Chapter 5

Climatic variability drives adaptive responses in the gills of Lake Victoria cichlids

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Abstract

Climate changes and global warming have severe consequences for aquatic ecosystems. In East Africa's Great Lakes, environmental perturbations are occurring as a result of global warming. Lake Victoria, famous for its stunning adaptive radiation of cichlids, has suffered from cultural eutrophication over the past decades. This eutrophication is thought to be partly responsible for the dramatically reduced cichlid biodiversity. However, environmental variability through climate-induced changes in mixing dynamics might also have contributed. To determine how both these changes have influenced the lake and its cichlids over the past 50 years, we gathered environmental and meteorological variables and related these to changes in gill surface of four cichlid species. We found a gradual increase of the maximum air temperature and a gradual decrease of lake water levels from the 1960s onwards. During the period of severe eutrophication (1980s), a change in wind direction in combination with reduced wind speeds and increased rainfall were observed. Concurrently, dissolved oxygen (DO) levels dropped as did water temperature and transparency. The gill surface in three out of the four cichlid species increased as a response to these environmental changes. During the 2000s, wind speed increased again to values higher than previously recorded. During the same time, an increase in DO levels, water transparency and water temperature and a decrease of cichlid gill surface were observed. Our results imply that climatic changes and especially wind speed and direction, and their variability, play a crucial role in tropical lake dynamics and might affect cichlid fish evolution. With continued eutrophication, we hypothesize that a sequential drop of wind speeds will be detrimental to Lake Victoria's unique ichthyofauna. Restrictions on anthropogenic nutrient inputs will be an important step to prevent future biodiversity crises in Lake Victoria and other African Great Lakes.

Introduction

Evidence that global warming can cause dramatic perturbations in freshwater ecosystems has rapidly accumulated over the past decade (Winder & Schindler 2004; Parmesan 2006; Woodward *et al.* 2010). In East-Africa, the warming of the Indian Ocean and the changes in the El Niño Southern Oscillation (ENSO) are believed to have resulted in an increase in air temperature and changes in rainfall (Nicholson & Kim 1997; Clark *et al.* 2003; Funk *et al.* 2008; Williams & Funk 2011). The effects of this warming on the physical properties of African Great Lakes have been documented (O'Reilly *et al.* 2003; Verburg *et al.* 2003; Lorke *et al.* 2004; Vollmer *et al.* 2005; Hecky *et al.* 2010; Tierney *et al.* 2010; Cozar *et al.* 2012). In Lake Tanganyika, the warming coincided with higher water temperatures (O'Reilly *et al.* 2003; Verburg *et al.* 2003) and lower wind speeds (O'Reilly *et al.* 2003) with inferred decreases in vertical mixing.

Periodic decreases in vertical mixing have been inferred for Lake Victoria as well during conditions of higher rainfall, reduced winds and higher relative humidity (Kolding *et al.* 2008; Hecky *et al.* 2010). The extent of vertical mixing is crucial for tropical lake dynamics and is an important driver of other environmental variables such as phytoplankton abundance, water transparency, and dissolved oxygen (DO) levels. These factors are important indicators of eutrophication. Eutrophication of freshwater lakes has become a worldwide issue in terms of biodiversity loss (Smith & Schindler 2009). The loss of species diversity can be inflicted in several ways, including demographic decline and introgressive hybridization. Though the latter can be beneficial for speciation during early adaptive radiation events (Seehausen 2004) it is more likely to result in loss of biodiversity through reversed speciation (Seehausen 2006). Evidence for reversed speciation through hybridization has been found in several freshwater fishes, including European whitefish, three-spined sticklebacks and East-African cichlids (Seehausen *et al.* 1997a; Taylor *et al.* 2006; Vonlanthen *et al.* 2012). Hybridization occurs as eutrophication hampers sexual selection by increasing the water turbidity (Seehausen *et al.* 1997a) or forces fish habitats and diets to overlap due to benthic oxygen depletion (Vonlanthen *et al.* 2012).

In Lake Victoria, several meteorological variables vary seasonally (Fish 1957; Talling 1966; Lehman 1998; Stager *et al.* 2009; Hecky *et al.* 2010). Stratification patterns have mostly been documented for the northern offshore waters. Stratification occurs after the southwest monsoon and has been attributed to reduced wind speeds (Talling & Lemoalle 1998; MacIntyre 2013). However, recent analyses using data from the National Center for Ecological Analysis and Synthesis indicate that stratification is due to flow of warmer water from the southern regions of the lake which experience greater rates of heating from September through March compared to other times of the year (S. MacIntyre, personal communication). This stratification results in lower DO levels near the bottom (Talling 1966; Hecky *et al.* 1994). During the stratified period, the phytoplankton biomass (which is light limited, Silsbe *et al.* 2006) tends to peak at inshore stations due to less vertical mixing and a high influx of nutrients from the land, created by heavy rains (Akiyama *et al.* 1977; Mugidde 1993; Cornelissen *et al.* 2013). These seasonal peaks are species dependent though, with some diatom species (e.g. *Melosira spp.*) dominating in the windy, mixing season (Talling 1966). Beginning at the end of the wet season, and in some years continuing in the dry season, the south-east trade winds cause the break-down of the stratification by internal wave motions and by increased evaporation and concomitant vertical mixing (Talling 1966; MacIntyre 2013; S. MacIntyre, personal communication). The vertical mixing influences nutrient and phytoplankton abundance that are reflected in a relatively high water transparency during the dry season. Recent variability in the seasonal patterns resulted in a change of these seasonal patterns and intensified the eutrophication of the lake (Wanink *et al.* 2001; Verschuren *et al.* 2002; Hecky *et al.* 2010).

The eutrophication of Lake Victoria has been attributed mainly to anthropogenic influences (Verschuren *et al.* 2002). However, recent studies imply that also climatic changes have contributed (Kolding *et al.* 2008; Hecky *et al.* 2010). The latter two studies suggest that the observed decrease in wind speeds during the 1970s and 1980s has enhanced the already inflicted eutrophication due to increased stability and persistence of seasonal stratification, which resulted in deoxygenation of the hypolimnion. During this time, the enhanced primary production and increase of cyanobacteria and algae resulted in a decrease in transparency and increased hypoxia (Seehausen *et al.* 1997a; Hecky *et al.* 2010). Together with the introduction of the piscivorous Nile perch, these dramatic ecological changes are thought to be responsible for the eradication of hundreds of endemic haplochromine cichlid species (Seehausen *et al.* 1997a; Witte *et al.* 2007).

