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Dams and fish morphology: effects on four endemic species in the Nestos River

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Abstract. Dams can significantly impact biodiversity upstream and downstream of their locations. The effects vary based on factors such as dam size, location, design, and management practices. The most common impacts include habitat alteration, changes in water quality, altered sediment transport, altered flow regimes, and the spread of invasive species and diseases among wildlife populations. It is postulated that such obstacles may alter the morphological characteristics of fish species. This research explored the impact of hydroelectric dam installations on the morphological variation of four endemic fish species—*Squalius orpheus* Kottelat and Economidis (2006); *Barbus strumicae* Karaman (1955); *Alburnoides strymonicus* Chichkoff (1940); and *Cobitis strumicae* Karaman (1955)—in the River Nestos, Greece. Using multivariate analysis of variance (MANOVA), the study assessed morphometric and meristic characteristics in fish populations residing upstream and downstream of the dams. Notable differences in morphometric variables were observed between *B. strumicae* and *A. strymonicus*, with downstream populations exhibiting a more streamlined body than upstream populations. Conversely, no significant differences were observed in *S. orpheus* and *C. strumicae*. In *A. strymonicus*, significant meristic differences were noted, specifically in the dorsal and caudal fins, with the downstream population having more caudal fin rays and fewer dorsal fin rays than the upstream group. These variations indicate adaptations that influence fish swimming capabilities, with downstream fish displaying a more hydrodynamic form due to increased water velocity.

Keywords: *Barbus strumicae*, *Squalius orpheus*, *Alburnoides strymonicus*, *Cobitis strumicae*, artificial lakes, morphological analysis, multivariate analysis.

Introduction

In recent decades, there has been a growing demand for the exploitation of both surface and underground water resources for hydroelectricity, recreation, agriculture, and municipal water supplies (Vogl & Lopes 2009). This demand has increased stress on ecosystems, sometimes causing irreversible damage, not only to hydrologic patterns but also to the diversity of fish fauna (Vogl & Lopes 2009, Yavno et al. 2013).

Dams, one of the most widespread human interventions in aquatic environments since ancient times (Jansson et al. 2000), have supported human development but also caused significant ecological consequences, including the decline or extinction of fish groups (Dynesius & Nilsson 1994, Daniels et al. 2005). Acting as traps for water, sediments, and nutrients, dams transform lotic environments into lentic ones and disrupt floodplain connectivity. They alter hydrological regimes, degrade spawning grounds, and modify key physico-chemical properties such as water temperature and dissolved oxygen, thereby affecting migration, reproduction, and survival of aquatic fauna (Jackson & Marmulla 2001, Morais et al. 2009).

These changes can reduce populations of stenothermic, endemic, or native species, shift trophic dynamics, and favor only tolerant taxa (Kawara et al. 1998).

In freshwater ecosystems, such impoundments isolate fish populations upstream and downstream, exposing them to divergent environmental conditions (temperature, turbidity, pollution, and flow rate), which in turn promote phenotypic differentiation (Vehanen & Huusko 2011). Phenotypic plasticity in body shape is considered a mechanism for survival in altered habitats (Vehanen & Huusko 2011), reflecting environmentally induced gene expression (Schmitt 1997, Schmitt et al. 1999, 2003, Agrawal 2001). Environmental variables, such as temperature and hydrodynamics, are known to influence meristic and morphometric traits (Lindsey 1962, Beacham 1990, Blob et al. 2008). Moreover, during ontogeny and evolution, habitat shifts drive morphological changes that reduce drag and improve locomotion (Webb 1984, Sagnes et al. 2000).

Morphometric analysis is a well-established tool for quantifying shape variation, assessing stock structure, and supporting fishery management (Cadrin & Friedland 2005, Turan 2004, Nama et al. 2022). It is widely adopted due to its affordability and ease of data collection. To address the limitations of traditional methods, the truss network system introduced by Strauss & Bookstein (1982) has been used.

The Truss network system (Strauss & Bookstein 1982) offers robust approaches for capturing body-shape variation and identifying population differences, with applications ranging from species discrimination (Palma & Andrade 2002) and ontogeny studies (Hard et al. 1999) to functional morphology research (Dean et al. 2006).

