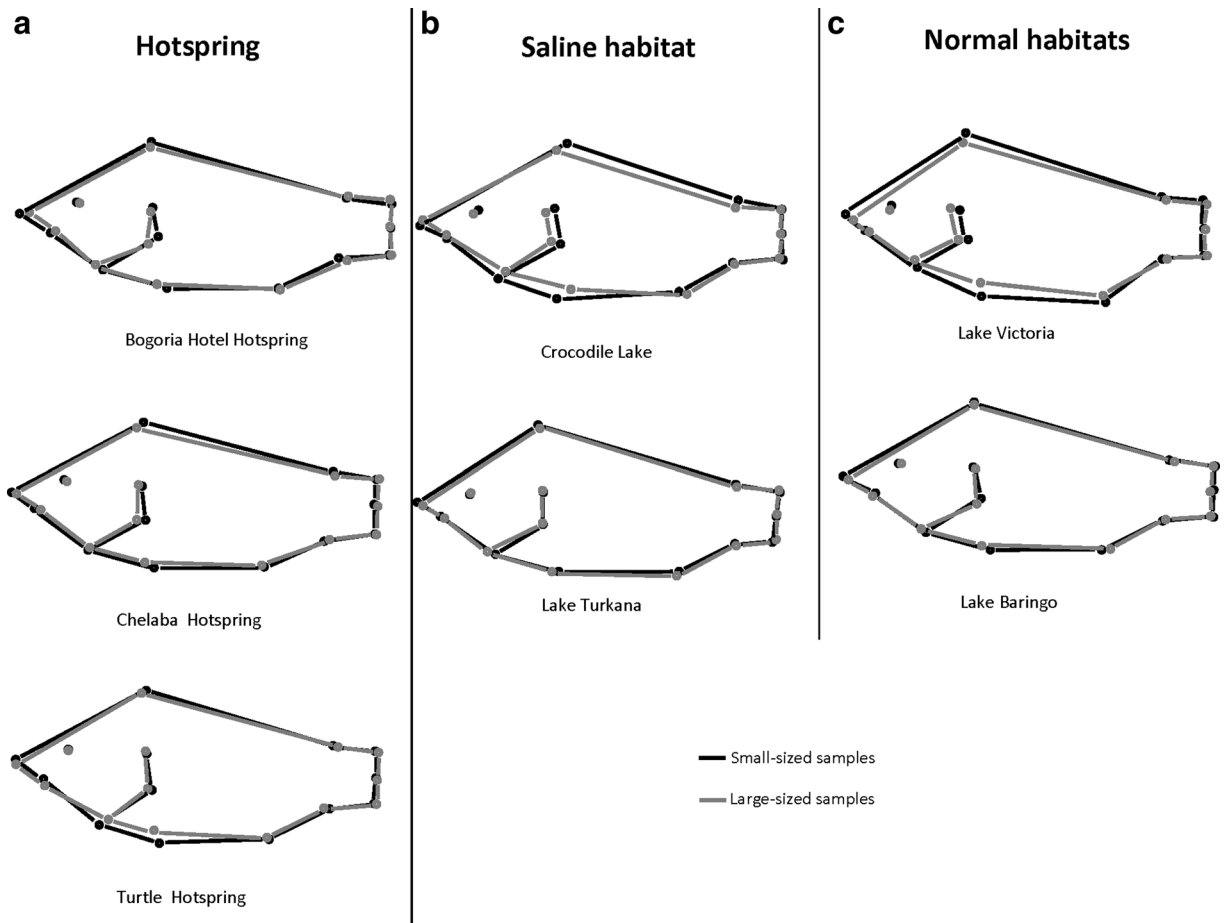


Fig. 5 Growth patterns in the seven populations of *Oreochromis niloticus*. Grey colour represents the shape of the large samples while the black colour represents the shape of the small sized individuals

to small sized individuals, while no differences were observed for Lake Bogoria Hotel Spring population (Fig. 5).

Growth patterns of the Lake Turkana system (Turkana and Crocodile) populations differed significantly among themselves, and among other *O. niloticus* populations. For instance, great change in shape was observed between the small sized samples and the large sized samples involving drastic decrease of the depth of the body and caudal peduncle. Dorsal displacement of the mouth and anterior displacement of the eye, dorsal fin and operculum were also noted. Smaller differences were observed between small and large samples in Lake Turkana; anterior elongation of the mouth for small sized individual, anterior displacement of the lower jaw and pelvic fin of mature individuals, and slight elongation of the caudal peduncle (Fig. 5).