Despite the severely changed environmental conditions, some haplochromines recovered (Witte *et al.* 2007; Kishe-Machumu 2012) and adjust morphologically to the altered environment in the Mwanza Gulf of Lake Victoria (Witte *et al.* 2008; Van der Meer *et al.* 2012 [Chapter 2 in this thesis]; Van Rijssel & Witte 2013 [Chapter 3 in this thesis]; Chapter 4 in this thesis). This is the site of a long term haplochromine monitoring study conducted by the Haplochromis Ecology Survey Team (HEST) since the 1970s. HEST found that, among other species, *Haplochromis (Yssichromis) pyrrhocephalus* increased its in gill surface by 64%, and suggested that this was a response to the low oxygen conditions (Wanink *et al.* 2001; Witte *et al.* 2008). Under laboratory conditions, several other cichlid species showed an increase in gill surface as a plastic response to low dissolved oxygen (DO) levels (Chapman *et al.* 2000; Rutjes 2006; Rutjes *et al.* 2009). The above-mentioned studies suggest that Lake Victoria cichlids are resilient to severe hypoxic conditions in the field as well as in the lab. It remains unclear, however, whether the morphological changes in the gills under natural conditions are the result of phenotypic plasticity or genetically based changes (in response to directional selection and/or resulting from hybridization (Chapman *et al.* 2000; Chapman *et al.* 2002; Witte *et al.* 2008).

The goal of this research was to study the effects of meteorological variables on the physical limnology of the Mwanza Gulf (Figure 1.1) and how these affect the gill morphology of four resurgent Lake Victoria haplochromines. We expected that climatic variability would have a major effect on the lake dynamics which influence environmental variables such as water transparency and DO levels. A decrease of these variables is expected to induce an increase of the gill surface of the four resurgent cichlid species (abbreviations of species in parentheses); *H.(Y.) pyrrhocephalus (pyr)*, *H. (Y.) laparogramma (lap)*, *H. tanaos (tan)* and *P. degeni (deg)*.

Materials and methods

Environmental variables

Monthly meteorological data was obtained from 1960-2012. We obtained data from the meteorological department measured at Mwanza Airport for minimum and maximum air temperatures (1960-2012), rainfall (1960-2012), wind speed (1972-2012) and wind direction (1977-2012). Wind speed and direction were measured during the day at 1200 h. Data for lake water levels (1965-2012) were obtained from the Lake Victoria Basin Water Office and were measured at a station located between Mwanza City and the village of Nyegezi (Figure 1.1). This station is located 1130 m above sea level which is extracted from the lake level measurements.

The Mwanza Gulf is a relatively long, narrow gulf which extends 60 km southward with an average width of 5km and which varies in depth from 1-2m in the southern part up to 20-25m at the entrance of the gulf (Figure 1.1; Goudswaard *et al.* 2002). Periodically, DO-levels, water transparency and the bottom and surface water temperatures (the difference between them was calculated) were measured monthly in 1973-74, 1980, 1983-85, 1986-87, 1988, 2002, 2006, 2008 and 2010-11. Some years were combined to obtain sufficient data for each period. Most of these measurements were conducted at station G (12-14m depth) of the research transect in Mwanza Gulf where most fishes used in this study were caught (see below; Figure 1.1). Daily DO levels were measured 1m above the bottom, together with water temperature, which was also measured at the surface. The surface and bottom temperature were subtracted from each other and this difference can be interpreted as an indicator of stratification. These measurements were made with a Yellow Springs Instrument (YSI) AQ model 57 while water transparency was measured with a Secchi disc (Table 5.1).

Table 5.1 Dissolved oxygen, bottom (1m above the bottom) and surface water temperature, and Secchi disc measurements at station G.

Year	**DO level**	**St. dev.**	**N**	**Bottom water temp.**	**St. dev.**	**Surface water temp.**	**St. dev.**	**N**	**Secchi depth**	**St. dev.**	**N**
1973-74	4.69	1.22	4	24.56	0.55	24.85	0.59	4	1.54	0.22	4
1980	4.41	1.52	8	25.13	0.52	26.51	0.83	8	2.48	0.22	4
1983-85	1.54	1.8	12	24.57	1.11	26.06	1.09	12	1.29	0.16	5
1986-87	1.18	0.78	4	23.63	0.31	24.4	0.60	9	1.13	0.12	8
1988	0.77	0.81	3	22.22	0.47	23.08	0.58	6	1.23	0.29	3
1995-96									1.38	0.23	3
2002	1.27	1.0	3	24.63	0.49	25.8	0.82	3	1.75	0.31	3
2006	3.4	1.7	2	25.0	0	25.47	0.46	3	2.0		1
2008	3.01	1.63	2	24.5	0.14	25.1	0.57	2	1.88	0.18	2
2010-11	4.62	0.95	4	25.3	0.69	26.09	1.53	3	1.23	0.15	4

Data from 1973-74 were obtained from Akiyama *et al.* (1977) who measured south of Nyamatala Island at a depth of 8m (five kilometers south of station G). To correct for the depth difference with station G, we compared the bottom water temperature, DO levels and

Secchi depth between station G and station E (also 8m depth) from the same time period. The average difference was subtracted or added from values reported by Akiyama *et al.* (1977). The data from 2010-11 were measured by SEDEC Wageningen UR, one kilometer south of station G (11m depth). We selected data from the wet season (February-April) with heavy rains but calm weather when thermal stratification and hypoxia occur in the lower part the water column in the open water of Lake Victoria (Talling 1966) as in the Mwanza Gulf (Akiyama *et al.* 1977; Witte & van Densen 1995; Wanink *et al.* 2001; Cornelissen *et al.* 2013). With lower visibility, lower DO levels and higher water temperatures, this seems to be the harshest period of the year for the cichlids.

Fish collection

Fishes were collected during the years 1981, 1984, 1987, 1991, 1993, 1999, 2002, 2006 and 2011, at the research transect in the northern part of the Mwanza Gulf, Lake Victoria, Tanzania. The species *pyr* and *lap* were mainly caught above mud bottoms at station G, while the species *deg* and *tan* were mainly caught at sand/mud bottom bays (Butimba and Kissenda, 4-8m depth) at opposite ends of the transect (Figure 1.1; Table 5.2).

We selected males only to avoid any effects of sexual dimorphism. In total, 214 adult fish of four species (an average of seven specimens per species per year) were selected for the gill morphology analysis. This is a subsample of the fish used in Van Rijssel & Witte (2013) [Chapter 3 in this thesis].

