Caudal region regeneration in a natural population of the morid fish *Physiculus cyanostrophus* in the tropical Eastern Atlantic Ocean.

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Abstract:

The present study describes a remarkable caudal regeneration capacity of *Physiculus cyanostrophus*, a benthopelagic fish from the twilight zone. This trait reveals the ability of this particular morid to survive attacks that would be lethal in other species. In teleosts, the most widespread sublethal predation is confined to scale-eating and fin-nipping by specialized predators. In *P. cyanosytrophus* the larger sized individuals the greatest amount of tissue loss occurred in the caudal region (maximum 28 vertebrae, corresponding to 45% lost body length). The amount of regenerated tissue was independent of body losses and ranged between 5-19% of total length for the entire range of sizes. The frequency of regenerated individuals in the population increases with ontogeny (16.8-67.5%). Tissue losses represent a significant allocation of costs in terms of growth and offspring production. Nevertheless, regeneration capability decreases mortality in the large breeding females of a population, maximizing maternal fitness.

Keywords: twilight zone, regeneration, tissue loss, growth, maternal fitness, *Physiculus cyanostrophus*

INTRODUCTION

In marine ecosystems, fishes are subject to injuries due to aggressive behaviour, predation and diseases (Winemiller 1990; Ziskowski *et al.* 2008; Sinclair *et al.* 2011). From a trophic viewpoint, some predators in aquatic ecosystems have specialized in sublethal attack mechanisms (Farmer & Bramish 1973; Papastamatiou *et al.* 2010). Among fishes (as prey), the most widespread sublethal predation, in both fresh and marine waters, is due to specialized scale-eating (lepidophagous) and fin-nipping predators (e.g., Sazima 1983; Winemiller 1990; Nakae & Sasaki 2002). Additionally, in marine ecosystems, some prey species have developed mechanisms to revert potential lethal attacks into sublethal injuries. This sublethal predation in marine ecosystems occurs among prey species with regenerative abilities and autotomy (Bely & Nyberg 2010), such as, crustaceans, polychaetes and echinoderms. In animals, sublethal predation is the predominant mechanism of tissue loss (Bely & Nyberg 2010). Nevertheless, it is widely accepted that sublethal predation may influence prey dynamics. The degree and consequences of these predatory effects depend on the amount of lost biomass or the loss of function resulting from sublethal attacks (Maginnis 2006).

Research studies on regeneration, based on the ability of fish to regenerate lost tissues, has largely focused on zebrafish and electric fishes (Unguez 2013). However, in nature, evidence of tissue regeneration is quite rare. From an ecological perspective, the population-level consequences of the regeneration of body parts are poorly understood in fishes (Maginnis 2006; Unguez 2013). Studies that incorporate the costs associated with the regeneration process into understanding population dynamics are necessary.

The genus *Physiculus* Kaup, 1858 currently comprises 41 valid species (see Gonzalez *et al.* 2018) exhibiting the highest degree of diversity within the family Moridae (Paulin 1989). The *Physiculus* species are mainly known from a taxonomic perspective, but few data are available concerning their ecology and population structure. The *Physiculus* species are distributed in all tropical, subtropical, and warm-temperate seas of the world at depths of 100–600 m (Paulin 1989). Some *Physiculus* species has the ability to regenerate the caudal fin. This newly regenerated fin develops a replacement skeletal structure known as the “pseudo-hypural plate” (Brüss 1986; Paulin & Matallanas 1990). Sublethal injuries (or regenerated caudal fin) frequently occur in Macrouridae and other Moridae genera to such an extent that total length has been replaced by preanal fin length in taxonomic and ecological studies (Atkinson 1991). Within benthopelagic species belonging to these groups (e.g., grenadiers, rattail fishes and deep-sea cods), a high percentage of individuals with sublethal injuries or regenerated caudal fins have been found (e.g., Paulin & Matallanas 1990; Massutí *et al.* 1995; Andrews *et al.* 1999). Nevertheless, sublethal predation in the deep-sea has not yet been explored, probably because ecological knowledge of these species is lacking and because most sublethal injuries are often attributed to the trawl net effect (Massutí *et al.* 1995). Regeneration of caudal fins has been described in macrourids and morids (Brüss 1986; Atkinson 1991) and tail loss occurs frequently in deep-sea gadiformes e.g *Bathysaurus mollis* specialises in biting tails off marcrourids and hence avoids parasitic infections (Campbell *et al.* 1980). Our study describes and evaluates the importance of caudal region regeneration in *Physiculus cyanostrophus*, including consequences in their population dynamics, growth and fitness.

MATERIALS AND METHODS

Biological data collection

Seven expeditions were undertaken to investigate the marine epi- and benthic macrofauna of the Cape Verde archipelago. Sampling was carried out off the islands of Boa Vista, Santiago, including the Bancona Bank, São Vicente, Santa Luzia, Sal, and São Nicolau in order to search for new living resources, between 2010-2012 and 2017, at depths of 66 m and 458 m. Expeditions were conducted in the spring (April and March), summer (June and July), autumn (November) and winter (January and February). In all cruises bottom traps and semi-floating traps (operated around 2.4 m above the seafloor) (see González *et al.* 2016 for trap design) were used as sampling system.

Morphology measures

Each fish was sampled (n=917) following standard measurements for family Moridae (Paulin 1989; Trunov 1989). The total length (TL), head length (HL) and regenerated total length (regTL) were measured to the nearest 0.01 mm. The theoretical total length (tTL) was obtained from the linear regression between HL and TL from the non-regenerated (NR) specimens to estimate the corresponding TL of regenerated individuals (R). Body lost length was estimated as the difference between tTL and the length after injury. Other measurements are explained in Figure S1. Gutted mass and gonad mass were recorded to the nearest 0.001 g. Sex determination was assessed according to macroscopic differences in gonads. Otoliths (n=767) were extracted, and otolith mass was recorded to the nearest 0.0001 g. Based on current ontogenetic data (maturity and fecundity), three ontogenetic groups were defined for both sexes: juveniles (females TL<210 mm; males TL<190 mm), young-adults (females 210 mm>TL< 300 mm; males 190 mm>TL< 240 mm) and old-adults (females TL>300 mm; males TL>240 mm). The specimens (n=120) were prepared with their fins fully extended and were then photographed with a digital camera for further image analysis to obtain fin surface estimates (mm^2). Similarly, specimens with a "pseudo-hypural plate", considered regenerated individuals (R), were photographed (n=73) to estimate their regenerated fin surface (body area from the beginning of the length regenerated to the end of the body, including fin surface; mm^2). Vertebral counts on a subsample of specimens (n=30) were obtained from radiographs by means of an X-ray machine and were then processed with eFilm Lite™ software. The percentage of body lost length was calculated along with the number of lost vertebrae (Fig. S4).

Growth

Otolith mass versus TL and tTL of R (n=215) and NR (n=562) females within the same size range were compared. Otolith sections from R (n=72) and NR (n=159) females were prepared according to protocols described by McCurdy *et al.* (2002). Age was estimated twice, with two researchers reading the otolith sections according to Wright *et al.* (2002). Observed length-at-age was described by the *von Bertalanffy growth model* using a non-linear least square procedure of a *Gauss-Newton algorithm* for R and NR females.

Fecundity outputs

The ovaries (n=764) were fixed and preserved in 4% buffered formaldehyde and were subsequently processed for histological analyses to verify the macroscopic maturity stage using H&E staining, following standard procedures for fish biology studies. Following the terminology of Brown-Peterson *et al*. (2011), spawning capable fish were selected from R (n=42) and NR (n=58), covering the entire range of sizes (Murua *et al.* 2003), to estimate the total fecundity (TF) and batch fecundity (BF) (Hunter *et al.* 1985). TF and BF were estimated using gravimetric methods (Murua *et al*. 2003). Each subsample was weighted to the nearest 0.0001 g and was then filtered via 100 µm mesh to exclude previtellogenic oocytes for the estimation of TF. The mesh size was estimated by calculating the diameter of previtellogenic oocytes with image analysis (Thorsen & Kjesbu 2001) from early-developed subphase ovaries, following the methods of Brown-Peterson *et al.* (2011).