Within Lakes Victoria and Baringo populations, marked differences were observed between small and large sized samples. Comparison between their growth patterns showed that Lake Victoria samples had deeper caudal peduncles that increased in length with maturity of the fish, while Lake Baringo samples had slender caudal peduncles, which also increased in length with fish growth (Fig. 5).

Additionally, correlation of F_{st} values and Mahalanobis distance based on Mantel test analysis showed existence of significant relationship ($P < 0.05$) (Fig. 6).

Minimum spanning network computed from Mahalanobis distances between localities showed close

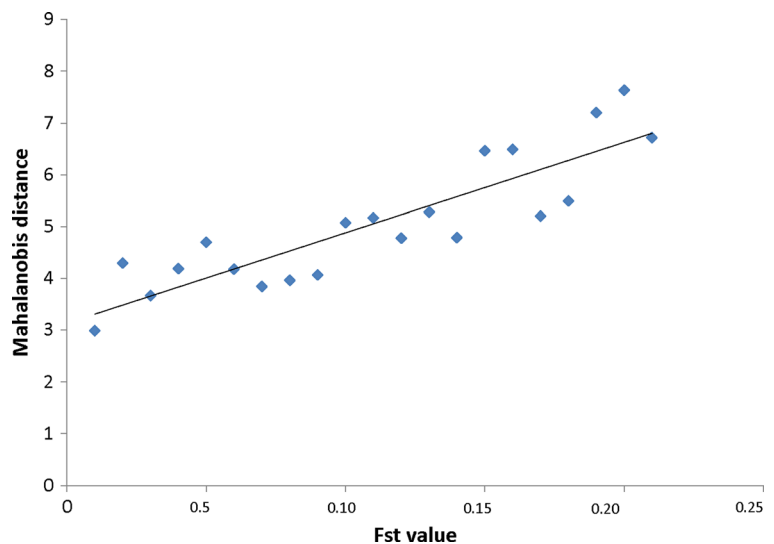
proximity between the three hot spring populations. Additionally, Lake Turkana population showed close relationship to Lake Victoria population, while Crocodile Lake and Lake Turkana populations were separated (Fig. 7).

Discussion

Morphological analysis revealed that specimens studied were first differentiated by their sizes. Fishes from Lakes Turkana and Victoria were the largest with a mean standard length sizes of 122.8 ± 4.38 mm (mean \pm standard error) and 163.3 ± 4.40 mm, respectively. The smallest specimens were from Turtle Spring and Crocodile Lake, and exhibited mean standard length sizes of 76.6 ± 6.11 mm and 70.1 ± 1.72 mm, respectively. Differences were also observed in terms of variability of sizes; some samples had higher size range (Lake Victoria) than others (Crocodile Lake). These observed differences may partly represent artefacts due to sampling, and may not completely reflect the actual differences of fish sizes in different localities despite the knowledge that Lakes Turkana and Victoria host large specimens of *O. niloticus* (Trewavas 1983) due to their larger sizes and greater depths, compared to biotopes investigated in the present study.

The PC analysis revealed a large amount of shape differences whereby variations of the head, caudal peduncle and anal fin accounted for 23 % (Fig. 4a). These variations allowed discrimination of the Lake Victoria

Fig. 6 Correlation of F_{st} values against Mahalanobis distance showing positive correlation