Table 5.2 Catch locations and number of specimens per species per year.

Year	***H. laparogramma***	**N**	***H. pyrrhocephalus***	**N**	***H. tanaos***	**N**	***P. degeni***	**N**
1978-1982	G, Transect	8	G	8	BB	8	BB, J	7
1984	G	8	G	8			BB	7
1987	G	8	Luanso Bay	7			BB, Transect	3
1991	J, P	8	J, P	8				
1993	G, H, I	8	H, I	8	I, J, K	4		
1999	Transect	6	Transect	8				
2001-2002	G	8	G	8	J, BB	8	J	7
2006	F-J	8	G	8	E	8	J	8
2011	F-J	8	F	7	J	8	F, J, K	6
Total		**70**		**70**		**36**		**38**

E-K, stations on the transect; P, Python Island-Nyamatala Island; BB, Butimba Bay; Transect, unknown station along the transect. The location of Python Island, Nyamatala Island and Luanso Bay are indicated on maps found in Bouton *et al.* (2002b), Witte *et al.* (1992b) and Goldschmidt *et al.* (1993), respectively.

Gill morphology

The first and second gill arch on the right side of each fish were dissected and photographed with a digital camera (Nikon Digital Sight DS-FI1) mounted on a dissection microscope. Both sides (hemibranches) of the second gill arch were photographed with a reference scale. The length (L) and number (N) of the filaments were measured from these photographs. Four filaments equally divided over the medial hemibranch were selected for measuring the secondary lamellae (sec. lam.). From each side, ten sec. lam. were selected from the middle of the filament through scanning electron microscopy (SEM). The gill surface of the second gill arch was calculated following Witte *et al.* (2008):

$$A = N \times L \times d \times a,$$

where A is the gill surface of the second gill arch (mm^2), N is the total number of filaments on both hemibranches, L is the average filament length (mm), d is the average sec. lam. density (mm^{-1}) and a is the average sec. lam. surface (mm^2). The d was calculated by dividing 10 by the distance between the first and the tenth sec. lam resulting in the number of sec. lam. per mm. The a was calculated considering the sec. lam. as a triangle $a = l \times \frac{1}{2}h \times 2$, where l is the sec. lam. length (mm) and h is the sec. lam. height (mm). Note that the surface area is multiplied by two because each sec. lam. has two functional sides. All measurements were conducted with ImageJ (1.47a).

Statistical analysis

Differences in environmental factors between years were tested with a One-way ANOVA unless the data were not normally distributed (tested with Shapiro-Wilk test). The latter was the case for minimum air temperature, wind speed, lake levels and the difference between surface and bottom water temperatures which were tested with a non-parametric Kruskal-Wallis test. A general linear model (GLM) with body volume (BV, measured by volumetric displacement) as covariate and year as independent factor was applied to test whether morphological characters of the gills differed in time following Van Rijssel & Witte (2013) [Chapter 3 in this thesis]. These data were log-transformed to achieve linearity, estimated marginal means (EMM) were anti-logged and plotted through time. A Pearson correlation test was used to see if the environmental factors were correlated with each other and with the EMM of the morphological characters. A Spearman correlation test was used for non-normally distributed data which was the case for minimum air temperature and bottom water temperature. The *P*-values of all tests were corrected with a sequential Bonferroni test. All statistical tests were performed with SPSS version 20.

Results

Environmental variables through time

The wind direction showed significant changes through time in the yearly dataset ($P = 0.003$, Figure 5.1). In the period 1978-1981, the wind roughly came from the west (253-266°) and from 1982 till 1994 mainly from the southwest (180-249°). Three years after the change in wind direction (1985), the wind speed dropped significantly (both in the yearly and periodical dataset, $P < 0.001$, Figures 5.1 & 5.2A). From 1985 onwards, the wind speed decreased and remained low until 1997, with the exception of a small peak in 1992-93 (Figure 5.2A). The wind direction changed back to mainly western winds from 1995 onwards. Three years later, in 1998, the wind speed increased again and during the 2000s reached speeds above those previously recorded (Figures 5.1 & 5.2A, B). Despite changes in wind speed and direction, rainfall did not change significantly through time (Figures 5.1 & 5.2A, B). However, there was an increase of rainfall in 1988 compared to the period 1983-85 (*P* = 0.041, not significant after sequential Bonferroni correction), which coincided with the change in wind direction and the decrease in wind speeds (Figure 5.2A, B). Even though there was no overall decrease in rainfall, the lake level decreased gradually over time with a steep decline during the early 2000s (P < 0.001; Figures 5.1 & 5.2B, C).