Data analysis

Differences in the HL–TL relationship between the sexes were evaluated by an *F-test*, and differences between *b* values and the expected value from isometric growth (*b*=1) were evaluated by a *t-test* (Sachs 1982).

Differences in mean TL and TM between sexes were analysed using *Student's t-test*. The *Kolmogorov–Smirnov non-parametric Z-test* was used to analyse differences in TL and TM distributions between groups male-female and R-NR (each sex).

The regeneration-ratio (R:NR) and the sex-ratio (male:female) were estimated for the whole population and for each of the three ontogenetic groups. A *Pearson Chi-square test* was conducted to test the null hypothesis of equality of frequencies between R and NR and by sexes (H_0=1:1 ratio) with a significance level of α=0.05 (Sachs 1982; Sokal & Rohlf 2012).

The bias and precision of annuli counts in otoliths were compared between readers, using paired *t-tests* (Campana 2001). Estimates of ageing precision were determined using the coefficient of variation (Chang 1982). The multivariate *Hotelling's T^2-test* was used to compare growth parameters between R and NR females (Bernard 1981).

Otolith mass, TF and BF for R and NR females were represented as a function of tTL and TL, respectively. Potential regressions for different groups were statistically compared with slope homogeneity and covariance of linearized potential regression applying an *F-test* (Sachs 1982). These tests evaluated the null hypothesis of equality of two regressions estimated by sexes with a significance level of α=0.05. The same procedure was conducted to compare TF and BF within R and NR against age.

RESULTS

Regeneration pattern

A total of 917 individuals classified into two groups were sampled: non-regenerated (NR, n=659) and regenerated (R, n=258). R individuals had fewer vertebrae than NR individuals as a consequence of sublethal attacks, causing body loss in the caudal region. Most R individuals had a thicker caudal peduncle, produced by the regeneration process. By means of X-ray, the absence of a caudal skeleton, as well as the transformation of the last present vertebra after injury into a "pseudo-hypural plate" for insertion of new C-rays, was observed (Fig. 1).

Prevalence of tail regeneration in a natural population

The NR females reached a mean size and a mean mass larger than males (TL, t=8.86 $p<0.05$; TM, t=7.92, $p<0.05$) (Table S1). R females presented a mean size and mean mass greater than R males (tTL, t=6.62 $p<0.05$; TM, t=6.36, $p<0.05$). The TL and TM distributions of NR and R also differed significantly between females and males (Fig. S4b; TL, Z=3.93 $p<0.05$; TM, Z=3.72, $p<0.05$). The distribution of tTL and TM of R individuals also differed significantly between females and males (tTL, Z=2.61, $p<0.05$; TM, Z=2.62, $p<0.05$). For R females, the mean size was larger than NR (t=3.30, $p<0.05$). Conversely, a similar mean size between R and NR males was found (t=0.799, $p>0.05$) (Fig. 2a). The size distribution of R and NR groups was different, with NR females being present in the small classes, but the R group was absent in these classes (Z=1.47, $p<0.05$). Male size distributions were similar in both groups (Z=0.80, $p>0.05$) (Fig. 2b).

NR females were more abundant than NR males (1:6.3, χ^2=348.17, $p<0.05$). Size classes less than or equal to 180–200 mm TL showed similar numbers of NR males and NR females (1:1, χ^2=0.923, $p>0.05$). However, NR females were more abundant in size classes larger than 200 mm TL (χ^2>7.14, $p<0.05$), mainly over 300 mm TL, where only females were observed. R Females were also more abundant than R males (1:5.14, χ^2=117.35, $p<0.05$). With size classes, R females and R males followed the same pattern as that observed for NR individuals. Female regeneration-ratio (R:NR) was 1:2.6 (χ^2=79.02, $p<0.05$). Statistical differences were found among ontogenetic groups, where dominance of NR females decreased from juveniles (1:5.6, χ^2=16.03, $p<0.05$) and young-adults (1:3.1, χ^2=65.05 $p<0.05$) to old-adults (1:1.6, χ^2=5.84 $p<0.05$). Moreover, NR males were more abundant than R males in the ratio 1:2.1 (χ^2=11.57, $p<0.05$). This ratio was similar through the ontogeny of males (1:2.6, 1:2.1 and 1:1.8; χ^2=0.297, $p>0.05$).

Tail loss

Estimations of tTL based on the HL–TL relationship of NR individuals yielded significant differences between sexes (F=29.1403, p<0.05), being isometric in females and males (t>7.69, p<0.05). The body lost length increased with ontogeny (F>18.154, p<0.05): being 43.21±18.37 mm (mean±s.d) for juveniles, 61.90±25.29 mm for young-adults and 77.33±28.36 mm for old-adults, with the last group exhibiting the highest losses (Fig. 3a). Young-adults showed the widest range of body lost length (0–45%), (Fig. 3b). A *post hoc test* showed differences in body lost length between young-adults and old-adults (p<0.05). Indeed, these groups showed differences within juveniles (p<0.05). The relationship between tTL and the number of vertebrae in regenerated specimens is shown in Figures S2 and S3. The highest vertebrae loss was 28, corresponding with a specimen of 45% body lost length. We also measured the net balance between lost fin surface and regenerated fin surface, and our results indicate that they were completely offset until losses reached 15% of tTL (Fig. 4).

The regenerated length (regL) is independent of body lost length and of total length regenerated in % of theoretical total length (tTL). Values of regL showed a mean increase of 13±3.31 mm (mean±standard deviation, in %) and ranged between 5-19% of tTL for the entire range of sizes (Fig. S4).

Growth

Statistical differences between NR and R females were found in the otolith mass–TL power function relationship (F=180.58, p<0.05). Indeed, mean otolith mass of R females (80.187±18.47 mg) was heavier than that of NR females (70.90±20.15 mg) (t=3.696, p<0.05) for the same size range distribution.

Based on the annual growth, ring counts of 173 (75%) readable female otoliths ranged from 2 to 18 years (age). Fish younger than 2 years were absent in the study sample. The youngest R individual was 8 years. The *von Bertalanffy growth curves* for observed TL and tTL at age of R and NR females are shown in Figure 5. *Hotelling's T^2-test* showed significant differences in the growth parameters between R and NR females (T^2=106.930, p<0.05). Differences in growth between both groups increased with longevity. NR and R individuals at 9 years exhibit differences of 18 mm in TL (NR, 252 mm TL; R, 234 mm tTL), and this value increased in individuals aged 17 years: 44 mm in TL (NR, 345 mm TL; R, 301 mm tTL).

Total fecundity, offspring and maternal fitness

The fecundity outputs by ontogeny are summarized in Table 1. There were no significant differences between mean values of TF and BF between the NR and R groups (t>0.840, p>0.05). Throughout ontogeny, mean fecundity outputs between NR and R groups showed no significant differences within young-adults. Nevertheless, R old-adults showed lower BF than NR old-adults (t=2,180, p<0.05) (Table 1). TF and BF plotted against TL and tTL for NR and R, respectively, showed significant differences between groups (Fig. 6a) (F>5.03, p<0.05), wherein the slope of TF plotted against size was lower in R females than NR females. This difference was greater when TF and BF data were plotted and compared against the ages of the individuals (Fig. 6b) (F>180.30, p<0.05).

DISCUSSION

Our study describes a remarkable capacity to withstand sublethal tail loss in *Physiculus cyanostrophus* a fish species not listed in previous reviews of sublethal tissue loss in freshwater or marine ecosystems (Maginnis 2006; Unguez 2013).

Regenerated individuals in natural populations

Regenerated individuals represent a large fraction of the population (27.0%). This is a high prevalence of tail loss in a marine species far exceeding the 3.1-15.5% observed in the winter flounder in which the cause is bacterial fin-rot disease (Ziskowski *et al.* 2008). However, the distribution of regenerated individuals and its importance change within the natural population. Thus, the frequency of R individuals increases throughout ontogeny (16.8-67.5%), whereas females dominate the long-lived fraction of the population. This phenomenon may be because the species studied shows a marked sexual dimorphism, where R or NR females are bigger and more abundant than R or NR males; a common strategy in deep-water species to avoid intraspecific competition for resources (Shine 1989; Fairbairn 1997). An important consequence of ontogenetic niche shifts is that a population can potentially be divided into ecologically distinct stages (Olson 1996). Concerning the subject of ontogeny, early stages and juveniles of our studied species exhibit a pelagic behaviour, in contrast with adults, which are benthopelagic (Okamoto *et al.* 2007), as has been described in closely related groups, such as macrourids (Bergstad & Gordon 1994). These differences in ontogenetic niches could explain the absence of regenerated juvenile specimens, because predation is more likely to be lethal.