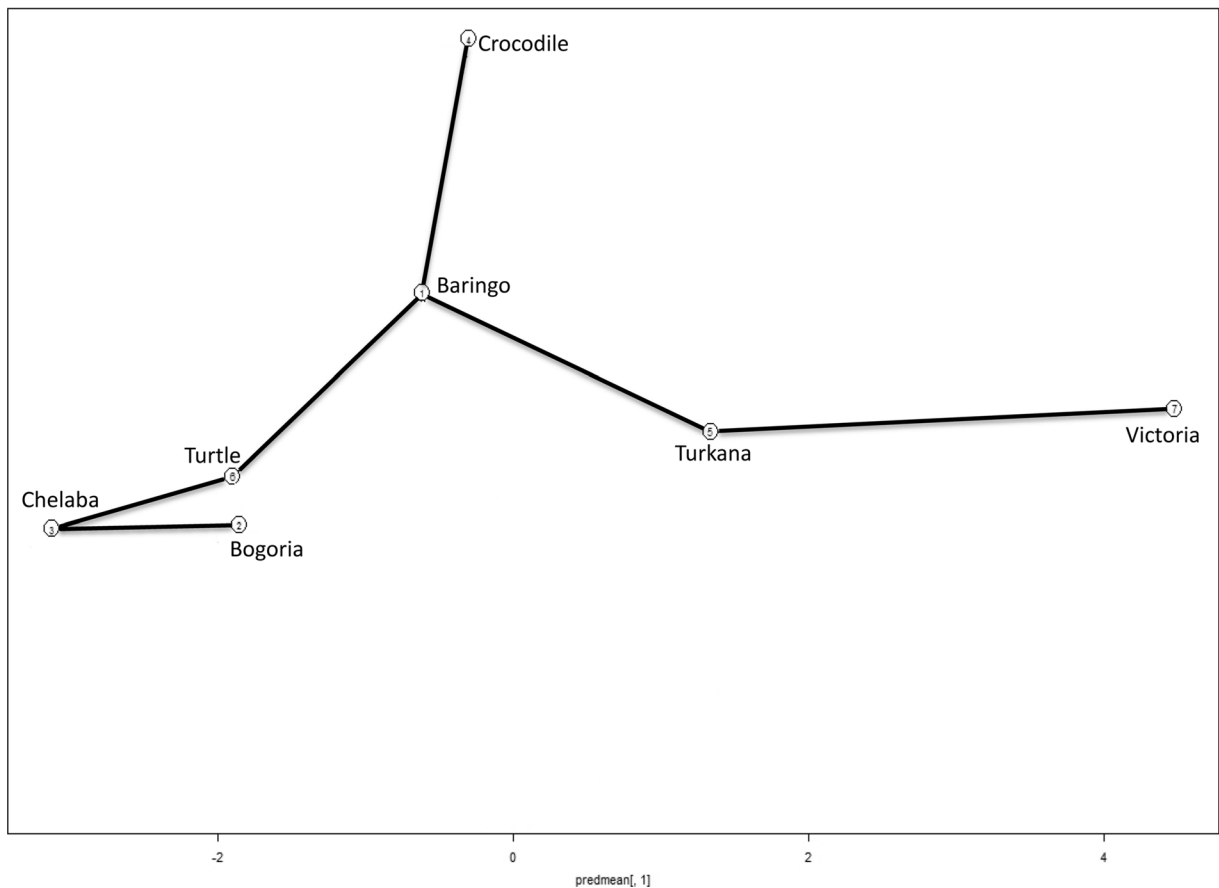


Fig. 7 Minimum spanning network showing overall similarity in body shape among the *O. niloticus* populations

specimens from those of other Lakes (Baringo, Turkana and the hot springs). Within these latter populations, fishes from Lake Turkana were placed between the two groups of fishes.

Differentiation of the Lake Victoria population from all other population based on linear discriminant analysis was even clearer (Fig. 4b). There was also a clear separation between the hot springs specimens on one hand, and Lake Turkana and Crocodile Lake specimens on the other hand. Specimens from Lake Baringo appeared to fall between these two groups of specimens. All observed variations on both LDA1 and LDA2 were related to differences within the head and the caudal region. These results are congruent with the genetic variation observed by Agnèse et al. (1997), who demonstrated that fishes from Lake Baringo and Lake Turkana are genetically close. Later on, Nyingi et al. (2009) and Ndiwa et al. (2014) observed that fishes from the hot springs are even more closely related to fishes from Lake Baringo. If one assumes that fishes from

Crocodile Lake should have a genetic background identical to the fishes of Lake Turkana, and that fishes from Lake Victoria introduced from Lake Albert (Fryer and Isles 1972; Trewavas 1983) should be less genetically related to the other fish studied, then morphological differentiation seems to be congruent with genetic differentiation. Indeed, our studies based on mantel test confirmed the role of genetic factors in influencing morphology. In addition, links between morphological and genetical differentiation have been reported by Stewart and Albertson (2010), who integrated both genetic and geometric morphometric approaches in studying scale eating cichlids of Lake Tanganyika, which had been reported to show frequency balanced polymorphism in the left-handedness and right-handedness of the mouth, an adaptation previously reported by Takahashi et al. (2007). Their morphometric results strongly supported the genetic basis of jaw laterality.