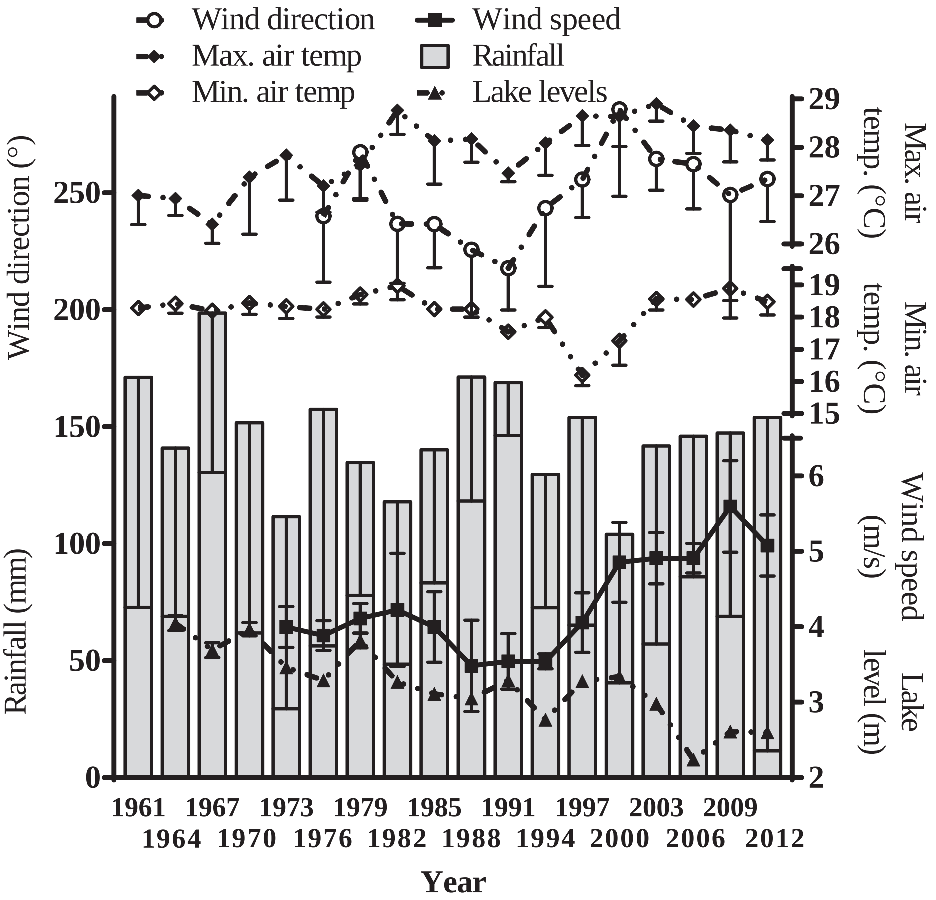

Figure 5.1 Meteorological variables and lake levels plotted through time measured at Mwanza airport and between Mwanza City and the village of Nyegezi respectively during the wet season (February-April). Lake levels are depicted as height values with the elevation of the Mwanza Gulf (1130m) extracted from them. For better visualisation, every three years were combined and averaged to one year with the middle year being depicted in the graph e.g. year 1961 represents years 1960, 1961 & 1962 etc. Depicted (lower end) standard deviations are averaged from yearly standard deviations.

The maximum air temperature increased significantly in 1980 compared to the 1960s-1970s (yearly dataset, *P* < 0.001, Figure 5.1). It remained high up till 2012 with the exception of a non-significant drop from 1985 till 1996 which also concurs with the period of major wind changes. The minimum air temperature remained stable during the 1960s and 1970s and

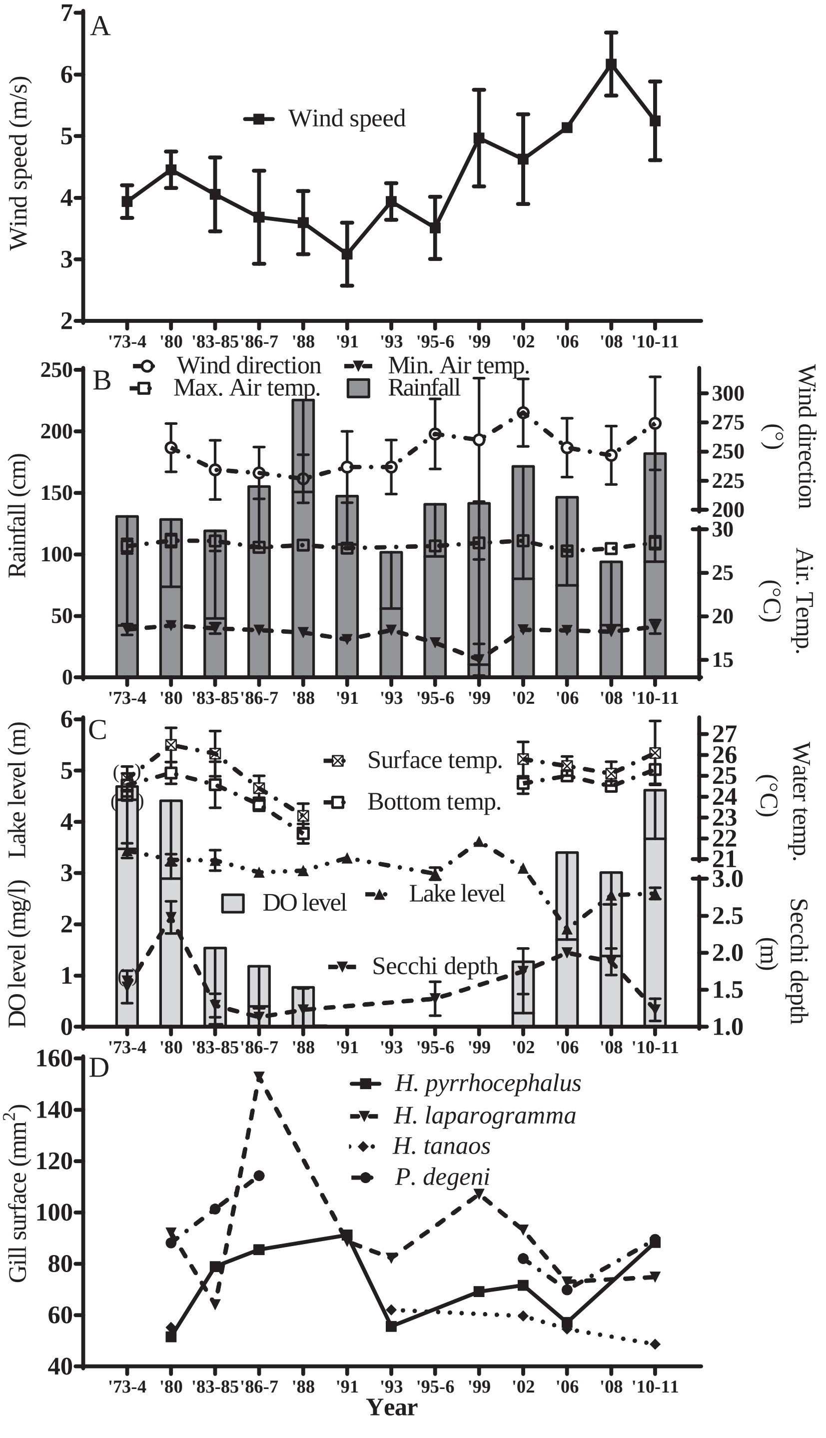
A
Wind speed (m/s)
Wind speed
7
6
5
4
3
2
'73-4 '80 '83-85 '86-7 '88 '91 '93 '95-6 '99 '02 '06 '08 '10-11
B
Wind direction
Min. Air temp.
Max. Air temp.
Rainfall
Rainfall (cm)
250
200
150
100
50
0
Wind direction (°)
300
275
250
225
200
Air. Temp. (°C)
30
25
20
15
'73-4 '80 '83-85 '86-7 '88 '91 '93 '95-6 '99 '02 '06 '08 '10-11
C
Lake level (m)
DO level (mg/l)
6
5
4
3
2
1
0
Surface temp.
Bottom temp.
DO level
Lake level
Secchi depth
Water temp. (°C)
27
26
25
24
23
22
21
Secchi depth (m)
3.0
2.5
2.0
1.5
1.0
'73-4 '80 '83-85 '86-7 '88 '91 '93 '95-6 '99 '02 '06 '08 '10-11
D
Gill surface (mm²)
160
140
120
100
80
60
40
H. pyrrhocephalus
H. laparogramma
H. tanaos
P. degeni
'73-4 '80 '83-85 '86-7 '88 '91 '93 '95-6 '99 '02 '06 '08 '10-11
Year