Numerous studies have shown that habitat fragmentation and altered flow regimes drive morphological divergence in fish populations (Storfer 1999, Pringle 2001, Schmitt et al. 2003, Haponski et al. 2007, Kerfoot & Schaefer 2006, Boquiren et al. 2023).

In this context, the River Nestos in Northern Greece is particularly important, as it harbors several endemic species strongly associated with environmental conditions. Four abundant endemic species with different biological niches were selected as model taxa: *Squalius orphe*us Kottelat & Economidis 2006, a rheophilic species common in running and standing waters; *Barbus strumicae* Karaman 1955, a benthic fish preferring rocky or sandy substrates; *Alburnoides strymonicus* (Bloch 1782), inhabiting clean, well-oxygenated streams; and *Cobitis strumicae* Karaman 1955, a benthic loach with cryptic behavior found in sandy and muddy bottoms (Economidis et al. 2009, Froese & Pauly 2025). *B. strumicae* is a benthic fish in rivers and creeks with rocky or sandy bottoms (Economidis et al. 2009). *A. strymonicus* inhabits clean, well-oxygenated, fast-flowing water (Economidis et al. 2009), while *C. strumicae* is benthic with cryptic behavior, hiding in muddy or sandy bottoms of slow-flowing springs and lowland waters (Economidis et al. 2009, Froese & Pauly 2025).

These species provide an excellent model system for assessing how altered hydrological conditions upstream and downstream of dams influence body-shape variation.

This study aims to evaluate whether lentic habitats created by dams drive morphological and meristic divergence in these endemic fishes. Specifically, we test the hypothesis that populations exposed to different environmental regimes upstream and downstream of dams exhibit significant differences in body shape and morphometric traits. Establishing such links is crucial for understanding the role of phenotypic plasticity in the persistence of fish under anthropogenic disturbance and for guiding conservation and management strategies in Balkan freshwater ecosystems.

Materials and methods

Study area

The River Nestos is one of the largest rivers in northern Greece, originating from the Rila Mountains in Bulgaria and flowing into the North Aegean Sea after covering 234 km (104 km in Bulgaria and 130 km in Greece) (Fig. 1). In the Greek part, two Hydro-Electric Power Plants (HEPPs) have been constructed by the Public Electricity Corporation (PEC): the Thisavros dam (operating since 1997) and the Platanovrysi dam (operating since 1999), forming two artificial lakes (Sylaios & Bournanski 2009) (Fig. 1). An irrigation weir, constructed in 1966, is located 30 km from the river's mouth near Toxotes village. According to Koutrakis et al. (2013), 22 fish species have been recorded in the Nestos River, of which 14 are endemic. The most abundant species belong to the Cyprinidae family, with *S. orpheus* and *B. strumicae* particularly prevalent. Smaller species with cryptic behavior, like *C. strumicae* and *Oxynoemacheilus bureschi*, were also noted.

To assess trait divergence, species with different habitat requirements were chosen: *B. strumicae*, *C. strumicae*, *S. orpheus*, and *A. strymonicus*. The dams have divided the river into three ecosystems: two lacustrine and one free-flowing downstream stretch, each with distinct physico-chemical and hydrological conditions (Psilovikos et al. 2006, Kamidis 2011). Water discharge from the bottom of the reservoirs during power production results in a rapid temperature drop downstream (Kamidis et al. 2007), with variations up to 12°C. The river system includes a lacustrine section from the Greek-Bulgarian border to the Thisavros dam, another from Thisavros to the Platanovrysi dam, and the riverine section from the Platanovrysi dam to the river mouth. Due to the lack of fish passes, these dams inhibit both upstream and downstream fish migration, though the weir in Toxotes allows for catadromous fish movement (not anadromous).

