Contemporary phenotypic change in correlated characters in the African cyprinid, *Rastrineobola argentea*

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Phenotypes reflect a complex interplay between the direct and indirect effects of multiple selective pressures; adaptive responses in individual traits may therefore be constrained by architectonic considerations and correlations with other traits. We explored whether and how three suites of neighbouring structures (respiratory, trophic and cranial) have changed over time in *Rastrineobola argentea*, a small pelagic cyprinid fish that is endemic to the Lake Victoria Basin in East Africa. We compared museum specimens from 1966 to modern (2010) specimens, spanning a period of almost 50 years of strong anthropogenic change in the lake, including increased frequency and extent of hypoxia and anoxia, and a dramatic restructuring of the lacustrine fish community and food-web. We found that modern *R. argentea* had significantly larger gills, wider heads, and shorter and more tightly packed gill rakers than their historical counterparts. Larger gill size probably represents a direct, adaptive response to more frequent bouts of hypoxia over time; the increase in head width and decrease in raker spacing may reflect correlated (and potentially maladaptive) responses to the increase in gill size. Integrative studies such as this are key to unravelling complex interactions between phenotypes and the environment.


**INTRODUCTION**

In nature, observed phenotypes often reflect a complex interplay between the direct and indirect effects of multiple selective pressures (Dewitt & Langerhans, 2003; Langerhans, Chapman & DeWitt, 2007; Hendry et al., 2011). First, multiple environmental factors may act simultaneously on a single trait, with either synergistic or antagonistic effects. This dynamic may be particularly important in anthropogenic contexts, because humans typically modify multiple aspects of ecosystems simultaneously. Second, as an added layer of complexity, selection on any one trait may result in indirect effects on correlated traits (Lande & Arnold, 1983; Barel et al., 1989; Ghalmambor, Walker & Reznick, 2003). Such correlated effects may be due to trait linkages either at the genetic (e.g. pleiotropy) or phenotypic level (e.g. spatial constraints).

Teleost fishes are an excellent model for the study of such ecomorphological trade-offs because of the unique evolutionary challenges posed by the aquatic environment. In particular, the need to move through a viscous medium has selected for streamlined, laterally compressed body forms in most fishes, within which all organs must be accommodated (Barel, 1983). Spatial constraints are thought to be particularly important in the head region, which tends to be very compact, but houses structures involved in both feeding and respiration (Smits, Witte & Van Veen, 1996; Chapman, Albert & Galis, 2008). The efficiency of respiration increases directly with gill surface area (Hughes & Morgan, 1973), and so, all else being equal, selection should favour larger gills. However, an increase in gill size must occur either at the expense of neighbouring trophic structures, potentially compromising feeding performance, or via an increase in overall head size, which could result in reduced streamlining and poorer swim performance. Thus, we should expect strong ecomorphological trade-offs between these structures.
The decline of the haplochromine cichlids (Mwebaza-Ndawula, 1998; Wanink, 1998; Wanink & Witte, 2000; Wanink et al., 2001). Concurrently, it broadened its trophic niche by incorporating benthic macroinvertebrate prey into its historically zooplanktivorous diet (Wanink, 1998; Sharpe & Chapman, 2014; Supporting Information, Table S1). Wanink & Witte (2000) proposed that these habitat and dietary shifts might have selected for rapid morphological changes in R. argentea. To test this hypothesis, they compared specimens from the Mwanza Gulf of Lake Victoria in 1983 and 1988 and found preliminary evidence for both an increase in gill filament number and a reduction in gill raker number over this 5-year period (Wanink & Witte, 2000). These intriguing trends warrant further analyses that examine multiple aspects of trophic and respiratory morphology across larger time scales. Furthermore, as selection on trophic and respiratory structures may be influenced by spatial constraints mediated through head size, ideally analyses would examine concurrent changes in all three structures.

The goal of our study was to explore whether and how these three groups of neighbouring structures (respiratory, trophic and cranial) have changed in R. argentea over almost 50 years of strong anthropogenic ecological change in Lake Victoria (1966–2010). We predicted first that increasing eutrophication over time, coupled with increased use of the hypoxic benthic zone, should favour an increase in total gill surface area (e.g. Chapman et al., 2000; Fig. 1). Second, we expected that the shift to a diet composed of larger benthic prey (Sharpe & Chapman, 2014; Supporting Information, Table S1) should favour a morphological shift to fewer, shorter, wider and/or more widely spaced gill rakers (e.g. Crowder, 1984; Fig. 1). Third, we expected to see evidence of a trade-off between these two suites of traits (e.g. Schaack and Chapman 2003, Binning et al., 2010). In other words, simultaneous changes in both sets of traits could only occur alongside an increase in head size. Conversely, if head size remained constant through time, then an increase in one set of traits (e.g. gills) would inhibit or limit any changes in the other set of traits (e.g. gill rakers).