Body losses in caudal region

The ability to withstand body losses varies with size and is higher in large individuals, which can survive losses of up to 28 vertebrae, equivalent to 45% of total length. However, the capacity to regenerate the caudal zone in our studied species is limited. The balance between body lost length in a sublethal attack and the length of the newly regenerated caudal region shows a limit of maximum recovery of 19% in tTL versus a maximum of 45% of the body lost length. This limited regeneration of the caudal zone could be an adaptive mechanism, as an imperfect or limited regeneration could represent an optimal investment into the regeneration process for the injured animal (see Bely & Nyberg 2010). This result is in line with those of studies on caudal fin regeneration in fishes (e.g., Fu *et al*. 2013).

Tissue loss allocation cost in growth.

Our results describe a smaller somatic growth in regenerated females than NR females. This statement was achieved through two approaches. On the one hand, a close relationship between otolith mass and age exists (e.g., Boehlert 1985; Pawson 1990; Fletcher 1991; Worthington *et al.* 1995; Cardinale *et al.* 2000). Taking into account that otolith mass continues to increase throughout the life of an individual, even if somatic growth is small or null (e.g., Fowler & Doherty 1992, Morita & Matsuishi 2003, Ashworth *et al.* 2016), a different context can be considered when comparing regenerated and non-regenerated individuals. We found that predation does not affect the increments in otolith mass with age. Nevertheless, injuries change the growth rate, resulting in the R individuals showing a lower phenotypic growth compared to non-regenerated individuals. On the other hand, estimates of age based on the number of growth increments also reveal differences between R and NR individuals. Our results showed that the youngest R individual was 8 years old. The magnitude and effects of the body loss on growth have a different degree of influence according to the percentage of body lost and the elapsed time from the injury. This can be seen in the disribution of age data. Therefore, the size of R individuals compared to NR individuals depends on the time since the injury, and on the percentage of body loss. When a natural population is studied, the period in which the sublethal attack occurred on individuals is unknown. Therefore, our results indicate that regeneration implies a reduction in long-term growth. The benefit of structure replacement in their lifetime will depend on the age of the animal at injury and its expected longevity (Vitt & Cooper 1986). The slow growth and long age (18 years) observed in our species compensates for the energy allocation during the regeneration process, since in the long term, it benefits from producing offspring during many seasons.

Tissue loss effects in offspring and maternal fitness

In nature, two well-differentiated strategies can be developed by a species depending on whether natural selection maximizes the maternal or offspring fitness. Species in which evolution has prioritized offspring fitness tends to produce a single, very large offspring (Bernardo 1996). In contrast, species wherein selection favours maternal fitness tend to have a greater number of offspring with low fitness. The ability of mothers to produce lower quality offspring (selfish maternal effect) does not imply a worsening of maternal fitness, but quite the opposite (Marshall & Uller 2007). Our studied species is a batch spawner. This reproductive strategy maximizes maternal fitness and produces numerous smaller offspring (batches). Our comparative results on fecundity outputs between R and NR groups showed no significant differences in mean values. However, across the ontogeny, the R older-adults showed low offspring fitness. Indeed, the slope of the relationship between fecundity or offspring and size for the whole population showed lower values in R individuals. If we add growth as a factor in offspring fitness, these differences become greater. Since there are regenerated individuals with less growth than individuals without regeneration allocations (NR), the ability to regenerate the lost caudal area allows the population to maintain a large number of mothers that contribute significantly to the next generation. It is important to bear in mind that, in this population group (old-adults), a higher frequency of regenerated specimens was found (67.5%). Some authors argued that the contribution of larger females to self-recruitment is significantly greater than the expected on the basis of the relationship between body size and fecundity, indicating that female size has an important maternal effect on traits in their offspring (see Beldade *et al*. 2012).

Survivorship of R individuals in the population of our studied species, even at the expense of a decrease in offspring, generates a relevant contribution to maternal fitness at the population level. Following Einum & Fleming (2000), selection acts to maximize maternal fitness. Mothers tend to make smaller, more numerous offspring, effectively sacrificing individual offspring fitness for maternal fitness.

Loss of the tail

While it is true that sublethal predation implies an important allocation cost in different groups of metazoans (Alvarado 2000), it should be noted that the most widespread sublethal predation in teleosts is reduced to scale-eating and fin-nipping specialized predators (e.g., Sazima 1983; Winemiller 1990). In this scenario, sublethal predation in fishes has been usually considered as an opportunistic mechanism to keep prey abundant as a rapidly renewable resource for specialized predators (Northcote *et al.* 1986; Nico & Taphorn 1988). Our study suggests a well developed capacity of *Physiculus cyanostrophus* to survive following a predator attack. However, the putative predator has not been identified. The loss of the tail and regrowth with a pseudo-hypural plate suggests the tail loss was traumatic event rather than disease as in the winter flounder (Ziskowski et al. 2008).

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COMPETING INTEREST

The authors declare no competing interests.

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SUPPORTING INFORMATION

Additional Supporting Information can be found online in the supporting information tab for this article.

TABLE CAPTIONS

Table 1. Mean values (± standard deviation) and range (minimum-maximum) of total fecundity (TF) and batch fecundity (BF) for non-regenerated (NR) and regenerated (R) individuals of *Physiculus cyanostrophus* in young-adults and old-adults ontogenetic groups. *Significant differences ($p<0.05$) between bold values.

FIGURE CAPTIONS

Figure 1. (a) *Physiculus cyanostrophus* with intact and regenerated tails. (b) Radiographs of three regenerated individuals (R) within 10 and 35% of body losses in total length (blTL). Examples of a regenerated caudal region "pseudo-hypural plate" with regenerated new c-rays.

Figure 2. Size distribution of males (a) and females (b) population of *Physiculus cyanostrophus*. Bars correspond to individuals for each size class of 10 mm TL. Green bars are regenerated individuals (R), and black bars non-regenerated ones (NR).

Figure 3. Box-plot showing ranges of body lost length (a) and in % of theoretical total length (tTL) (b) for juveniles, young-adults and old-adults in regenerated individuals of *Physiculus cyanostrophus*. Different letters above bars denote statistically significant differences ($p<0.05$) between ontogenetic groups. The line within each box is the median value, box ends represent the inner and outer quartiles, and whiskers are the inner and outer tenths.