Sampling

To explore the potential impact of dams on fish morphology, four seasonal samplings were conducted during two years (2012-2014) in sampling stations both upstream and downstream of the installations using a portable electrofishing device (Hans Grassl Direct Pulse Current Electrofishing Device IG200/2) at 80 Hz, following CEN standards for electric fishing (CEN 2003). Sampling continued until the target number of specimens was reached. Specimens were preserved in a 7% (w/v) formalin solution. To reliably test whether dam-induced environmental differences shape fish morphology, A total of 40 specimens of each species from both upstream and downstream populations were collected (Table 1). Adequate sample size ensures stable estimates of body shape, sufficient statistical power in multivariate analyses, and accounts for natural variability among individuals (Kocovsky et al. 2009, Rummel et al. 2024). Previous morphometric studies recommend a minimum of 30–40 specimens per group to obtain robust and reproducible results, particularly when using geometric morphometrics with multiple landmarks (Zelditch et al. 2004).

Figure 1. Map of the River Nestos and the sampling stations in the main course and tributaries of the river. The sampling stations designated with the letter M and a number refer to the stations on the main course of the river, while those designated as T and a number denote the tributary stations.

Table 1. Number of specimens of *S. orpheus*, *B. strumicae*, *A. strymonicus*, and *C. strumicae* caught at each sampling station in the upstream and downstream sections of River Nestos.

	Squalius orpheus	*Barbus strumicae*	*Alburnoides strymonicus*	*Cobitis strumicae*
Station	Upstream			
M1	12	6	18	0
M4	0	0	17	11
T1	25	5	5	4
T2	3	14	0	7
T3	0	4	0	13
T4	0	11	0	5
	Downstream			
T5	5	6	6	9
T6	11	3	12	2
M2	2	2	8	8
M3	20	20	9	10
M4	2	9	5	11

To minimize artifacts in morphometric analyses caused by preservation, all specimens were preserved for the same period before analysis (Franssen 2011). Sex determination was performed by optical observation of the gonads. The main characteristics of each sampling station, including temperature and water flow, were measured using a Valeport 105 current meter (Table 2). The mean monthly fluctuation in water flow downstream of the Platanovrysi dam, as recorded by Kamidis (2011), ranged from 12.1 m^3/s to 172.5 m^3/s (Kamidis et al. 2007). Sampling stations were categorized into upstream and downstream groups based on their position relative to the dams. The Analysis of Similarity (ANOSIM) was applied to verify this separation (Clarke & Warwick 1994).

The morphometric variables of the specimens were measured using a modified Strauss & Bookstein (1982) truss network protocol, involving 25 landmarks on the fish body, yielding 39 morphometric distances (D1 to D39) (Fig. 2, Table I.1). Measurements were recorded with a NIKON Digital Sight DS – L2 system, featuring a NIKON DS-Fi1 camera and a NIKKOR AF 1, 2.8D lens, accurate to 0.01 mm. Small insect mounting pins helped locate landmarks such as fin tips.

Five meristic characters were recorded for *S. orpheus, B. strumicae*, and *A. strymonicus*, including scales in the lateral line and ray counts for dorsal, caudal, anal, and ventral fins. For *C. strumicae*, instead of scales, the number of black spots in Gambetta's longitudinal pigmentation zones was noted.

Table 2. Mean annual water flow measured in the sampling stations. The bottom substrate of each station is also provided.

	Station	Water flow (m/s)	Substrate
Upstream stations	M1	6.67	Rocky bottom with no vegetation.
	T1	4.51	Rocky and muddy substrate and no vegetation.
	T2	0.94	Rocky bottom with no vegetation.
	T3	1.51	Rocky and sandy substrate.
	T4	0.31	Rocky and muddy substrate with some vegetation.
Downstream stations	M2	26.44	Sandy bottom.
	T5	2.32	Rocky substrate with no vegetation.
	T6	0.02	Sandy bottom with some rocks.
	M3	19.41	Sandy bottom with some rocks and spots with mud and vegetation.
	M4	32.86	Sandy bottom.
	M5	24.34	Sandy bottom.