MATERIAL AND METHODS

STUDY DESIGN

To test for changes in respiratory, trophic and cranial structures of R. argentea through time, we compared historical specimens from 1966 (N = 43) to contemporary specimens from 2010 (N = 49). Historical specimens were obtained, with permission, from collections at the Biodiversity Museum of the National Fisheries Resources Research Institute (NaFIRRI) in Jinja, Uganda.
Uganda. Contemporary specimens were collected using a 5-mm surface seine during the dry season (May–June) in 2010, and immediately killed with clove oil. Both historical and contemporary specimens were preserved in 10% formalin, and all specimens were collected from the same location: the Napoleon Gulf, northern Lake Victoria. Like other parts of the lake, the Napoleon Gulf now becomes seasonally stratified (Mugidde & Hecky, 2004), during which time hypoxic conditions can develop in the hypolimnion (Mwebaza-Ndawula, 1998; Supporting Information, Fig. S1). *R. argentea* make use of the entire water column in the Napoleon Gulf, including the benthic zone, only shifting their vertical distribution upwards to just above the oxycline during periods of strong stratification (Mwebaza-Ndawula, 1998), as has been observed elsewhere across the lake (Kaufman & Ochumba, 1993; Wanink et al., 2001).

We aimed to compare adult specimens across time periods, but because size at maturity has declined significantly over this same period (Sharpe, Wandera & Chapman, 2012), this meant that historical specimens were larger on average than contemporary specimens (mean length: 52.3 vs. 38.5 mm standard length). However, we ensured there was sufficient size overlap to perform a common size-standardization (Supporting Information, Figs S2–S4).
each specimen in the lateral, ventral and dorsal planes. To quantify gill morphology, we dissected out the first gill arch from the left side of each fish, placed it on a microscope slide and photographed it on both sides using a Lumenera Scientific Infinity camera attached to a dissecting microscope. We used Motic Images Plus (v.1.3, 2000) to digitally measure five gill traits on the outer surface of the first hemibranch: total gill filament length (TFL), average gill filament length (AFL), total number of gill filaments (GN), total hemibranch area (THA) and total hemibranch perimeter (THP), following Chapman et al. (2008) and Crispo & Chapman (2010) (Table 1; Supporting Information, Fig. S5). Previous work has shown that these metrics, and in particular TFL, are a good proxy for total gill surface area (Chapman et al., 2000, 2008; Timmerman & Chapman, 2004).

To quantify the precision of our gill measurements from the first hemibranch (above), we randomly selected a subset of 20 specimens for which we repeated all gill measurements on both sides of all four hemibranchs. We then ran Pearson correlation tests comparing the value of each trait as measured from the first hemibranch only to the same trait as measured from all four hemibranchs. There was very high degree of agreement between the two sets of measurements: TFL $(r = 0.98, P < 0.01)$, AFL $(r = 0.97, P < 0.01)$, GN $(r = 0.87, P < 0.01)$, THA $(r = 0.98, P < 0.01)$ and THP $(r = 0.98, P < 0.01)$.

Using the same gill arch, we then measured four gill raker traits: total number of gill rakers (RN), mean gill raker length (RL), mean gill raker width (RW) and mean inter-raker space (IRS). The last three measurements were repeated on the 2nd, 3rd and 4th rakers of the lower arch, and the mean of the three measurements was used in the analysis (Table 1; Supporting Information, Fig. S6). Finally, we used the external photographs to digitally measure nine different head traits: head length (HL), head depth in two places (HD1, HD2), interorbital width (IO), dorsal and ventral head width (DHW, VHW), dorsal and ventral post-head width (DPHW, VPHW), and operculum area (OPA) (Table 1; Supporting Information, Fig. S7). HD1 is the most traditional way to measure head depth, but includes soft tissue and so can vary depending on stomach fullness, and fixation deformation. HD2 on the other hand is more closely related to gill size as it includes mainly the operculum and neurocranium. We included both measurements because one was more closely related to our functional hypotheses (HD2), but the other (HD1) would permit comparisons with other researchers.

**Statistical analysis**

To test for morphological differences through time, we performed separate multivariate analyses of covariance (MANCOVAs) on the three suites of traits (gills, rakers, heads). In each model, we included year as a fixed factor, and body size (standard length for heads and rakers, and mass for gills) as a covariate, as well as the interaction between them. MANCOVAs were followed by separate ANCOVAs on each trait to examine specific differences in more detail. All continuous variables were log$_{10}$-transformed to satisfy the assumptions of normality and linearity (Supporting Information, Table S2); however, even after transformation, the two traits that involved discrete counts, gill number (GN) and gill raker number (RN), were not normally distributed. Therefore, these traits were excluded from the above MANCOVAs, and differences across years were instead investigated using non-parametric Wilcoxon tests.