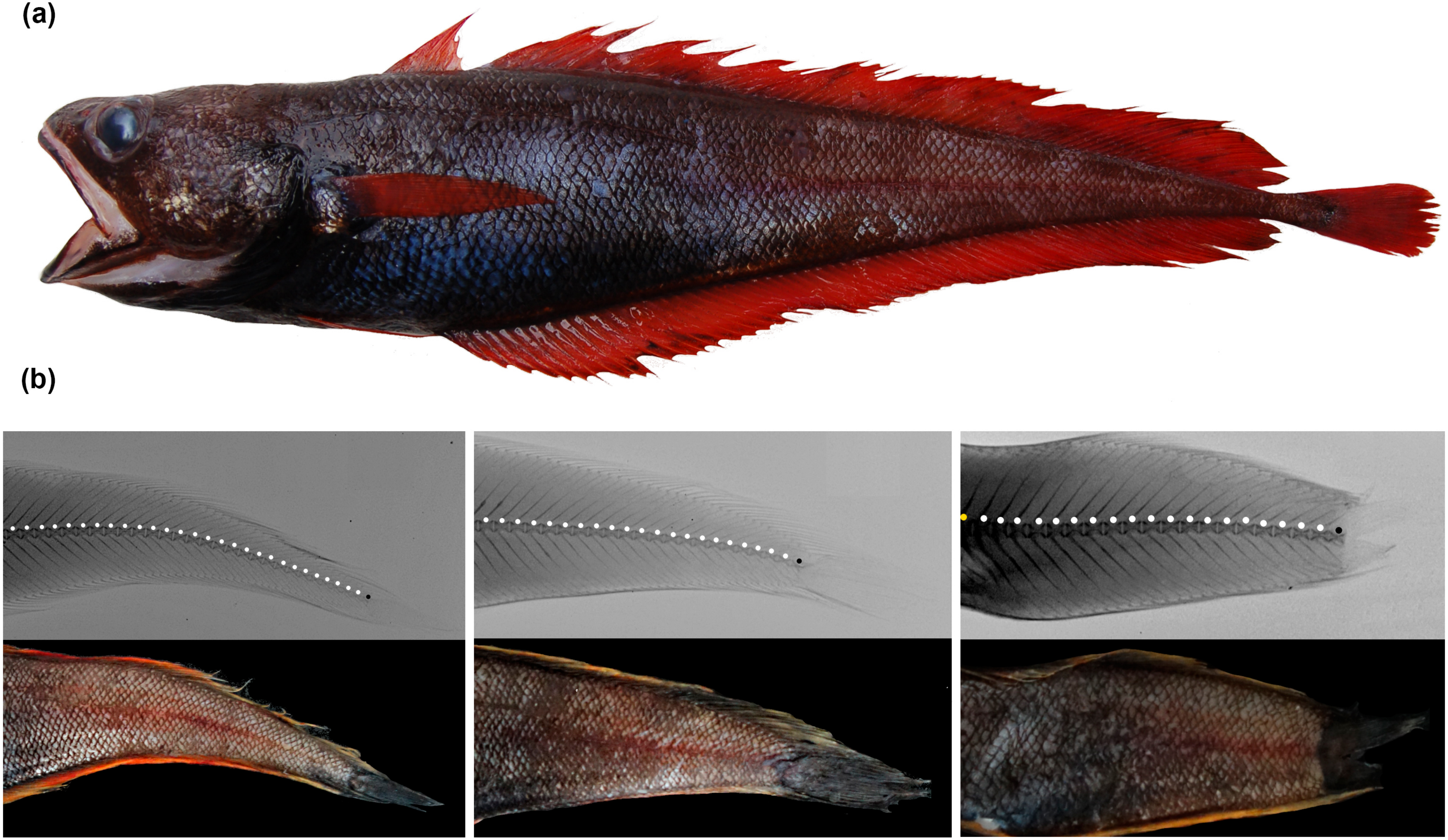
Figure 4. (a) Schematic representation of *Physiculus cyanostrophus* lost fin surface (red) and regenerated fin surface (green) for each % in total body lost length. (b) Net balance in fin surface for each % of body lost length of *P. cyanostrophus*. The green line denotes regenerated fin surface, the red line represents lost fin surface, and the grey line represents the balance between both measurements. Net balance means values for each interval of body lost length have been indicated. Net balance was completely offset up to losses of 15% of the total length.

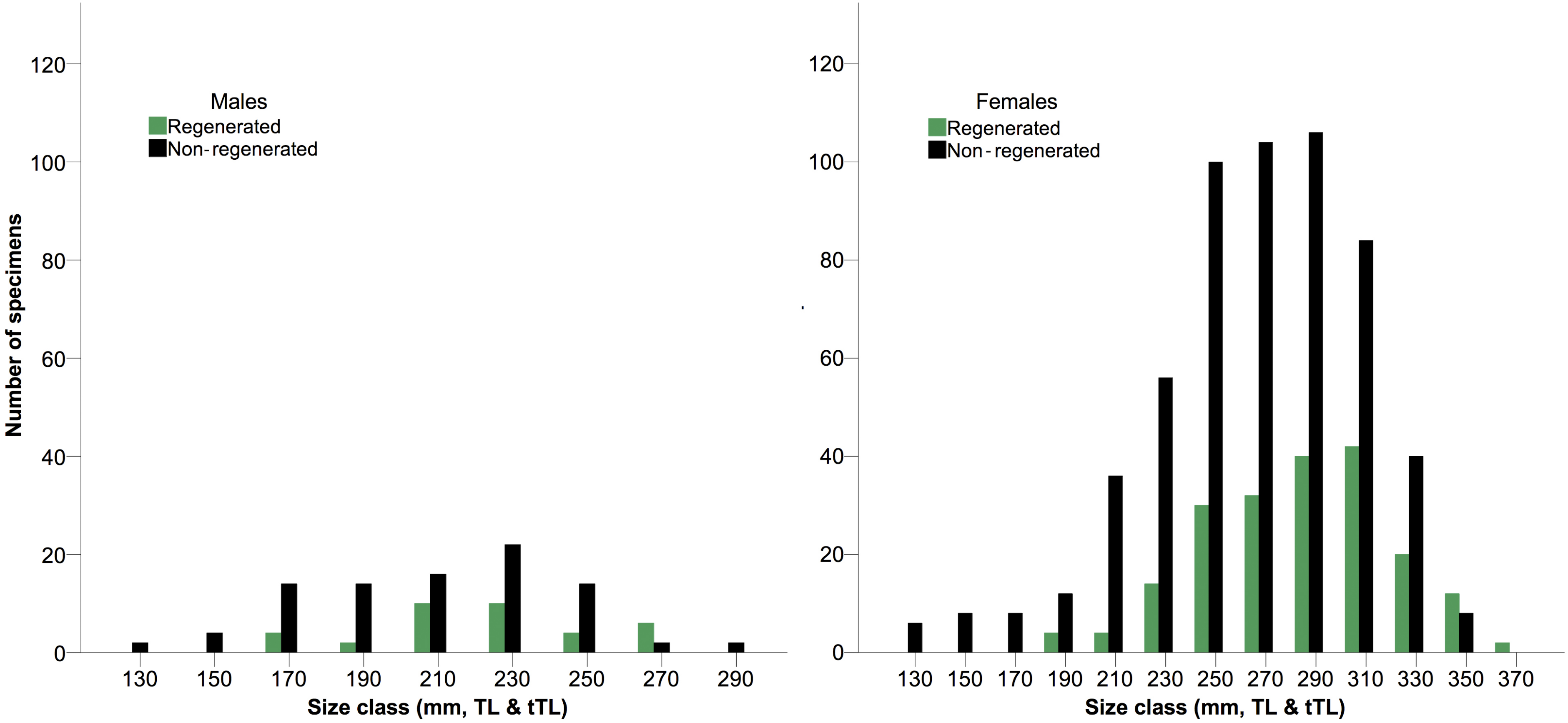
Figure 5. Length-at-age data for regenerated (R) and non-regenerated (NR) females of *Physiculus cyanostrophus* with fitted von Bertalanffy growth curves: $LT = L_\infty [1 - \exp(- k(t - t_0))]$, where LT is the length of fish at age t, L_∞ is the estimated asymptotic length, k is a constant that determines the rate at which TL approaches L_∞, and t_0 is the hypothetical age at zero length. Age-range for regenerated individuals was 8-18. No individuals younger than 2 years were located in the studied population. Percentage agreement between otolith readings of Reader 1 and Reader 2 (83.6-86.2%) was similar. Moreover, the exact match of the average readings between both readers was high (85.3%) with a quite low variability of IAPE (t=1.654 p<0.001) and CV indices (3.9% and 3.4%, respectively).