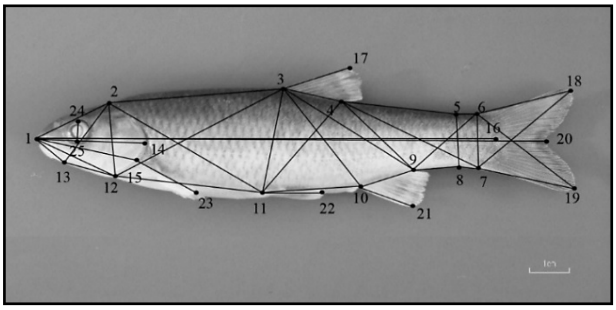

Figure 2. The arrangement of 25 landmarks on a fish's body resulting in a set of 39 morphometric distances.

Statistical Analysis

A Multivariate Analysis of Variance (MANOVA) was initially used to assess morphometric differences associated with sexual dimorphism. MANOVA was also employed to compare morphological variables between upstream and downstream fish, with Discriminant Function Analysis (DFA) used to calculate discrimination functions. SIMPER analysis identified the morphometric parameters contributing most to population dissimilarities.

All morphometric data were standardized to eliminate size effects (Beacham 1990, Anvarifar et al. 2011). Standardization was based on Reist's (1985) method:

$$M_{adj}=\log M - b^{*}(\log SL - \log SL_{mean})$$

where M_{adj} is the standardized value, M is the initial measurement, SL is the standard length, SLmean is the mean value of the standard length of all specimens, and b is a coefficient that is calculated from the overall linear regression of logM against logSL (Reist 1985).

Statistical analyses were performed using StatPoint STATGRAPHICS Centurion XVI and Primer v.6, with significance set at $\alpha = 0.05$.

To interpret the results and examine the hydrodynamic profile of the fish, the Fineness Ratio (FR) was calculated as the ratio of total body length to maximum body depth, excluding fins (Scarnecchia 1988). The variation in FR was assessed using the coefficient of variation (CV), and significance between upstream and downstream FR was tested with multiple t-tests.

Results

The ANOSIM test indicated that the “upstream” sampling stations were significantly different from the “downstream” station (Global R = 0.564, p > 0.005).

All specimens of each species, irrespective of sampling station, were analyzed using MANOVA to evaluate morphometric differences attributable to sexual dimorphism. The analysis revealed no significant differences for any species (*S. orpheus*: Wilks' λ = 0.211, F(37, 42) = 1.303, p = 0.121; *B. strumicae*: Wilks' λ = 0.403, F(37, 42) = 1.682, p = 0.052; *A. strymonicus*: Wilks' λ = 0.494, F(37, 42) = 1.162, p = 0.317; *C. strumicae*: Wilks' λ = 0.481, F(37, 42) = 1.225, p = 0.261). As such, data from both sexes were pooled for each species.

No significant differences were found concerning morphometric variables of the upstream and downstream groups of *S. orpheus* and *C. strumicae* (*S. orpheus*, Wilks' λ = 0.465, F (37, 42) = 1.307, p = 0.199; *C. strumicae*, Wilks' λ = 0.513, F (37, 42) = 1.076, p = 0.407). Moreover, the MANOVA test showed that there were no significant differences regarding the meristic variables of the fishes (*S. orpheus*, Wilks' λ = 0.959, F (5, 74) = 0.636, p = 0.673; *C. strumicae*, Wilks' λ = 0.904, F (8, 71) = 0.946, p = 0.485). The mean FR for *S. orpheus* ranged from 3.98–4.82 (mean = 4.37) for the upstream group and 3.07–5.04 (mean = 4.35), while for *C. strumicae* it ranged from 2.96–3.74 (mean = 3.30) for the upstream and 3.02–3.59 (mean = 3.31) for the downstream group (Table 3). In both species, multiple t-tests did not indicate any significant differences between the FR of fish of the upstream and downstream groups (p = 0.66

for *S. orpheus* and p = 0.88 for *C. strumicae*).