To evaluate the extent to which populations from the two years could be discriminated based on their morphology, we performed linear discriminant analysis (LDA) on the three suites of size-standardized traits. Traits were standardized to a common body size, using the following allometric equation (Hendry & Taylor, 2004):

$$Z_{\text{std}} = Z_s \left( \frac{\text{mean body size}}{\text{observed body size}} \right)^b$$

where $Z_{\text{std}}$ is the size-standardized trait value, $Z_s$ is the observed trait value, SL is the observed standard length, and $b$ is the common slope from a regression of log($Z_s$)~log(SL) + year for each trait. Before fitting a common slope, we tested for heterogeneity of slopes across years, which was not significant for 17 of the 18 traits examined. There was a significant interaction between year and standard length for one trait (HD2), so size-adjusted values for this one trait should be interpreted more cautiously. Body mass was used as the size covariate for gills, and standard length for the head and gill raker traits. Gill raker number did not vary with standard length ($F_{1,43} = 0.013, P = 0.908$), and so was left untransformed. All size-standardized traits were no longer correlated with standard length or mass ($P > 0.05$). Classification success in the LDA was evaluated using leave-out-out cross-validation implemented using the lda() function in R.

Finally, to test for co-variation between suites of traits, we performed separate principal components analyses (PCAs) on the three suites of size-standardized traits. For each suite of traits, we retained the principal components that explained at least 85% of the total variance (PCs 1 and 2 for heads and gills, and PCs 1–3 for gill rakers; Supporting Information, Table S3). We then performed pair-wise Pearson correlation tests on these PCs.
RESULTS

MORPHOLOGICAL CHANGE THROUGH TIME

From 1966 and 2010, *R. argentea* differed in terms of gill traits (MANCOVA: $F_{4,82} = 65.61$, $P < 0.001$), gill raker traits (MANCOVA: $F_{3,43} = 39.20$, $P < 0.001$) and head traits (MANCOVA: $F_{8,29} = 99.56$, $P < 0.001$).

*Rastrineobola argentea* exhibited an overall increase in gill size over time (Fig. 2A, B). Specifically, after adjusting for body mass, we observed significant increases in TFL (6.6%), AFL (1.2%), GN (1.5%), THA (9.2%) and THP (2.8%; Table 2). Gill raker morphology also differed substantially between time periods (Figs 2C, D, 3). Specifically, we observed decreases in RL (9.0%) and IRS (13.2%), but increases in RW (3.2%; Table 2). The number of gill rakers did not change over time. Finally, modern *R. argentea* had larger heads overall (Fig. 2E, F). After controlling for differences in body size, contemporary *R. argentea* had heads that were substantially longer, deeper and wider than historical specimens (Table 2). Operculum area also increased substantially (18.5%) over time.

LDA consistently classified *R. argentea* to year at a substantially higher rate than expected by chance alone (50%). For gill traits, *R. argentea* were correctly classified 74% of the time for both years. For rakers, correct classification rates were 70% for 1966 and 96%

<table>
<thead>
<tr>
<th>Category</th>
<th>Trait name</th>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gills</td>
<td>Total gill filament length</td>
<td>TFL</td>
<td>Sum of the lengths of all gill filaments on the outer surface of the first left hemibranch</td>
</tr>
<tr>
<td></td>
<td>Average gill filament length</td>
<td>AFL</td>
<td>Mean of the lengths of all gill filaments on the outer surface of the first left hemibranch</td>
</tr>
<tr>
<td></td>
<td>Total number of gill filaments</td>
<td>GN</td>
<td>Total number of gill filaments on the outer surface of the first hemibranch</td>
</tr>
<tr>
<td></td>
<td>Total hemibranch area</td>
<td>THA</td>
<td>Total area of the outer surface of the first hemibranch</td>
</tr>
<tr>
<td></td>
<td>Total hemibranch perimeter</td>
<td>THP</td>
<td>Perimeter of the first left hemibranch</td>
</tr>
<tr>
<td>Rakers</td>
<td>Total number of gill rakers</td>
<td>RN</td>
<td>Total number of gill rakers on the first gill arch</td>
</tr>
<tr>
<td></td>
<td>Mean raker length</td>
<td>RL</td>
<td>Mean length of the 2nd, 3rd and 4th rakers on the lower part of the first gill arch</td>
</tr>
<tr>
<td></td>
<td>Mean raker width</td>
<td>RW</td>
<td>Mean width of the 2nd, 3rd and 4th rakers on the lower part of the first gill arch</td>
</tr>
<tr>
<td></td>
<td>Mean inter-raker space</td>
<td>IRS</td>
<td>Mean distance between the centres of the 2nd, 3rd and 4th rakers on the lower part of the first gill arch</td>
</tr>
<tr>
<td>Head</td>
<td>Head length</td>
<td>HL</td>
<td>From the rostral tip of the upper jaw to the caudal end of the gill cover</td>
</tr>
<tr>
<td></td>
<td>Head depth 1</td>
<td>HD1</td>
<td>Straight line from the topmost neurocranium margin through the point of operculum connection to the skull to the most ventral point of suboperculum</td>
</tr>
<tr>
<td></td>
<td>Head depth 2</td>
<td>HD2</td>
<td>Straight line from the topmost neurocranium margin through the point of operculum connection to the skull to the most ventral point of urohyal</td>
</tr>
<tr>
<td></td>
<td>Interobtial width</td>
<td>IO</td>
<td>Distance between points on the dorsal, bony margin of the left and right orbit</td>
</tr>
<tr>
<td></td>
<td>Dorsal head width</td>
<td>DHW</td>
<td>Distance between points of operculum connection to the skull in the dorsal plane</td>
</tr>
<tr>
<td></td>
<td>Ventral head width</td>
<td>VHW</td>
<td>Distance between points of operculum connection to the skull in the ventral plane</td>
</tr>
<tr>
<td></td>
<td>Dorsal post-head width</td>
<td>DPHW</td>
<td>Distance between the bony margins of the left and right supracleithrum in the dorsal plane</td>
</tr>
<tr>
<td></td>
<td>Ventral post-head width</td>
<td>VPHW</td>
<td>Distance between the bony margins of the left and right supracleithrum in the ventral plane</td>
</tr>
<tr>
<td></td>
<td>Operculum area</td>
<td>OPA</td>
<td>Operculum area</td>
</tr>
</tbody>
</table>