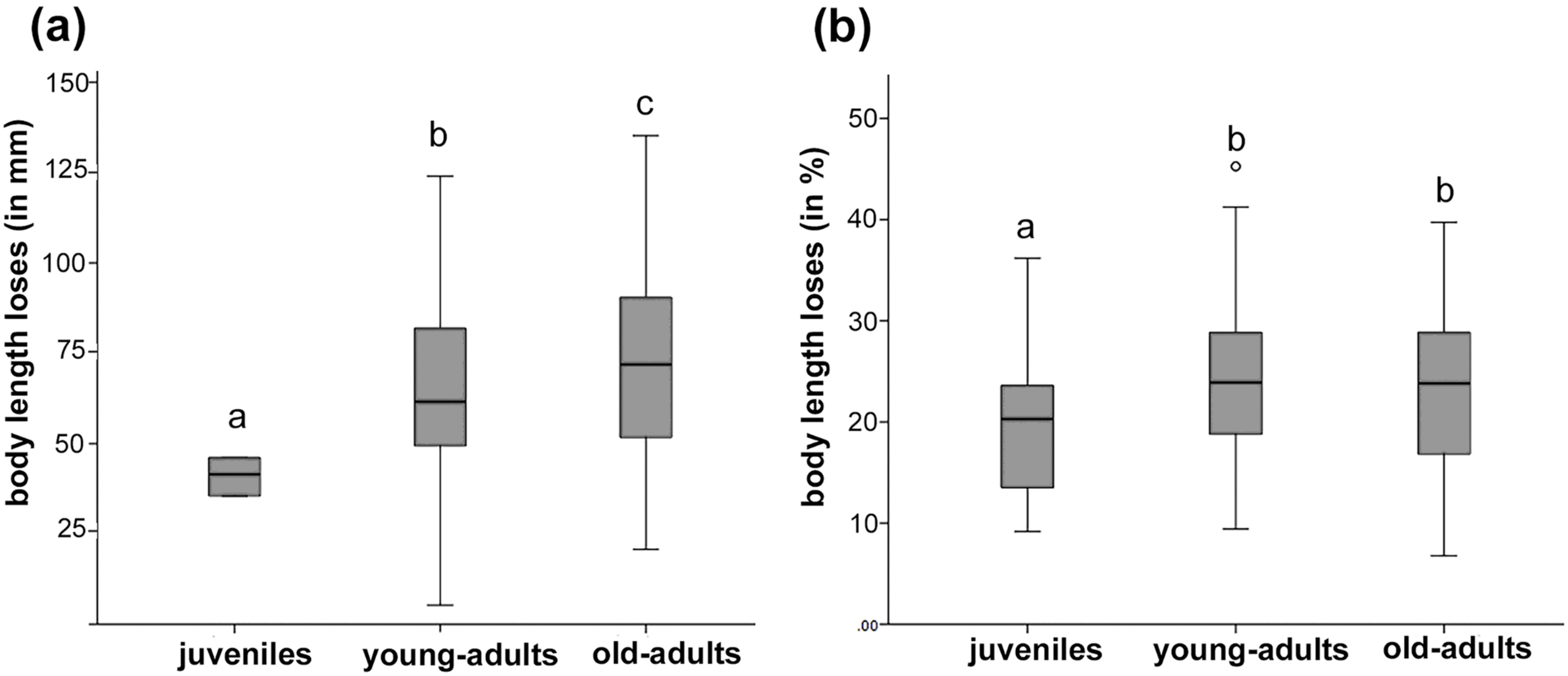
Figure 6. Power function relationship of total (TF; a and b) and batch fecundity (BF; c and d) between length (a and c) and age (b and d) of *Physiculus cyanostrophus*. Black circles are non-regenerated (NR) individuals, whereas the green triangles are regenerated (R) individuals.

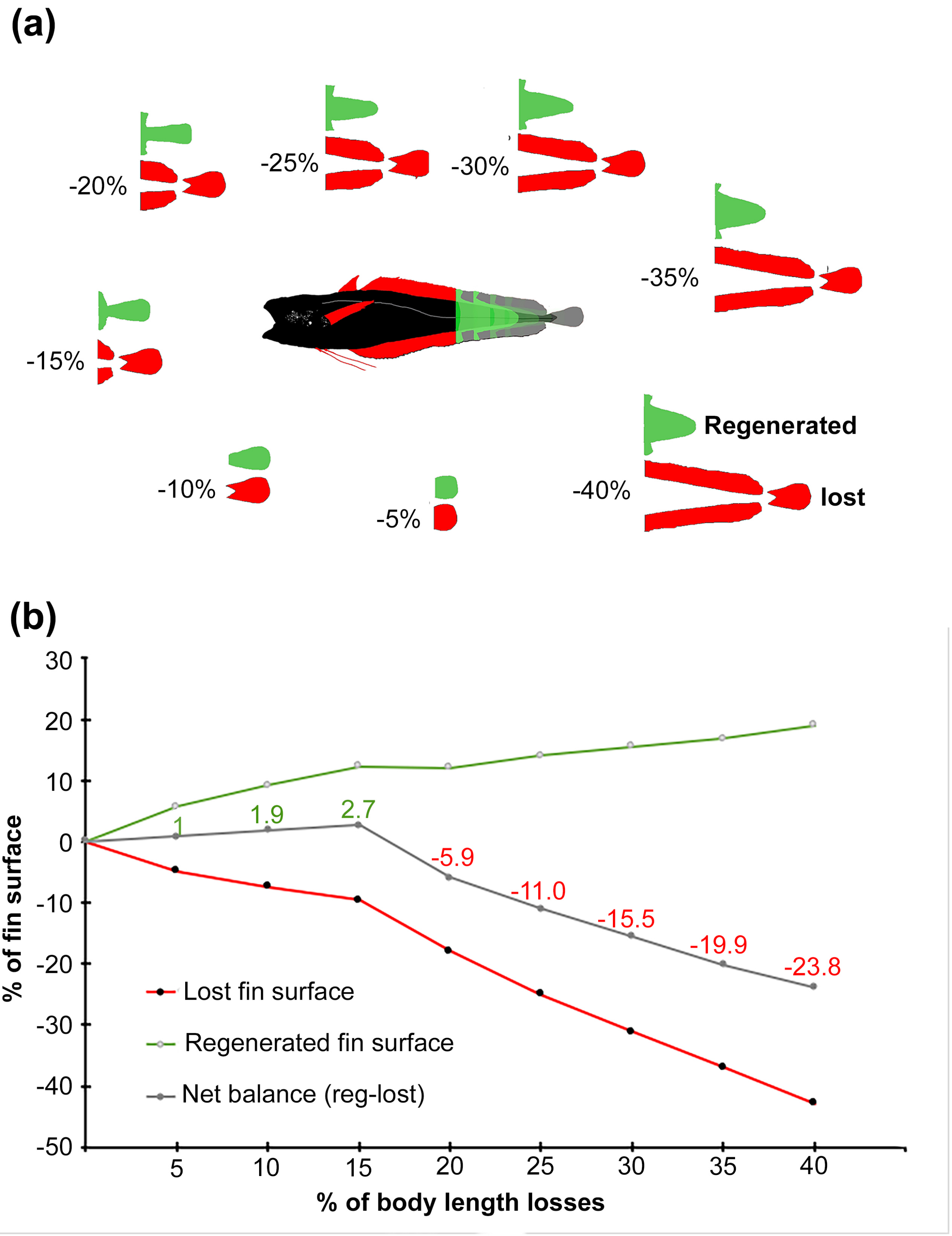
Table 1.