Figure 5.2 Environmental variables of the periodic dataset (A, B, C) and gill surfaces of four haplochromine species (D) plotted through time measured during the wet season (February-April). A: Wind speed, B: rainfall, minimum and maximum air temperature and wind direction, C: dissolved oxygen (DO) level, surface and bottom water temperature, Secchi depth and lake level, D: estimated marginal means of gill surfaces of four haplochromine species. Secchi depth, DO levels and water temperature have been measured at station G. Corrected values from Akiyama *et al.* (1977) are indicated in parentheses. Maximum air temperature and lake level are lacking for the year 1993. Dissolved oxygen levels and water temperature have not been measured in the period 1991-1999. From 1988 till 2001 there were no specimens available for *P. degeni* and from 1982-1992 no specimens were available for *H. tanaos*. Only lower end standard deviations are depicted for rainfall and DO levels.

then dropped gradually from 1980 onwards, reaching its lowest point in 1999 (both datasets, $P < 0.001$; Figure 5.1 & 5.2B). In 2000, the minimum air temperature increased again to the level of the 1960s and 1970s and remained at that level up till 2012.

Bottom and surface water temperature, Secchi depth and DO levels all show a significant drop during the mid and late 1980s compared to 1973-74 and 1980 ($P < 0.001$, Figure 5.2C) which concurs as well with the observed wind changes. Compared to 1973-74, the difference between bottom and surface temperature increased significantly in 1980 and 1983-1985 ($P < 0.01$) indicating stratification. The increased difference was maintained, albeit at a less pronounced level, from 1987 onwards and in 2006 the difference decreased ($P < 0.05$) to a similar level as in 1973-74. Although data from the 1990s are mostly missing, from 2002 onwards water temperature, Secchi depth and DO levels increased again reaching the same level as they were in 1973-74 and 1980 ($P < 0.001$). Secchi depth, however, showed again a significant drop in 2011 ($P < 0.001$).

Gill morphology through time

In the mid and late 1980s when DO levels decreased, three out of four species (*pyr, lap* and *deg*) showed a significant increase in gill surface ($P < 0.01$, Figure 5.2D) with a significant effect of the covariate BV ($P < 0.001$, Appendix Table 5.1). The increase was mainly due to a significant increase in filament length, sec. lam. surface (*pyr* and *lap*) and sec. lam. density (*deg*, Appendix Figure 5.1). The gill surface of the three species decreased significantly during the 2000s when DO levels had increased again ($P < 0.05$, Figure 5.2C, D). In 2011, however, there was once more a significant increase in gill surface for *pyr* and *deg* while DO levels were high ($P < 0.05$).

Correlations in yearly dataset

Wind speed shows a significant positive correlation with wind direction (r = 0.396, $P = 0.017$), which means that wind speed is higher when the wind is coming from the west and lower when the wind is coming from the south and southwest. Wind speed also shows significant correlations with minimum air temperature (r = 0.380, $P = 0.014$), maximum air temperature (r = 0.352, $P = 0.032$), rainfall (r = -0.353, $P = 0.023$) and lake level (r = -0.386, $P = 0.011$, Table 5.3). However, only the correlation with lake level was significant after sequential Bonferroni correction. Wind direction showed an almost significant negative correlation with rainfall ($r = -0.314$, $P = 0.062$), which would mean that there may be more rainfall with wind coming from the south and southwest and less rainfall with wind coming from the west. Rainfall also shows a significant negative correlation with the maximum air temperature ($r = -0.347$, $P = 0.015$), which is partly caused by the increased cloud cover reducing solar radiation with increased rainfall. The maximum air temperature shows an almost significant positive correlation with minimum air temperature ($r = 0.253$, $P = 0.079$) and a significant negative correlation with lake level ($r = -0.386$, $P = 0.011$, Table 5.3).

Table 5.3 Correlations of environmental parameters from the yearly dataset. Significant correlations are indicated in bold. Only the correlation between wind speed and lake level was significant after sequential Bonferroni correction.

Environmental variable	Rainfall	Min. air temp.	Max. air temp.	Lake level	Wind direction	Wind speed
Rainfall	X					
Min. air temp.	$r = 0.005$ $P = 0.973$	X				
Max. air temp.	**$r = -0.347$** **$P = 0.015$**	$r = 0.253$ $P = 0.079$	X			
Lake levels	$r = -0.02$ $P = 0.898$	$r = -0.138$ $P = 0.366$	**$r = -0.386$** **$P = 0.011$**	X		
Wind direction	$r = -0.314$ $P = 0.062$	$r = 0.078$ $P = 0.651$	$r = 0.200$ $P = 0.272$	$r = -0.060$ $P = 0.737$	X	
Wind speed	**$r = -0.353$** **$P = 0.023$**	**$r = 0.380$** **$P = 0.014$**	**$r = 0.352$** **$P = 0.032$**	**$r = -0.322$** **$P = 0.046$**	**$r = 0.396$** **$P = 0.017$**	X

Correlations in periodic dataset

The DO levels did not show a significant correlation with the gill surfaces of the four species (Appendix Table 5.2). Wind speed did show a negative correlation with the gill surface of *tan* (not significant, $r = -0.821$, $P = 0.088$) and *deg* ($r = -0.829$, $P = 0.041$). As found in the yearly dataset, wind speed also showed a positive correlation with wind direction, though not significant ($r = 0.569$, $P = 0.068$). The wind direction is also positively correlated with the bottom water temperature ($r = 0.826$, $P = 0.011$), which means that southern wind results in lower bottom water temperatures and western wind results in higher bottom water temperatures. These bottom water temperatures also show a positive correlation with minimum air temperature ($r = 0.733$, $P = 0.025$) and, although not significantly, with DO levels ($r = 0.650$, $P = 0.058$) and are negatively correlated with the gill surface of *tan* ($r = -0.949$, $P = 0.051$). Surface water temperatures showed strong positive correlations with minimum air temperature ($r = 0.817$, $P = 0.007$) and bottom water temperature ($r = 0.923$, $P < 0.001$). The difference between surface and bottom water temperature was positively correlated with the maximum air temperature ($r = 0.819$, $P = 0.007$). Secchi depth showed a strong negative correlation with the gill surface of *pyr* ($r = -0.967$, $P = 0.002$, Appendix Table 5.2).