Please see Supporting Information, Fig. S5-S7 for more details.
For head traits, *R. argentea* were correctly classified 100% of the time for 1966 and 90% of the time for 2010. Inspection of the distribution of discriminant function scores showed strong discrimination between years, particularly for head and raker traits (Fig. 4).

**Trait Correlations**

There was a significant positive correlation between head PC1 and gill PC1 ($r = 0.37$, d.f. = 38, $P = 0.019$). That is, individuals with larger gills (greater TFL, AFL, GN, THA and THP) also had larger (longer, deeper and wider) heads. Along this axis of co-variation, contemporary *R. argentea* tended to have both larger gills and larger heads relative to historical specimens (Fig 5A; Supporting Information, Table S3).

We also found a significant positive correlation between gill PC2 and raker PC3 ($r = 0.32$, d.f. = 45, $P = 0.026$). Contemporary *R. argentea* had more gill filaments, and also wider rakers and smaller inter-raker spaces than historical *R. argentea* (Fig. 5B).

**Discussion**

Our goal was to explore contemporary phenotypic change in *R. argentea* in three suites of related traits: gills, gill rakers and head shape. We found evidence for substantial change in all three over the past 50 years. Specifically, modern *R. argentea* had larger gills, larger and wider heads, and shorter and more tightly packed gill rakers. Below we review these findings in more detail, and then discuss their potential significance in the context of the ecological changes that have occurred in Lake Victoria over the past century.

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**Figure 2.** Trait variation through time. Changes in representative gill (A, B), raker (C, D) and head (E, F) traits between 1966 (open circles) and 2010 (filled circles). Data shown are means of size-standardized traits ± 1 SE.
We observed a modest (1–9%) increase in all five measured gill traits between 1966 and 2010. Increases in each of these traits is likely to result in an overall increase in gill surface area, which is one of the strategies that water-breathing fishes that inhabit hypoxic habitats use to increase oxygen transfer from the environment to their tissues (Hughes & Morgan, 1973). Gill size (total gill surface area and/or gill filament length) has been shown to correlate with environmental dissolved oxygen levels at both the interspecific (Hughes & Morgan, 1973; Galis & Barel, 1979; Galis & Smit, 1979; Crampton, Chapman & Bell, 2008; Friedman, Condon & Drazen, 2012) and the intraspecific level (Chapman et al., 2000, 2007, 2008; Chapman & Hulen 2001; Timmerman & Chapman 2004; Crispo & Chapman 2010; Tobler et al., 2011). Increases in gill size have been reported for several other fishes within Lake Victoria in response to the decrease in environmental oxygen over time. For instance, Witte et al. (2008) compared archived specimens of the endemic haplochromine (Haplochromis pyrrhocephalus) collected between 1977 and 1981 to conspecifics collected between 1993 and 2001 in Mwanza Gulf, an area of Lake Victoria where hypoxia became more widespread between these two periods. Total gill surface area was 64% greater in the 1977–1981 collection, which they attributed to increased exposure to hypoxia. In a more recent study, van Rijssel et al. (2016) analysed the gill surface area of four species of haplochromine cichlids in Lake Victoria relative to dissolved oxygen (DO) levels, and found that gill surface in three of the species increased in the 1980s coincident with a decrease in DO levels and decreased in the 2000s associated with increased DO levels, suggesting a rapid gill morphological change over the past 33 years. Finally, as mentioned above, Wanink & Witte (2000) reported a small, but statistically significant, increase in gill filament number in *R. argentea* in the Mwanza Gulf between 1983 and 1988, in response to increased hypoxia in their benthic habitat (Wanink et al., 2001). Overall, the increase in gill size that we observed in *R. argentea* between 1966 and 2010 is consistent with a direct, adaptive response to the increased hypoxia that has been observed in Lake Victoria (Hecky et al., 1994) and in the Napoleon Gulf (Mwebaza-Ndawula, 1998). In addition to these morphological changes, *R. argentea* probably copes with low oxygen stress through behavioural means, such as by shifting its vertical distribution to avoid hypoxic waters (Mwebaza-Ndawula, 1998; Wanink et al., 2001).