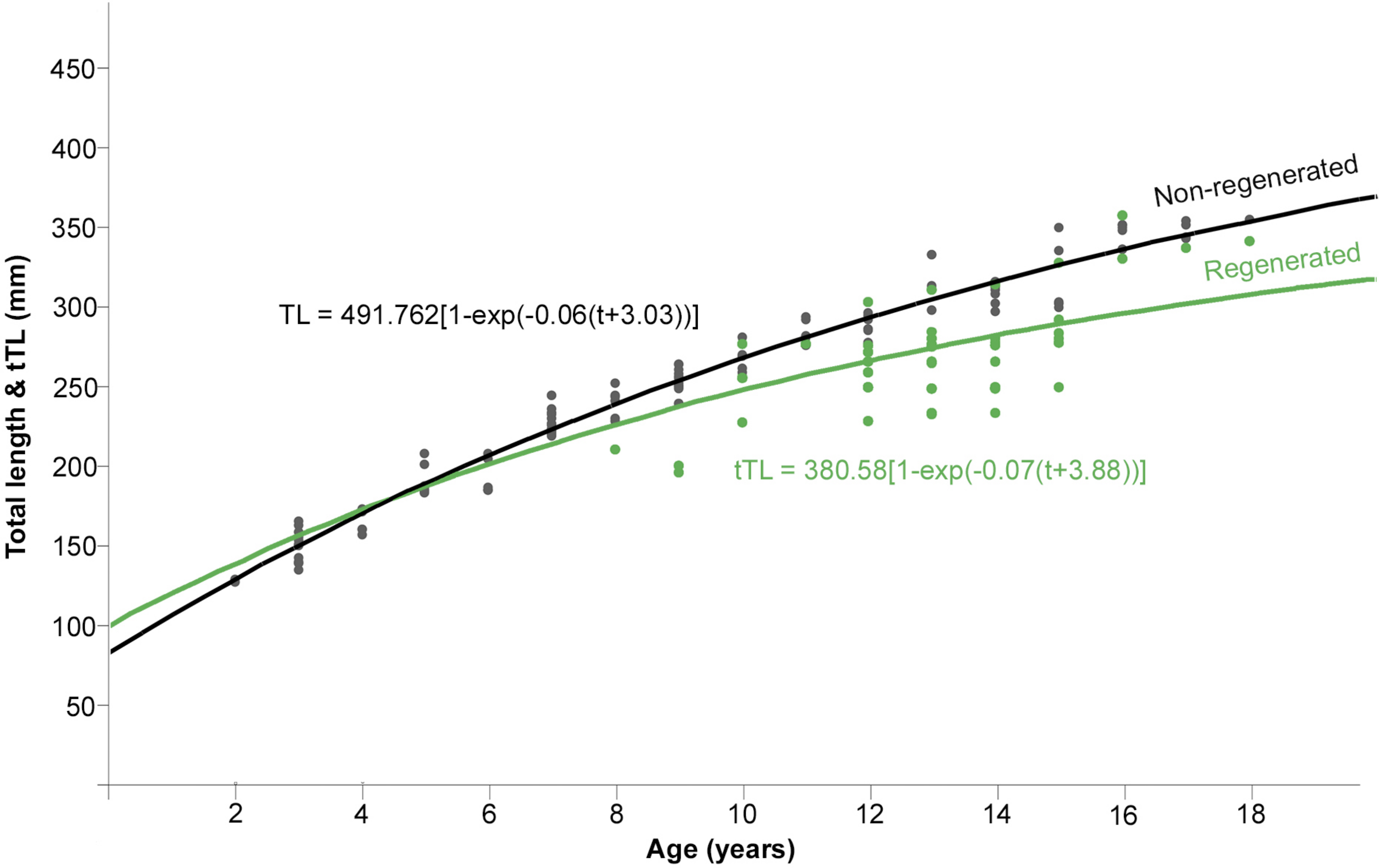
	young-adults (200 < TL < 300 mm TL)								**old-adults (TL > 300 mm TL)**							
	Non-regenerated (n=31)				**Regenerated (n=27)**				**Non-regenerated (n=20)**				**Regenerated (n=25)**			
	Min	**Max.**	**Mean**	**SD**	**Min**	**Max.**	**Mean**	**SD**	**Min**	**Max.**	**Mean**	**SD**	**Min**	**Max.**	**Mean**	**SD**
TF	12,545.19	69,902.06	35,558.39	2,993.72	23,700.58	46,756.23	31,143.98	1,400.24	33,056.43	137,322.37	65,999.81	5,821.83	8,320.83	117,309.64	53,907.20	6,623.64
BF	2,212.05	29,766.23	10,018.10	1,080.44	4,651.03	14,930.94	9,140.64	783.87	8,488.68	44,959.84	**18,254.08***	1,705.28	1,632.03	27,698.11	**12,473.30***	1,758.57

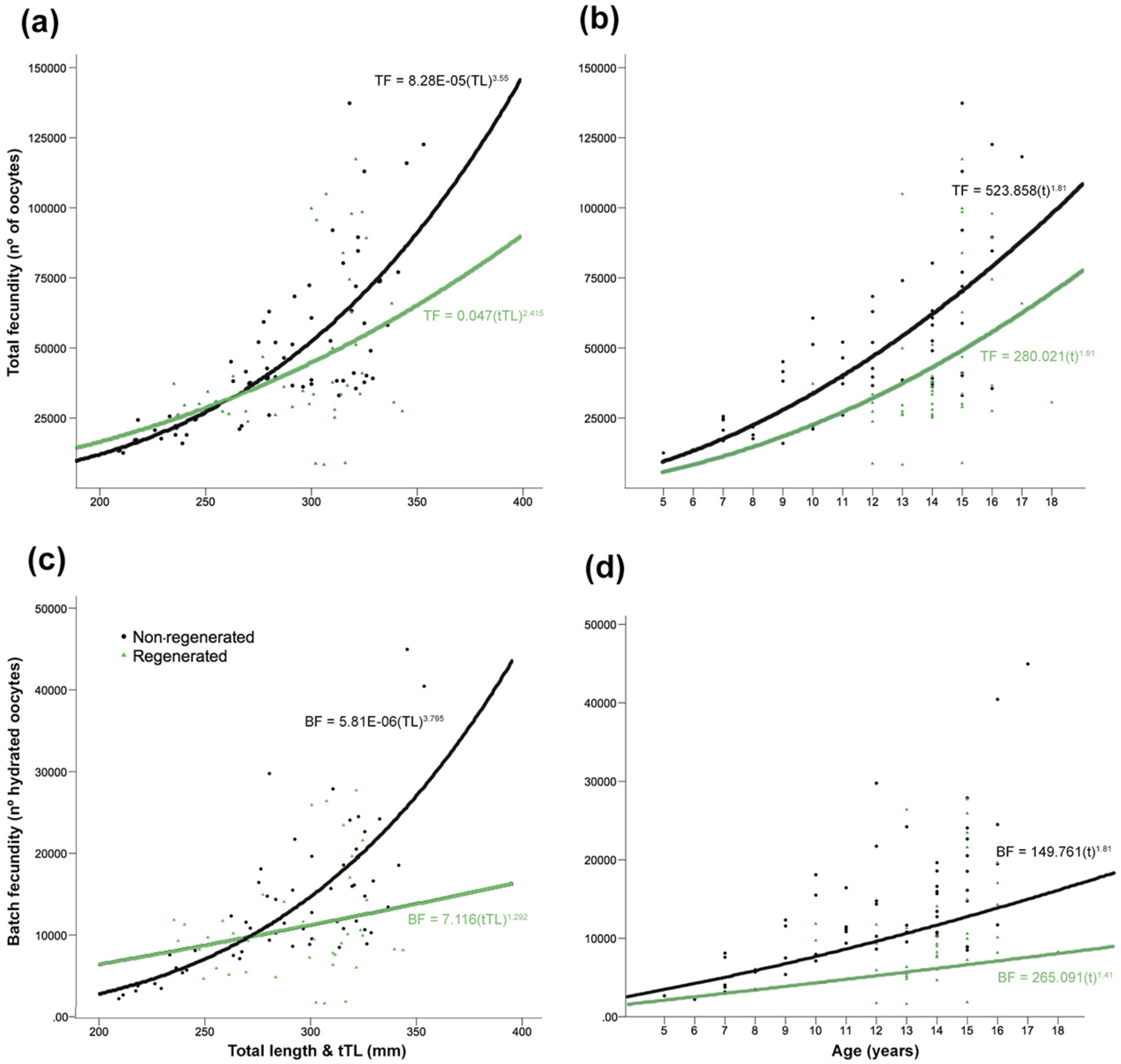

(a)
(b)


Males
Regenerated
Non-regenerated
Females
Regenerated
Non-regenerated
Number of specimens
0
20
40
60
80
100
120
130
150
170
190
210
230
250
270
290
310
330
350
370
Size class (mm, TL & tTL)
Size class (mm, TL & tTL)


(a)
(b)
body length loses (in mm)
150
125
100
75
50
25
a
b
c
juveniles
young-adults
old-adults
body length loses (in %)
50
40
30
20
10
.00
a
b
b
juveniles
young-adults
old-adults


(a)
-20%
-25%
-30%
-35%
-15%
-10%
-5%
-40%
Regenerated
lost
(b)
% of fin surface
30
20
10
0
-10
-20
-30
-40
-50
% of body length losses
5
10
15
20
25
30
35
40
1
1.9
2.7
-5.9
-11.0
-15.5
-19.9
-23.8
Lost fin surface
Regenerated fin surface
Net balance (reg-lost)


450
400
350
300
250
200
150
100
50
Total length & tTL (mm)
2
4
6
8
10
12
14
16
18
Age (years)
Non-regenerated
Regenerated
TL = 491.762[1-exp(-0.06(t+3.03))]
tTL = 380.58[1-exp(-0.07(t+3.88))]


(a)
(b)
(c)
(d)
Total fecundity (nº of oocytes)
Batch fecundity (nº hydrated oocytes)
150000
125000
100000
75000
50000
25000
50000
40000
30000
20000
10000
.00
200
250
300
350
400
5
6
7
8
9
10
11
12
13
14
15
16
17
18
TF = 8.28E-05(TL)^3.55
TF = 0.047(tTL)^2.415
TF = 523.858(t)^1.81
TF = 280.021(t)^1.91
● Non-regenerated
▲ Regenerated
BF = 5.81E-06(TL)^3.795
BF = 7.116(tTL)^1.292
BF = 149.761(t)^1.81
BF = 265.091(t)^1.41
Total length & tTL (mm)
Age (years)

Supporting information

Caudal region regeneration in a natural population of the morid fish *Physiculus cyanostrophus* in the tropical Eastern Atlantic Ocean.