For *B. strumicae*, the standardized coefficients revealed the traits for each variable: which variable had higher values in the upstream population and lower in the downstream population, and vice versa. It must be mentioned that the standard and fork length (D17 and D35, respectively) were not used in the statistical analysis, after elimination of the size effect (Table I.2). MANOVA revealed the presence of significant differences (Wilks' λ = 0.397, F (37, 42) = 1.726, p < 0.05) between the upstream and downstream populations. The variables that discriminated the two groups were D1, D5, D6, D7, D8, D9, D20, D28, D30 and D38 (Table 2). Combining the MANOVA results and the standardized coefficients, the upstream fish had higher values for M5, M6, M20, M28, M30, and M38, and lower values for M1, M7, M8, and M9, whereas the opposite was true for the downstream fish. Regarding the meristic variables, the DFA test showed no significant differences (Wilks' λ = 0.965, F (5, 74) = 0.535, p = 0.749). The SIMPER analysis indicated that the parameters D28, D38, D30, and M8 contributed most to the discrimination between the two populations. The average dissimilarity was 0.17 for D28, 0.15 for D38, 0.15 for D30 and 0.14 for D8. The FR ranged from 4.05–5.20 (mean = 4.61) for the upstream fish and 4.16–5.35 (mean = 4.69) for those downstream (Table 3). However, multiple t-tests did not indicate any significant difference between these two groups (P = 0.22).

Table 3. The Fineness Ratio (FR) of the four species under investigation in the River Nestos. The mean value for the FR and Coefficient of variation (CV) are shown in parentheses.

	Fineness Ratio		
	Total	Upstream	Downstream
Squalius orpheus	3.70 - 5.04 (4.36 / 0.06)	3.98 - 4.82 (4.37 / 0.04)	3.07 - 5.04 (4.35 / 0.07)
Barbus strumicae	4.05 - 5.35 (4.64 / 0.06)	4.05 - 5.20 (4.61 / 0.06)	4.16 - 5.35 (4.69 / 0.06)

For *A. strymonicus*, the standardized coefficients revealed the traits for each variable, except for the standard and fork length (D17 and D35, respectively) (Table 3 and Appendix I Table I.3). MANOVA showed the presence of significant differences (Wilks' λ = 0.245, F (37, 42) = 3.496, p < 0.0001) between the upstream and downstream populations. The morphometric distances that discriminated the two groups were D3, D4, D5, D8, D9, D18, D19, D21, D28, D31, D33 and D37 (Table I.3). Combining the results of the MANOVA test and the standardized coefficients, the upstream fish had higher values for the measurements D8, D18, D21, D28 and D33 and lower measurements for D3, D4, D5, D9, D19, D31 and D37. The SIMPER analysis indicated that the parameters D37, D18, D33, and M4 contributed most to the discrimination of the two populations. The average dissimilarity was 0.27 for D37, 0.26 for D18, 0.25 for D33 and 0.24 for D4. The FR was also significantly different between the upstream and the downstream fish (P < 0.0001) and ranged from 3.25–4.34 (mean = 3.65) for upstream fish and 3.39–4.42 (mean = 3.89) for downstream fish (Table 3). The higher FR of the downstream fish indicates a more streamlined body shape in contrast to the upstream fish.

As well as differences in the morphometric variables, the DFA revealed significant differences in the meristic variables of the two groups (Wilks' λ = 0.759, F (5, 74) = 4.678, p < 0.001). The meristic variables that discriminated the two groups were the number of rays in the dorsal and the caudal fins. The standardized coefficients revealed the traits for each variable. According to the standardized coefficients, the downstream population has more caudal fin rays and fewer dorsal fin rays than the upstream population, and vice versa.

Discussion

Statistical analyses revealed significant morphometric differences between upstream and downstream groups for one of the studied species, *B. strumicae* and *A. strymonicus*. However, no significant differences were observed for *S. orpheus* and *C. strumicae*. The morphometric variables distinguishing *B. strumicae* and *A. strymonicus* appear to influence their swimming abilities. Specifically, downstream fish exhibit a more hydrodynamic shape. According to Gilbert et al. (2020), this shape divergence may result from changes in the riverine environment, particularly the introduction of deep, still-water (lentic) habitats (see Table 1).