**Table 2. Morphological change in *Rastrineobola argentea* through time**

<table>
<thead>
<tr>
<th>Category</th>
<th>Trait</th>
<th>Test statistic (F or W)</th>
<th>P</th>
<th>Trend over time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gills</td>
<td>TFL</td>
<td>$F_{1,85} = 132.01$</td>
<td>&lt; 0.001</td>
<td>Increased 6.6%</td>
</tr>
<tr>
<td></td>
<td>AFL</td>
<td>$F_{1,85} = 200.52$</td>
<td>&lt; 0.001</td>
<td>Increased 1.2%</td>
</tr>
<tr>
<td></td>
<td>GN</td>
<td>$W = 593$</td>
<td>&lt; 0.001</td>
<td>Increased 1.5%</td>
</tr>
<tr>
<td></td>
<td>THA</td>
<td>$F_{1,85} = 159.88$</td>
<td>&lt; 0.001</td>
<td>Increased 9.2%</td>
</tr>
<tr>
<td></td>
<td>THP</td>
<td>$F_{1,85} = 158.98$</td>
<td>&lt; 0.001</td>
<td>Increased 4.2%</td>
</tr>
<tr>
<td>Rakers</td>
<td>RN</td>
<td>$W = 230$</td>
<td>0.313</td>
<td>No change</td>
</tr>
<tr>
<td></td>
<td>RL</td>
<td>$F_{1,85} = 92.66$</td>
<td>&lt; 0.001</td>
<td>Decreased 9.0%</td>
</tr>
<tr>
<td></td>
<td>RW</td>
<td>$F_{1,85} = 27.25$</td>
<td>&lt; 0.001</td>
<td>Increased 3.2%</td>
</tr>
<tr>
<td></td>
<td>IRS</td>
<td>$F_{1,85} = 63.49$</td>
<td>&lt; 0.001</td>
<td><strong>Decreased 13.2%</strong></td>
</tr>
<tr>
<td>Head</td>
<td>HL</td>
<td>$F_{1,85} = 741.47$</td>
<td>&lt; 0.001</td>
<td>Increased 7.8%</td>
</tr>
<tr>
<td></td>
<td>HD1</td>
<td>$F_{1,85} = 580.55$</td>
<td>&lt; 0.001</td>
<td>Increased 2.9%</td>
</tr>
<tr>
<td></td>
<td>HD2</td>
<td>$F_{1,85} = 619.75$</td>
<td>&lt; 0.001</td>
<td>Increased 9.6%</td>
</tr>
<tr>
<td></td>
<td>IO</td>
<td>$F_{1,85} = 199.48$</td>
<td>&lt; 0.001</td>
<td>Increased 10.0%</td>
</tr>
<tr>
<td></td>
<td>DHW</td>
<td>$F_{1,85} = 415.95$</td>
<td>&lt; 0.001</td>
<td>Increased 10.9%</td>
</tr>
<tr>
<td></td>
<td>VHW</td>
<td>$F_{1,85} = 404.24$</td>
<td>&lt; 0.001</td>
<td>Increased 11.6%</td>
</tr>
<tr>
<td></td>
<td>DPHW</td>
<td>$F_{1,85} = 331.32$</td>
<td>&lt; 0.001</td>
<td>Increased 11.0%</td>
</tr>
<tr>
<td></td>
<td>VPHW</td>
<td>$F_{1,85} = 147.13$</td>
<td>&lt; 0.001</td>
<td>Increased 22.7%</td>
</tr>
<tr>
<td></td>
<td>OPA</td>
<td>$F_{1,85} = 143.46$</td>
<td>&lt; 0.001</td>
<td>Increased 18.5%</td>
</tr>
</tbody>
</table>

Results from ANCOVAs or Wilcoxon tests examining changes in traits through time. Statistics are shown for the year effect in each model. Body size was also a significant co-variante in each model (results not shown). Note that differences were examined using the non-parametric Wilcoxon test for gill number (GN) and raker number (RN). The proportional change in each trait was calculated from the difference between mean size-standardized values for each time period. Substantial (>10%) changes are highlighted in bold.