Discussion

Environmental variables through time

This study shows how climatic variability can influence tropical lake dynamics on the relatively small scale of the Mwanza Gulf. Based on our results, we hypothesize that wind stress might be one of the major factors responsible for the observed environmental changes in the Mwanza Gulf (and Lake Victoria at large, Lehman 1998) and rapid morphological responses observed in the Lake Victoria cichlids.

During the 1980s, the wind changed to a more southwest direction in combination with lower wind speeds. These lower wind speeds are likely to have resulted in reduced mixing of the water (Fish 1957; Talling 1966; Lehman 1998; Stager *et al.* 2009; Hecky *et al.* 2010; MacIntyre 2013). This reduced mixing would have resulted in thermal stratification which in turn led to lower DO levels and lower water temperature. The change in wind speed and direction coincided with increased rainfall. Both the lower wind speed and higher rainfall are likely to have influenced the water transparency by reduced mixing and increased nutrient and sediment influx from the shores and watershed (Cornelissen *et al.* 2013).

In addition, as suggested by Hecky *et al.* (2010), the lower wind speeds created optimal conditions for buoyant phytoplankton taxa (e.g. cyanobacteria) compared to rapidly sinking taxa such as diatoms (Reynolds 2006) which may have reinforced the resulting decline in Secchi disc transparency. The increase of cyanobacteria has contributed to the decrease in water transparency and DO levels in deeper waters as well (Verschuren *et al.* 2002; Hecky *et al.* 2010).

Despite no overall change in rainfall over the period of record, the lake level decreased gradually over time reaching similar values as those observed in 1961-1962 (Yin & Nicholson 1998). This decrease is, based on our data, likely due to increased evaporation as a result of increased maximum temperatures. Next to the observed climatic changes, human management also regulates lake levels (Yin & Nicholson 1998). Especially the sharp lake level decline during the 2000s can for a large part be attributed to the operation regime, initiated in 1999, of the expanded hydropower facility in Uganda, the Kiira Dam (Swenson & Wahr 2009).

The cichlids in Lake Victoria have withstood substantial climatic changes for at least 15,000 years (Johnson *et al.* 1996), and are apparently able to cope with such fluctuations. However, recent anthropogenic perturbations are likely to have exacerbated the effects of climatic changes which together resulted in eutrophication of the lake (Verschuren *et al.* 2002; Hecky *et al.* 2010). Lake Victoria is regarded to be in a new, relatively stable state (Hecky *et al.* 2010). However, so far, phosphorous loadings continue to rise and signs of improvement of lake conditions are derived from environmental variables (e.g. increased oxygen levels and water transparency) which are symptoms of eutrophication rather than improvement in rising nutrient concentrations (Hecky *et al.* 2010; Sitoki *et al.* 2010). The reduction of phosphorous input is considered to be the only successfully proven method for reducing eutrophication (Schindler 2012). However, in highly eutrophic situations such as Lake Victoria, phytoplankton abundance as well as deep water oxygen conditions are under hydrodynamic control and not driven by nutrients alone (Silsbe *et al.* 2006), though phytoplankton abundance in the Mwanza Gulf might still partly be nutrient limited (Cornelissen *et al.* 2013).

In the case of the Mwanza Gulf, the wind speed increased during the 2000s with levels higher than recorded in the past 40 years. These high wind speeds are likely to have increased evaporation rates which probably have resulted in complete vertical mixing (Talling 1966; MacIntyre 2013). This mixing seems to be responsible for the improvement of environmental variables (higher DO levels, generally higher Secchi depth except for 2011) to their values from before severe eutrophication. Therefore, we predict that a future change in wind direction and a sequential drop in wind speeds would result in the recurrence of hypereutrophication with even harsher conditions for the haplochromine cichlids than experienced during the late 1980s (Kolding *et al.* 2008).

Although we base our predictions and conclusions on the Mwanza Gulf only, it might well be that the Mwanza Gulf is representative for many other gulfs and bays along the Lake Victoria shoreline which show similar signs of eutrophication (Ochumba & Kibaara 1989; Mugidde 1993; Gikuma-Njuru & Hecky 2005; Hecky *et al.* 2010; Ngupula *et al.* 2012). In fact, the enormous cichlid biodiversity is mainly determined from catches along the lake's shoreline, in and around the Mwanza Gulf as well as from several other gulfs and bays (Witte *et al.* 2007). These areas are not only important in terms of biodiversity but over two million people depend directly on Lake Victoria's fisheries which are mainly exploited along the shoreline (LVFO FMP 2, 2008). In addition, hypereutrophication might not be limited to the inshore gulfs and bays (Hecky *et al.* 2010) which would mean that the complete lake and the species therein will have to face the harsh environmental changes while they might not be able to cope with these conditions.

Adaptive responses of the cichlids

Unexpectedly, the DO levels did not correlate with the gill surfaces of the four studied species. The lack of significant correlations is probably due to shortcomings of the collected dataset. The environmental variables collected are presented as being static while many of these variables (e.g. Secchi depth, DO level) can vary substantially within a day. Despite the lack of correlation between gill surfaces and DO levels, certain predicted trends can be recognized especially during the 1970s and 1980s. A significant increase of gill surface was observed in three species when oxygen levels dropped in the 1980s (we lack sufficient data in the 1980s for the fourth species, *tan*). During the 2000s, the gill surfaces of these species decreased again while DO levels reached their former levels from before the severe eutrophication. Although the gill surfaces fluctuated during the 1990s and 2000s, they tend to stay beneath the high surface areas observed when hypoxic conditions were most severe (late 1980s). However in 2011, when water turbidity increased again, so did the gill surfaces of the species *pyr* and *deg*. The highly significant correlation of Secchi depth and the gill surface of *pyr* might reflect a causal relation. As the water transparency is largely controlled by the influx of sediment and increased cyanobacterial abundance, the increase in turbidity may make it more difficult for fish to extract oxygen from turbid water. In other words, the gill surface area might be sensitive to fouling by suspended material as has been found for fish gill cells by Campbell *et al.* (1997) and Galvez *et al.* (2008). So, as well as DO levels in the ambient water *per se*, suspended material (which is partly reflected in Secchi depth Cornelissen *et al.* 2013) might determine the need for larger gill surface areas.