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Applied Marine Ecology and Fisheries Group (EMAP), University Research Institute for Environmental Studies and Natural Resources (i-UNAT), Universidad de Las Palmas de Gran Canaria, Campus de Tafira, Las Palmas de Gran Canaria, 35017, Las Palmas, Spain.

This supporting information contains the following:

Table S1. Total length, theoretical total length (TL & tTL in mm) and total mass (TM in g) values for males and females of none regenerated (NR in TL) and regenerated (R in tTL) individuals of *Physiculus cyanostrophus*. Min, minimum; max, maximum; n, number of specimens; mean, mean value followed by ± standard deviation.

		n	size range (min-max)	mean size	mass range (min-max)	mean mass
NR	Females	569	129.00-353.09	269.09±2.51	11.82-278.51	124.68±3.25
	Males	90	130.00-290.00	211.28±4.86	12.88-132.71	57.95±3.92
R	Females	216	191.73-370.68	287.20±3.74	34.43-268.50	144.58±5.38
	Males	42	175.04-279.00	225.40±7.16	12.88-132.71	61.33±5.69
	total	917				

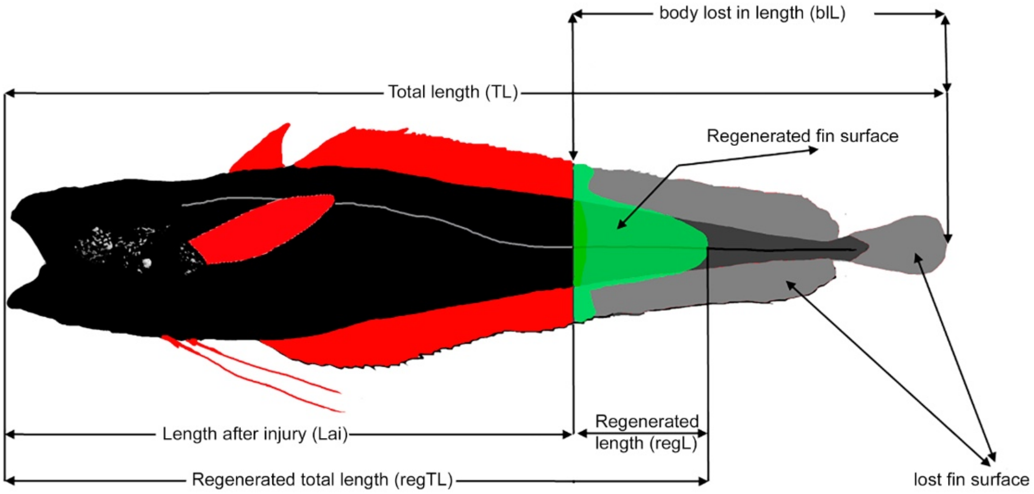

Figure S1. Scheme of the main morphological data used in the study on *Physiculus cyanostrophus*. Total length (TL), length after injury (Lai, body length from snout to the beginning of regenerated area), regenerated length (regL, body length from the beginning of regenerated area to the end of the body), regenerated total length (regTL, length after injury + regenerated length) were measured to the nearest 0.01 mm. Body lost length (blL) was estimated as the difference between tTL – Lai. Regenerated fin surface (area from the beginning of regenerated area to the end of the body, including fin surface, mm^2). For R individuals, the theoretical area (in %) was calculated by the relationship between TL and Tba (in %) from the NR individuals. Specimens (n=120) were prepared with fully extended fins and then photographed with a digital camera for further image analysis to obtain fin surface (mm^2). Similarly, specimens with “pseudo-hypural plate”, considered as regenerated individuals (R), were photographed (n=73) to calculate the regenerated fin surface (Body area from the beginning of the length regenerated to the end of the body, including fin surface, mm^2).

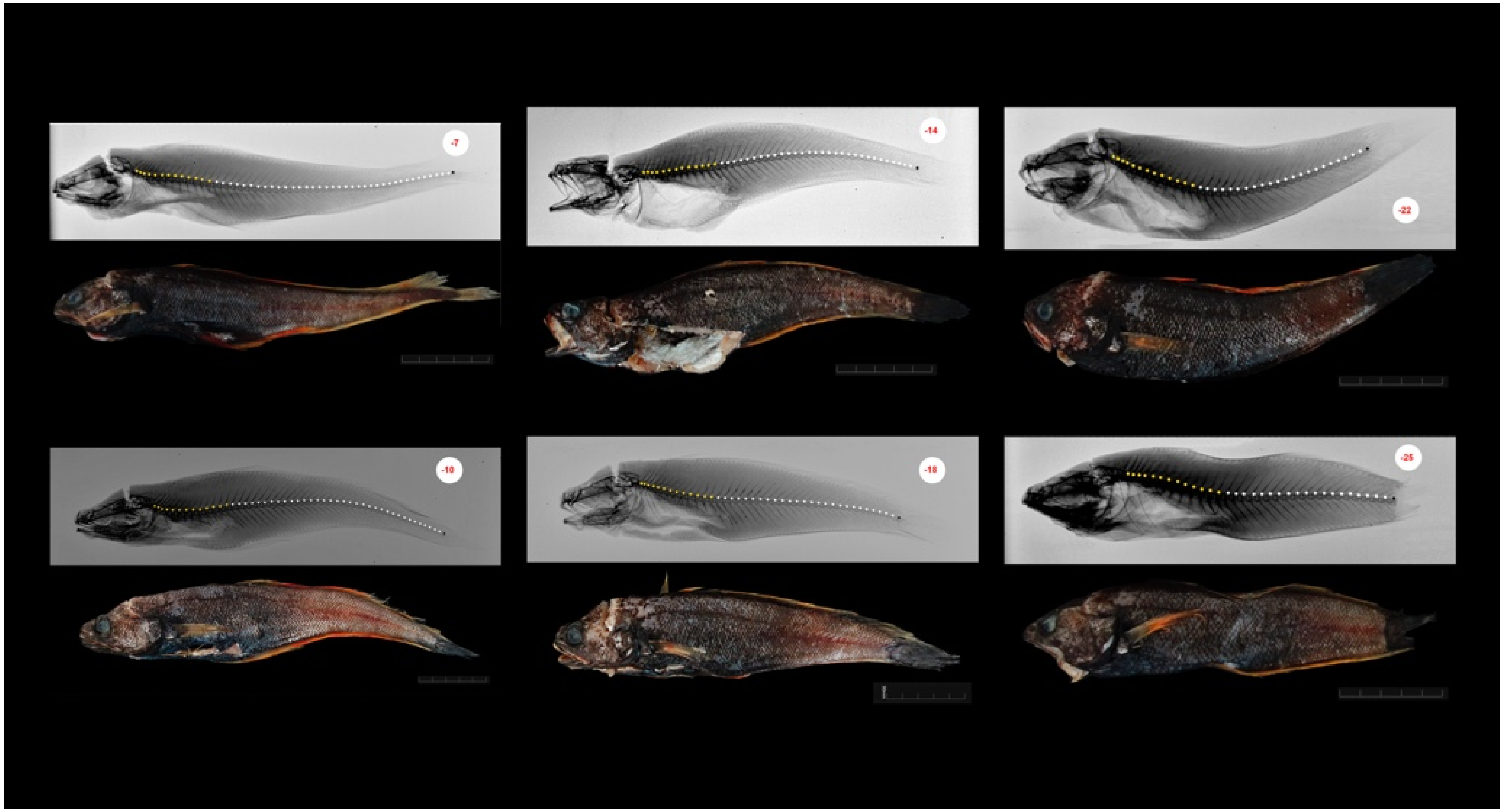

Figure S2. Some examples of *Physiculus cyanostrophus* with regenerated caudal zone. Numbers in red indicate the number of lost vertebrae for each individual.