In our study, we also observed different allometric patterns of growth in all the seven populations of Nile

tilapia. Considering the fact that most of the localities differ greatly in their environmental conditions, the observed allometries may be partly attributed to environmental differences, which might have acted on developmental stages, and shaped the differences observed between *O. niloticus* populations. Whether differences are adaptive or the by-product of phenotypic plasticity is still speculative at that stage. However, common garden experiments are necessary in order to establish the role of various environmental factors on fish morphology.

Shape changes found in our study could be functional and environmentally related. Indeed, our observations are congruent to findings by Ramler et al. (2014) who investigated the effect of different temperatures on body size and shape on stickleback (*Gasterosteus aculeatus*). The authors reported significant differences between individuals across different temperature groups. Similarly, study on zebrafish (*Danio rerio*) juveniles reported occurrence of highly variable phenotypes induced by diverse thermal conditions during the early ontogenic stages (Sfakianakis et al. 2011). However, results obtained by Fujimura and Okada (2008) comparing (during their developmental stages) the lower jaw bones of a cichlid fish (*Haplochromis chilotes*) from Lake Victoria and another Riverine cichlid (*O. niloticus*) contradicted our findings. Irrespective of the habitat differences, the authors noted that most shape changes had a similar pattern during the growth of the two species showing that the development of these fish were relatively robust.

When looking at closely genetically related populations, one can observe a certain amount of morphological differentiation. The first case concerned specimens from the three hot springs. Specimens from Bogoria and Chelaba or Bogoria and Turtle springs were unevenly distributed along the first and second axis of the LDA analysis respectively (Fig. 4b). Differences between Chelaba and Bogoria Springs were mainly due to variations in body length and body depth, while differences between Bogoria/Chelaba and Turtle were as a result of variations in head length and body depth.

If we consider that the genetic background of these three populations is the same (Ndiwa et al. 2014), then these observed morphological differentiations could be attributed to phenotypic plasticity of the species. Very likely, environmental characteristics of these hot springs are slightly different. Bogoria Spring is the biggest of the three hot springs and runs a few hundred meters before

entering the swamp. The two other springs have a smaller flow rate and enter in the swamp after only few meters. Nevertheless, as only a few genes can strongly act on morphological differentiation, one cannot exclude that morphological differences observed between the three hot spring populations was due to genetic differences. Ndiwa et al. (2014) observed that gene flow between these populations is limited by the swamp, which is covered by a dense vegetation of *Typha domingensis* and *Cyperus papyrus*. Decomposition of organic matter, and shading provided by these vegetation is responsible for low oxygen concentration and low temperatures, which act as barriers to free movement of the fish from one place to another. Similarly, Ndiwa et al. (2014) also observed that these fish are slightly introgressed by *O. leucostictus* genes from escapees of this species originating from aquacultural ponds within the drainage. The account of possible introgression of alien genes acting on morphological differentiation cannot be excluded.

The second case of genetically related populations in our study concerns fish from Lakes Turkana and Crocodile. There was a clear morphological differentiation between these two groups of fish as shown on Fig. 4b. Specimens from Crocodile Lake were generally smaller than those from Lake Turkana, and differed in body depth, head size and caudal length.

These two populations are genetically very close (unpublished results) since Crocodile Lake population originated from Lake Turkana about one century ago. Most likely, the observed differences can be as a result of phenotypic plasticity. These lakes offer two quite different environments to the fish. Lake Turkana is a large alkaline (pH 9.3) and slightly saline lake (conductivity 3420 $\mu\text{S}/\text{cm}$) hosting numerous fish species (48 following Hopson 1982). Comparatively, Crocodile Lake is a small size lake (about 0.32 km^2), with high alkalinity (pH 10) and salinity (conductivity 10,590 $\mu\text{S}/\text{cm}$). Only three species of fish are present, two Cichlids (*Aplochromis* sp., *O. niloticus*) and one Clariid (*Clarias gariepinus*).

In conclusion, our study observed that populations of Nile tilapia inhabiting different localities displayed differentiated body shapes. The study also noted correlation between genetic distance and morphological distance. Despite these findings, further laboratory studies are necessary in order to confirm the importance of genetic and environmental factors in influencing morphological development. This can be achieved by breeding genetically identical siblings in different environmental conditions:

first under low versus high salinity, and secondly under low versus high temperature.

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