**Phenotypic change through time**

**Gills**

We observed a modest (1–9%) increase in all five measured gill traits between 1966 and 2010. Increases in each of these traits is likely to result in an overall increase in gill surface area, which is one of the strategies that water-breathing fishes that inhabit hypoxic habitats use to increase oxygen transfer from the environment to their tissues (Hughes & Morgan, 1973). Gill size (total gill surface area and/or gill filament length) has been shown to correlate with environmental dissolved oxygen levels at both the interspecific (Hughes & Morgan, 1973; Galis & Barel, 1979; Galis & Smit, 1979; Crampton, Chapman & Bell, 2008; Friedman, Condon & Drazen, 2012) and the intraspecific level (Chapman et al., 2000, 2007, 2008; Chapman & Hulen 2001; Timmerman & Chapman 2004; Crispo & Chapman 2010; Tobler et al., 2011). Increases in gill size have been reported for several other fishes within Lake Victoria in response to the decrease in environmental oxygen over time. For instance, Witte et al. (2008) compared archived specimens of the endemic haplochromine (Haplochromis pyrrhocephalus) collected between 1977 and 1981 to conspecifics collected between 1993 and 2001 in Mwanza Gulf, an area of Lake Victoria where hypoxia became more widespread between these two periods. Total gill surface area was 64% greater in the 1977–1981 collection, which they attributed to increased exposure to hypoxia. In a more recent study, van Rijssel et al. (2016) analysed the gill surface area of four species of haplochromine cichlids in Lake Victoria relative to dissolved oxygen (DO) levels, and found that gill surface in three of the species increased in the 1980s coincident with a decrease in DO levels and decreased in the 2000s associated with increased DO levels, suggesting a rapid gill morphological change over the past 33 years. Finally, as mentioned above, Wanink & Witte (2000) reported a small, but statistically significant, increase in gill filament number in *R. argentea* in the Mwanza Gulf between 1983 and 1988, in response to increased hypoxia in their benthic habitat (Wanink et al., 2001). Overall, the increase in gill size that we observed in *R. argentea* between 1966 and 2010 is consistent with a direct, adaptive response to the increased hypoxia that has been observed in Lake Victoria (Hecky et al., 1994) and in the Napoleon Gulf (Mwebaza-Ndawula, 1998). In addition to these morphological changes, *R. argentea* probably copes with low oxygen stress through behavioural means, such as by shifting its vertical distribution to avoid hypoxic waters (Mwebaza-Ndawula, 1998; Wanink et al., 2001).

**Gill rakers**

We found striking differences in gill rakers between modern and historical samples of *R. argentea*. This
A shift in gill raker morphology was driven largely by a decrease both in raker length (9%) and in inter-raker spacing (13%). We observed a statistically significant but small (3%) increase in raker width, but no change in the total number of gill rakers.

Traditionally, it has been thought that gill rakers are used to filter small particulate prey items from ingested water in suspension-feeding fishes, and thus that their spacing is related to the size of prey that can be consumed. This view was largely fuelled by observations of the repeated, parallel evolution of benthic–limnetic pairs that diverged both in diet and in gill raker counts and spacing (reviewed by Robinson & Wilson, 1994). For example, studies of intraspecific resource polymorphisms in threespine stickleback, Gasterosteus aculeatus (Schluter & McPhail, 1992), pumpkinseed sunfish, Lepomis gibbosus (Robinson et al., 1993), and Arctic char, Salvelinus alpinus (Snorrason et al., 1994), have found that benthic ecotypes typically have fewer, shorter and/or more widely spaced gill rakers than their pelagic conspecifics. However, the precise role of gill rakers remains poorly understood, and the view that they act as passive sieves in prey retention is probably oversimplified. While some experimental studies have shown that gill raker spacing is a key factor determining the minimum size of prey that can be retained during filter-feeding (e.g. rainbow trout, Oncorhynchus mykiss, Budy, Haddix & Schneider, 2005), others have found little or no relationship between gill raker spacing and prey size (e.g. Langeland & Nøst, 1995). In common bream (Abramis brama, Cyprinidae), movements of the palatal organ and of the lateral gill rakers are thought to enable the fish to adjust the selectivity of the branchial sieve. However, larger particles (>2 mm) do not even reach the gill rakers, but are instead retained in the pharyngeal slit between the palatal organ and the branchial basket (Hoogenboezem et al., 1991). In blackfish (Orthodon microlepidotus, Cyprinidae), Sanderson, Cech & Patterson (1991) used fibreoptic endoscopy to film suspension-feeding, and found that the majority of prey bypassed the gill rakers entirely and were instead carried dorsally by currents and trapped in mucus on the palatal organ.

In the present study, we found no change in gill raker number and a decrease (rather than increase) in gill raker spacing over time. These changes are the opposite of what would be expected a priori following a shift to larger, benthic prey (Crowder, 1984; Wanink & Witte, 2000). An alternative hypothesis is that the observed decrease in inter-raking spacing is a response to changes in the zooplankton community, which appears to have shifted towards smaller-bodied taxa over the past half century (Mwebaza-Ndawula, 1994; van Rijssel et al., 2015), potentially also as a result of eutrophication (Wanink et al., 2002). Another intriguing possibility is that the decrease in raker length and inter-raking spacing is the result of crowding from larger gills – a possibility we discuss in greater detail below.

Our results did not support previous assertions that a shift to benthic feeding has coincided with a decrease in gill raker number in R. argentea (Wanink & Witte, 2000; Ahnelt, Keckeis & Mwebaza-Ndawula, 2016). We think this may be because variation detected previously, although statistically significant, was small. For example, Wanink & Witte (2000) reported a decrease in mean raker number from 17.5 to 17.0 between 1983 (N = 23) and 1988 (N = 16). Ahnelt et al. (2016) reported a difference in mean raker number of 13 ± 1 and 14 ± 1 between contemporary littoral (N = 31) and pelagic (N = 26) populations of R. argentea. Given the high lability of raker number in fishes in general (Lindsey, 1981),
additional work is needed to clarify the extent of temporal and spatial variation in this trait in *R. argentea*, and its potential ecological significance. Our findings do suggest, however, that variation in raker number is smaller than variation in raker length and spacing – which were not measured in previous studies.