The increase of gill surface as a response to hypoxic conditions is quite common in fish including several cyprinids (crucian carp, goldfish), and cichlids (Chapman *et al.* 2000; Sollid *et al.* 2003; Sollid *et al.* 2005; Rutjes *et al.* 2009). In these studies, the gill surface increased as a result of phenotypic plasticity induced by hypoxic conditions in the lab.

Differences between morphological responses under natural and laboratory conditions can shed more light on the mechanism behind these responses under natural conditions. Chapman *et al.* (2000) found that natural populations of the Lake Victoria cichlid *Pseudocrenilabrus multicolor victoriae* at low-oxygen sites had longer filaments and larger secondary lamellae, while fishes experimentally raised under hypoxia showed an increase in filament length and number only. They attributed this disparity in response to differences in selection pressure and morphological constraints. Plastic responses to hypoxia in the lab were also found for *H. pyrrhocephalus* (Rutjes 2006). Somewhat different from the findings of Chapman *et al.* (2000), these fishes increased their gill surface not only by space occupying means (longer filaments) but also by means not related to space occupation (larger secondary lamellae).

The current study showed that all three species had longer gill filaments and two species (*pyr* and *lap*) larger secondary lamellae, while *deg* had an increased density of the secondary lamellae. Since the head volume of these fish decreased or remained the same during the hypoxic period in the 1980s (J.C. van Rijssel, unpublished data), space occupying changes were expected to be limited by morphological constraints. On the other hand, the reduction in eye size (Witte *et al.* 2008; Van der Meer *et al.* 2012 [Chapter 2 in this thesis]; Van Rijssel & Witte 2013 [Chapter 3 in this thesis]), and the possible reduction of the muscle used for suction feeding (*musculus sternohyoideus*, Witte *et al.* 2008), might have acted as morphological trade-offs. Whether phenotypic plasticity or genetically based changes underlie the morphological changes remains unknown. As suggested by Chapman *et al.* (2000), it is likely that both mechanisms are involved in the observed responses.

Future effects of eutrophication on fish species

The Lake Victoria cichlids have shown to adjust to a variety of environmental changes (Witte *et al.* 2008; Van der Meer *et al.* 2012 [Chapter 2 in this thesis]; Van Rijssel & Witte 2013 [Chapter 3 in this thesis], Chapter 4 in this thesis). However, a state of hypereutrophication is likely to be detrimental for cichlid biodiversity for two major reasons. Firstly, hypereutrophication might result in a very hostile environment in which cichlids and other species cannot cope with the changed environment (Smith & Schindler 2009). The new environment might demand morphological, physiological or behavioural adaptations that cannot be achieved by the fish through either genetic changes or plasticity. Though cichlids have been observed to be very plastic in the lab (Meyer 1987; Chapman *et al.* 2000; Stauffer & Van Snik Gray 2004; Rutjes 2006; Rutjes *et al.* 2009; Muschick *et al.* 2011) there are limits to this plasticity which might be reached through the changed environment. Secondly, hypereutrophication is likely to co-occur with low water transparency and low oxygen levels which has been shown to reduce fish biodiversity by hybridization (Seehausen *et al.* 1997a; Taylor *et al.* 2006; Vonlanthen *et al.* 2012).

Although the other African Great Lakes as a whole have not reached the level of eutrophication of Lake Victoria (Bootsma & Hecky 1993; Hecky 1993), eutrophication has been observed in some regions of these lakes too (Chale 2003; Hecky *et al.* 2003; Otu *et al.* 2011). The increased eutrophication poses a major threat to biodiversity and to the people depending for their income or food supply on the fisheries of these lakes. As the African population continues to expand, especially in areas surrounding the African Great Lakes (UNEP 2008), anthropogenic influences are likely to increase eutrophication by cumulative nutrient loading. In combination with climatic fluctuations such as reduced winds and increased rainfall which favour eutrophication, in time, these lakes, or regions within these lakes, may undergo similar losses of biodiversity as observed in Lake Victoria. For these reasons, we consider restrictions on anthropogenic nutrient inputs into the lakes as the most important task for ecosystem management. In addition, it is imperative that environmental variables such as nutrient loadings, chlorophyll, Secchi depths and DO levels will be monitored on a regular basis along with continued collection of meteorological data. By adequately monitoring these variables, we can improve our understanding of the effects of eutrophication on biodiversity and, with reductions in nutrient loading, moderate biodiversity losses in Lake Victoria and prevent biodiversity crises in other African Great Lakes.

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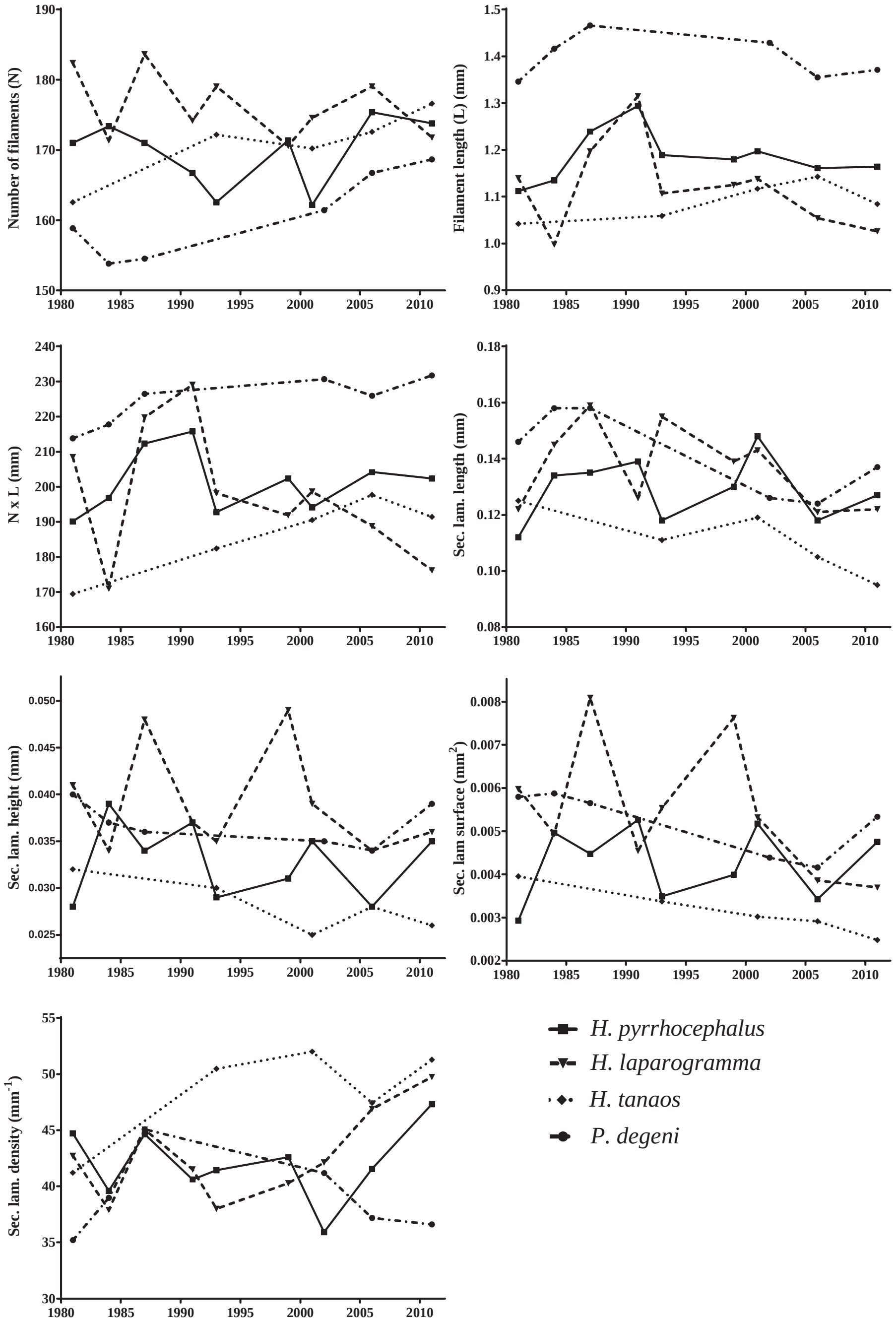
Appendix Table 5.1 Results of the GLM on morphological gill parameters. Significant *P*-values after sequential Bonferroni correction are depicted in bold. BV, body volume.