Many studies have demonstrated that both biotic and abiotic conditions, such as water flow and trophic environments, can drive morphological differentiation (Hendry et al. 2006, Bhagat et al. 2006). Research by Mclaughlin & Grant (1994), Brinsmead & Fox (2002), and Kerfoot & Schaefer (2006) shows that fish in high-velocity water environments, like streams, tend to develop more hydrodynamic, fusiform body shapes. Conversely, in lentic (still water) environments, fish tend to have deeper bodies, while in lotic (flowing water) environments, streamlined forms are favored, which reduce drag (Webb 1984, Mclaughlin & Grant 1994, Gaston & Lauer 2015, Geladi et al. 2019). Haas et al. (2010) observed that *Cyprinella venusta* in the Mobile River Basin had more hydrodynamic shapes than those in reservoirs. Similarly, Blob et al. (2008) found that *Sicyopterus stimpsoni* adapted to fast-flowing waters with a body shape that minimizes drag.

These morphological differences might be due to adaptive phenotypic plasticity, allowing species to adapt to new environments, as noted in studies on *Lepomis gibbosus* by Robinson & Wilson (1996). In the River Nestos, native species have adapted to both the lacustrine environment of the reservoir and the high flow downstream.

For *A. strymonicus* in the River Nestos, key distinguishing features between upstream and downstream populations include the length of caudal fin lobes and the base of median fins (caudal and anal fins). Elongated caudal fin lobes enhance thrust, improving swimming efficiency (Simonovic et al. 1999), which is advantageous in high-velocity environments. An increased base length of median fins reduces drag in such conditions (Webb 1984, Sagnes et al. 2000). Additionally, a longer caudal peduncle in downstream *A. strymonicus* further reduces energy loss and maximizes thrust (Robinson & Parsons 2002).

Significant variation was also observed in meristic variables for *A. strymonicus*, with the downstream population having more caudal fin rays but fewer dorsal fin rays compared to the upstream population. This variation might be an adaptation to minimize drag and energy loss, but environmental factors, such as water temperature during juvenile development, can also influence these traits (Beacham 1990, Lindsey 1962).

B. strumicae similarly developed a more streamlined body, with the downstream population having a larger anal fin base, akin to adaptations observed in *Cottus carolinae* (Kerfoot & Schaefer 2006). These changes likely help maintain position in higher-flow conditions.

Significant morphological differences were observed only in *B. strumicae* and *A. strymonicus*, while *S. orpheus* and *C. strumicae* showed no clear divergence between upstream and downstream populations. This pattern suggests that the observed changes may not necessarily represent fixed evolutionary adaptations but rather reflect phenotypic plasticity or accommodation to contrasting hydrological regimes. In fishes, body-shape plasticity is often a first response to altered flow and habitat structure (Webb 1984, Robinson & Wilson 1996), providing a functional advantage without requiring genetic differentiation. Given that less than two decades have passed since dam construction, it is perhaps more realistic to interpret these differences as evidence of high adaptive potential and rapid morphological adjustment, rather than fully established evolutionary adaptations. The absence of divergence in *S. orpheus* and *C. strumicae* also highlights species-specific responses, potentially related to ecological niche breadth, behavior, or habitat use. Thus, the findings of the current study underscore both the limits and the potential of morphological plasticity in endemic freshwater fishes subjected to anthropogenic habitat alteration.

The absence of significant morphometric or meristic differences between *S. orpheus* and *C. strumicae* suggests that these species either possess ecological and behavioral traits that buffer them against hydrological alterations or that their responses occur over longer evolutionary timescales. *S. orpheus* shows broad ecological tolerance, thriving in both lentic and lotic habitats (Matthews 1998, Blanck & Lamouroux 2007), while the cryptic behaviour of *C. strumicae* reduces direct exposure to hydrodynamic pressures (Kottelat & Freyhof 2007). Therefore, their apparent stability may reflect adaptive resilience rather than a lack of response. Alternatively, these species may be undergoing slower, subtler changes not yet detectable within 15 years after dam construction (Franssen 2011). This finding is biologically important, as it highlights interspecific differences in adaptive capacity and provides insight into which species may be more vulnerable or resilient to anthropogenic habitat modifications. In other fish species from different regions, predation has been shown to shape body form and drive morphological divergence (Schluter 1996, Langerhans & DeWitt 2004, Langerhans 2008). Although predation was not examined in this study, it may represent one of the environmental pressures that could partly explain the absence of morphological differentiation in these two species.