Overall, regardless of gill raker morphology, contemporary *R. argentea* are feeding successfully on both zooplankton and benthic macro-invertebrates in the wild (Mwebaza-Ndawula, 1998; Budeba & Cowx, 2007; Sharpe & Chapman, 2014; Supporting Information, Table S1). As in other cyprinids, movements of the palatal organ, adjustments of the buccal cavity, and mucous may work together with gill rakers to facilitate prey selection and retention in *R. argentea* (Sibbing & Nagelkerke, 2000). More detailed study of the functional morphology of all these structures in *R. argentea* would help shed light on how this species is adapting to a changing resource base in Lake Victoria.

**Head size**

We observed a substantial (2–22%) increase in all nine of the measured head traits between 1966 and 2010. Furthermore, *R. argentea* from the two periods could be very strongly discriminated based on head morphology alone. In contrast, in a previous study that used geometric morphometrics to explore
morphological variation in \textit{R. argentea} from Lake Victoria, Sharpe et al. (2015) did not find any evidence for a change in lateral head size or shape. This apparent discrepancy probably stems from methodological differences between the two studies. First, geometric morphometrics approaches analyse changes in the position of a relatively small number of landmarks, and therefore are best suited to exploring overall changes in gross morphology. Second, Sharpe et al. (2015) only examined changes in lateral size and shape, whereas in the present study, the largest changes in head dimensions occurred in width (rather than length) and thus were detectable only from photographs and measurements taken in the dorsal and ventral planes.

At face value, an increase in head size, particularly width, appears maladaptive, as it could increase drag. In a viscous medium, drag is minimized at certain values of the fineness ratio (FR = maximum length/maximum width, optimum FR: 3–7) (Webb, 1975; Fish, 1998). A relative increase in width could shift an organism away from the optimum FR, increasing drag and thus the energetic costs of propulsion. This might be especially costly for a species such as \textit{R. argentea}, which is a schooling species that actively forages in open-water habitats (Kaufman & Ochumba, 1993). However, as we discuss below, changes in head size may be better understood when considered in conjunction with concurrent changes in respiratory and trophic structures.

\textbf{Trait correlations and trade-offs}

We found evidence for a strong positive correlation between gill and head size, with contemporary (2010) \textit{R. argentea} having both larger gills and larger heads. A similar pattern of larger gills and larger heads in hypoxic conditions has been observed in other African fishes from divergent oxygen environments, such as the cichlid \textit{Pseudocrenilabrus multicolor victoriae} (Chapman et al., 2000, 2008; Crispo & Chapman, 2011) and the cyprinid \textit{Barbus neumayeri} (Langerhans et al., 2007). Bouton, De Visser & Barel (2002) estimated the volume of three different compartments in the head (oral, suspensorial and opercular) in six species of rock-dwelling haplochromine cichlids from Lake Victoria, and quantified ecological correlates of head compartment variation. They found that DO explained most of the variation in the volume of the head compartments, in particular the opercular compartment containing the gills, suggesting that DO can influence head shape via its effects on gill size.

In all these species, the fitness benefit of developing larger gills (and thus larger heads) in low-oxygen environments must outweigh the fitness costs of having bulkier, less streamlined heads.

We found evidence for a negative correlation between some aspects of gill and gill raker morphology. Specifically, individuals with more gill filaments tended to also have wider rakers and smaller inter-raker spaces. Given that both structures are located on opposite sides of the same gill arch, spatial constraints may explain this pattern. For example, an increase in gill size could force the gill arch to bend inward, crowding the gill rakers on the opposite side together, and resulting in a decrease in inter-raking spacing. Similar trade-offs between respiratory and trophic morphology have been observed in other African cichlids, such as \textit{Pseudocrenilabrus multicolor victoriae} (Chapman et al., 2000, 2008) and \textit{Astatoreochromis alluaudi} (Binning et al., 2010).

\textbf{Underlying basis of trait variation}

The design of our study does not allow us to infer the extent to which the observed phenotypic changes in \textit{R. argentea} represent genetic change, phenotypic plasticity or a combination of the two. Several studies across a phylogenetically diverse suite of fishes have demonstrated a high degree of plasticity in gill size in response to rearing under hypoxic versus normoxic conditions (Chapman et al., 2000, 2007, 2008; Saroglia et al., 2002; Rutjes, 2006; Crispo & Chapman, 2010; Dabruzzi & Bennett, 2014; Chapman, 2015). Gill raker morphology has also been shown to be plastic in some species. For example, Day, Pritchard & Schluter (1994) revealed that benthic and limnetic stickleback showed substantial adaptive plastic variation in gill raker length (but not raker count) when raised on either benthic or planktonic diets. Finally, head size and shape have shown phenotypically plastic variation in response to rearing under divergent diets (e.g. \textit{Geophagus} spp., Wimberger 1992), divergent oxygen regimes (e.g. \textit{Pseudocrenilabrus multicolor victoriae}, Crispo and Chapman 2011) and divergent flow regimes (e.g. \textit{Salmo salar} and \textit{S. trutta}, Pakkasmaa & Piironen, 2001). Overall, although the mechanisms underlying the observed changes in morphology remain unknown for \textit{R. argentea}, it seems likely there may be a strong plastic component.