Species	**Factor Covariate**	**Number of filaments (N)**	**Filament length (L)**	**N x L**	**Sec. lam. length**	**Sec. lam. height**	**Sec. lam. surface**	**Density**	**Gill surface**
H. pyrrhocephalus	P year	**0.002**	**0.001**	**0.015**	**<0.001**	**0.004**	**<0.001**	**<0.001**	**<0.001**
	P BV	**<0.001**	**<0.001**	**<0.001**	0.003	0.297	0.042	0.160	**<0.001**
H. laparogramma	P year	**0.008**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	P BV	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.911	0.068	0.212	**<0.001**
H. tanaos	P year	**0.010**	**0.025**	**0.011**	**<0.001**	0.003	**<0.001**	**<0.001**	0.194
	P BV	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.032**	**<0.001**
P. degeni	P year	0.018	0.096	0.725	0.011	0.154	**0.004**	**<0.001**	**0.008**
	P BV	0.055	**<0.001**	**<0.001**	**<0.001**	0.817	**0.005**	0.204	**<0.001**



Appendix Figure 5.1 Estimated marginal means of morphological gill characters through time of four species where N is filament number and L is filament length.

Appendix Table 5.2 Correlations (r) of environmental parameters and gill surfaces from the periodic dataset. Significant correlations (after sequential Bonferroni correction) are indicated in bold.

Environmental variable / gill surface		**Rainfall**	**Min. air temp.**	**Max. air temp.**	**Wind direction**	**Wind speed**	**Bottom water temp.**	**Surface water temp.**	**Difference in water temp.**	**Secchi depth**	**DO level**	**Lake level**	**Gill surface *pyr***	**Gill surface *lap***	**Gill surface *tan***	**Gill surface *deg***
Rainfall	r															
	P	X														
Min. air temp.	r	-0.126														
	P	0.697	X													
Max. air temp.	r	0.126	0.582													
	P	0.711	0.060	X												
Wind direction	r	0.114	0.251	0.393												
	P	0.739	0.456	0.261	X											
Wind speed	r	-0.276	0.189	-0.043	0.569											
	P	0.385	0.556	0.900	0.068	X										
Bottom water temp.	r	-0.033	0.733	0.400	0.826	0.600										
	P	0.932	0.025	0.286	0.011	0.088	X									
Surface water temp.	r	-0.506	**0.817**	0.470	0.610	0.425	**0.923**									
	P	0.165	**0.007**	0.202	0.109	0.254	**< 0.001**	X								
Difference in water temp.	r	0.005	0.484	**0.819**	0.012	-0.226	0.063	0.441								
	P	0.990	0.187	**0.007**	0.978	0.558	0.873	0.235	X							
Secchi depth	r	-0.458	0.142	0.012	0.311	0.428	0.452	0.499	0.118							
	P	0.215	0.715	0.975	0.454	0.251	0.222	0.172	0.762	X						
DO level	r	-0.364	0.600	-0.056	0.448	0.415	0.650	0.504	-0.346	0.451						
	P	0.335	0.088	0.886	0.265	0.267	0.058	0.166	0.362	0.223	X					
Lake level	r	-0.038	-0.418	0.553	-0.132	-0.522	-0.239	-0.053	0.418	-1.65	-0.136					
	P	0.912	0.201	0.078	0.716	0.099	0.536	0.892	0.263	0.672	0.727	X				
Gill surface *pyr*	r	0.550	-0.183	-0.107	-0.069	-0.349	-0.143	-0.404	-0.092	**-0.967**	-0.362	0.197				
	P	0.125	0.637	0.800	0.861	0.357	0.787	0.427	0.863	**0.002**	0.481	0.641	X			
Gill surface *lap*	r	0.207	-0.300	-0.182	-0.199	-0.298	-0.257	-0.777	-0.221	-0.248	-0.419	0.290	0.236			
	P	0.295	0.433	0.666	0.608	0.437	0.623	0.069	0.674	0.636	0.409	0.485	0.540	X		
Gill surface *tan*	r	-0.606	-0.410	0.134	-0.349	-0.821	-0.949	-.264	0.378	0.460	-0.868	0.315	-0.554	0.483		
	P	0.278	0.493	0.866	0.565	0.088	0.051	0.736	0.622	0.540	0.132	0.685	0.333	0.410	X	
Gill surface *deg*	r	-0.148	0.200	0.182	-0.613	-0.829	-0.486	-0.451	0.275	-0.643	-0.455	0.612	0.657	0.625	-0.391	
	P	0.780	0.704	0.729	0.196	0.041	0.329	0.370	0.597	0.169	0.365	0.197	0.157	0.184	0.609	X