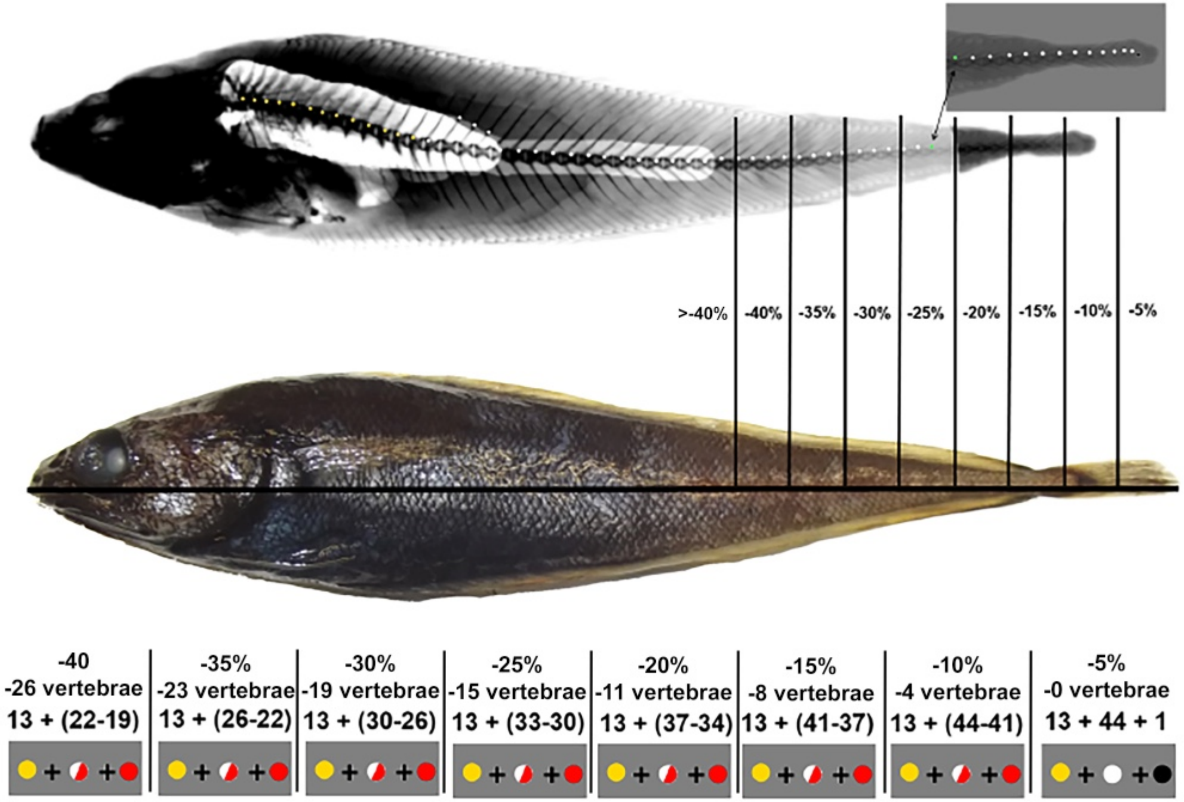

Figure S3. Relationship between % of body lost length (BlL) and losses in vertebrae in *Physiculus cyanostrophus*. Yellow spots represent number of abdominal vertebrae, white spots represent caudal vertebrae, and black spots represent hypural plate (13, abdominal vertebrae + 44, caudal vertebrae + 1, hypural plate). Numbers in parentheses represent the range of vertebrae lost for each %. Red spots represent the vertebra type lost. For a 5% BlL no vertebrae were lost, while for a 40% BlL represents the loss of 26 vertebras

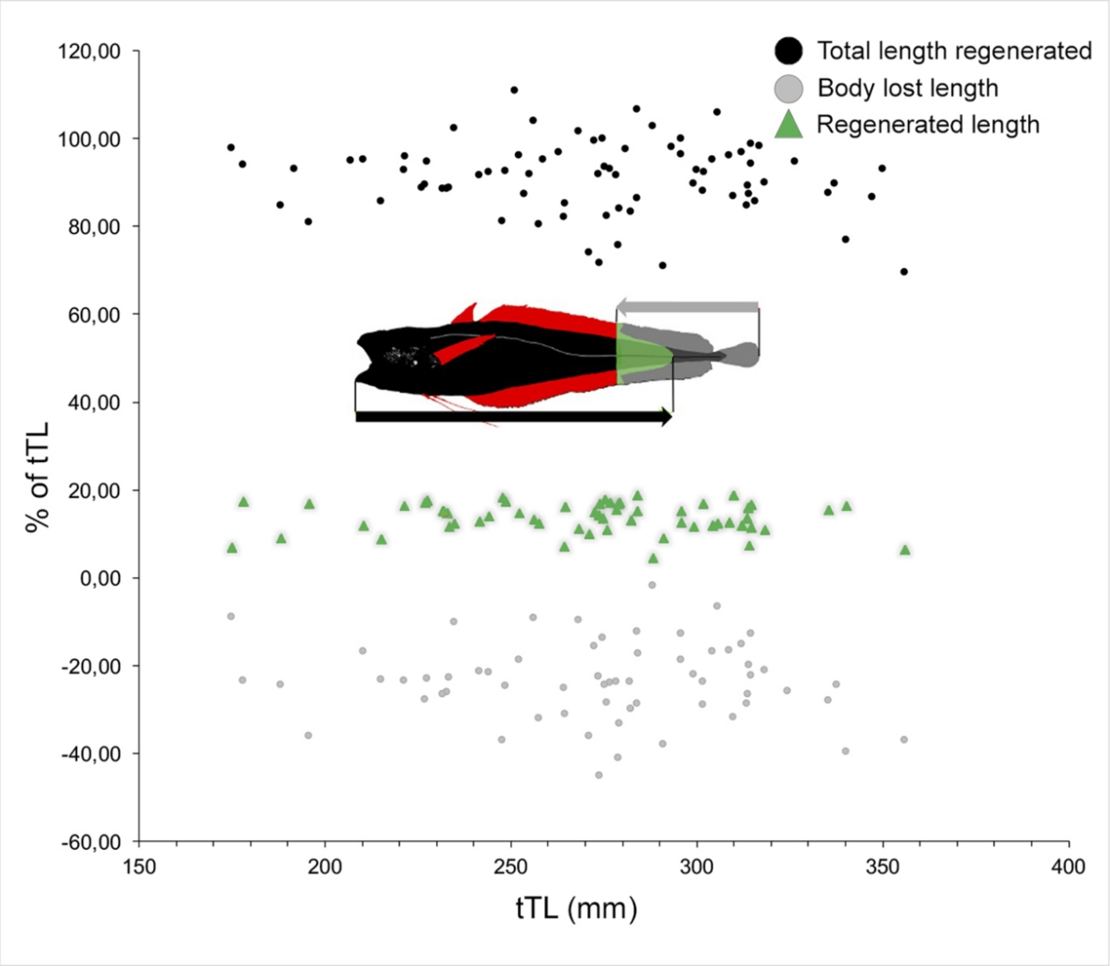

Figure S4. The regenerated length (regL) is independent of body lost length and total length regenerated in % of theoretical total length (tTL). regL showed a mean increase of 13±3.31 mm (mean ± standard deviation, in %) and ranged between 5-19% of tTL for the entire size range. Regeneration in *Physiculus cyanostrophus* is limited to 19% of tTL.