\textbf{CONCLUSIONS}

We found that gill size increased over time in \textit{R. argentea}, probably driving an increase in head size, despite potential costs in terms of streamlining. Gill rakers became more crowded over time – a morphological change that is not consistent with a shift to larger benthic prey, but is consistent with the expected trade-off with gill size. Interestingly, although head size increased, this seemed insufficient to uncouple the trade-off between gills and gill rakers. Overall,
R. argentea has undergone rapid morphological change in a suite of characters, some of which are likely to reflect direct, adaptive responses to increased exposure to hypoxia, and others which may reflect indirect (potentially maladaptive) responses to the former.

Our study adds to a growing number of examples of Lake Victoria fishes that have undergone rapid phenotypic changes in response to anthropogenic changes over the past half century (Witte et al., 2008; Sharpe et al., 2012; Van Der Meer et al., 2012; van Rijssel & Witte, 2013; van Rijssel et al., 2014, 2016), and more broadly to the increasing body of evidence that humans are major drivers of contemporary phenotypic change (Hendry, Farrugia & Kinnison, 2008). Lake Victoria is typical of many lakes in densely populated catchments that have experienced pollution, eutrophication, over-fishing, and the introduction of non-indigenous species either simultaneously or in close succession (Dudgeon et al., 2006). Such suites of stressors can sometimes drive complex, counterintuitive and even maladaptive changes in natural populations due to indirect effects and trait correlations. More integrative studies that examine the consequences of multiple stressors on multiple traits are needed to fully understand how species may be adapting to contemporary environmental change.

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

**Figure S1.** Dissolved oxygen profiles from the Napoleon Gulf of Lake Victoria. Data are from our unpublished data (panels A and B), and from the National Fisheries Resources Research Institute (NaFIRRI)’s 2015 CTD Acoustic survey (panel C). Symbols indicate different sampling locations within the Napoleon Gulf.

**Figure S2.** Allometric relationships for gills. Scatterplots show relationships between body weight (W) and gill traits, for Rastrineobola argentea from 1966 (grey points, dotted lines) and 2010 (black points, solid lines). See Table 1 for abbreviations. There were no significant interactions between mass and year for any trait.

**Figure S3.** Allometric relationships for gill rakers. Scatterplots show relationships between standard length (SL) and gill raker traits, for Rastrineobola argentea from 1966 (grey points, dotted lines) and 2010 (black points, solid lines). See Table 1 for abbreviations. There was no relationship between SL and total number of gill rakers (RN). There were no significant interactions between SL and year for any trait.

**Figure S4.** Allometric relationships for head traits. Scatterplots show relationships between standard length (SL) and head traits, for Rastrineobola argentea from 1966 (grey points, dotted lines) and 2010 (black points, solid lines). See Table 1 for abbreviations. There were no significant interactions between SL and year for any trait except head depth 2 (HD2).

**Figure S5.** Gill measurements. Each gill filament (blue lines) was measured for length, and these data were summed and averaged to calculate total filament length (TFL) and average filament length (AFL), respectively. Filament lengths were measured without lamellae. Gill filaments were also counted, starting from the top (1) to the bottom, for total number of gill filaments (GN). The blue polygon connecting the tips of all gill filaments was used to calculate total hemibranch area (THA) and total hemibranch perimeter (THP).

**Figure S6.** Gill raker measurements. Traits were measured on the second (R2), third (R3) and fourth (R4) rakers on the lower arch. Raker length (L) was measured as the distance from the centre of each raker at the point of its insertion into the gill arch to the distal tip. Raker width (W) was measured at the base of the raker, perpendicular to the line used to measure length. Inter-raker spacing (IRS) was measured from the centre of one raker to the centre of the adjacent raker. **Figure S7.** Head measurements. Dorsal plane (Panel A): interorbital width (IO): distance between point on the dorsal, bony margin of the left and right orbit – G1G2; dorsal head width (DHW): between points of operculum connection to the skull – C1C2; dorsal posthead width (DPHW): distance between bony margin of the left and right supracleithrum – D1D2. Lateral plane (Panel B): head length (HL): distance from the rostral tip of the upper jaw (A) to the caudal end of the gill cover (B) – AB; head depth1 (HD1): straight line from the topmost neurocranium margin (E) through the point of operculum connection to the skull (C) to the most ventral point of suboperculum (F1); it is drawn as a perpendicular to the line from rostral tip of upper jaw to operculum connection point (AC) – (ECF1); head depth2 (HD2): straight line from the topmost neurocranium margin (E) through the point of operculum connection to the skull (C) to the most ventral point of urohyal (F2) – (ECF2). Ventral plane (panel C): ventral head width (VHW): between points of operculum connection to the skull – C1C2; ventral posthead width (VPHW): between bony margin of the left and right cleithrum – D1D2.

**Table S1.** Diet of R. argentea from the Napoleon Gulf in 1966 and 2011

**Table S2.** Results of the Shapiro-Wilk’s test for normality for each trait

**Table S3.** Principal component loadings