These variations are likely expressions of genetic plasticity, allowing species to adapt to different environmental conditions (Schmitt 1997, Schmitt et al. 1999, 2003, Agrawal 2001).

Such studies are significant because they reveal how fish populations can respond rapidly to anthropogenic alterations, such as dam construction, which is among the most widespread global pressures on freshwater ecosystems (Nilsson et al. 2005, Grill et al. 2019). In the River Nestos, only 15 years after dam construction, up to the period of sample collection, two species had already exhibited significant morphological divergence, demonstrating how quickly hydrological changes can drive adaptive shifts. This underscores both the sensitivity and variability of species' responses, highlighting the balance between resilience and vulnerability. With dam numbers continuing to increase worldwide, understanding such rapid adjustments at the local scale provides valuable insight into broader conservation challenges. Therefore, the River Nestos serves as a case study that illustrates not only the biological plasticity of fish but also the urgent need to manage altered hydrosystems to safeguard biodiversity.

In conclusion, despite only 15 years since dam construction, upstream and downstream groups of *B. strumicae* and *A. strymonicus* show significant morphological adaptations related to hydrodynamic efficiency and swimming performance, reflecting adjustments to the altered hydrological conditions resulting from the operation of two HEPPs on the River Nestos.

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Appendix I

Table I.1. Morphometric characteristics analysed for the species in the current study (after Holcik et al. 1989).

Code	Distance	Description
D1	1-2	Length of head.
D2	2-3	Predorsal distance.
D3	3-4	Length of dorsal fin base.
D4	4-5	Distance from the end of the dorsal fin base to the upper point of minimum body depth.
D5	5-6	Distance from the upper point of minimum body depth to the upper point of caudal fin beginning.
D6	6-7	Caudal peduncle height.
D7	7-8	Distance from the lower point of minimum body depth to the lower point of caudal fin beginning.
D8	8-9	Distance from the end of the anal fin base to the lower point of minimum body depth.
D9	9-10	Length of anal fin base.
D10	10-11	Distance from insertions of pelvic and anal fin.
D11	11-12	Distance from pectoral fin base and ventral fin base.
D12	12-13	Length of throat.
D13	13-1	Lower jaw length.
D14	1-12	Maxillary length.
D15	1-14	Distance between snout and operculum.
D16	1-15	Prepectoral distance.
D17	1-16	Standard length.
D18	2-11	Distance from the rear end of skull to the beginning of ventral fin.
D19	2-12	Head depth.
D20	2-13	Distance from the rear end of skull to the beginning of lower jaw.
D21	3-9	Distance from the beginning of the dorsal fin to the end of anal fin base.
D22	3-10	Distance between the insertion of dorsal fin and the insertion of anal fins.
D23	3-11	Distance between insertions of pelvic and dorsal fins.
D24	3-12	Distance from the insertion of dorsal fin to the isthmus.
D25	3-17	Dorsal fin depth.
D26	4-7	Distance from the end of the dorsal fin base to the lower point of caudal fin beginning.
D27	4-9	Distance from the end of the dorsal fin base to the end anal fin base.
D28	4-11	Distance from the end of dorsal fin to the beginning of ventral fin.
D29	5-8	Minimum body depth.
D30	6-9	Distance from the upper point of caudal fin beginning to .the end of anal fin base
D31	6-18	Length of upper lobe of caudal fin.
D32	6-19	Distance from the upper point of caudal fin beginning to the posteriormost point of the lower caudal fin lobe.
D33	7-18	Distance from the lower point of caudal fin beginning to the posteriormost point of the upper caudal fin lobe.
D34	7-19	Length of lower lobe.
D35	1-20	Fork length.
D36	10-21	Anal fin depth.
D37	11-22	Length pelvic fin.
D38	15-23	Length of pectoral fin.
D39	24-25	Eye diameter.

Table I.2. The canonical means and the standardized coefficients for the upstream and downstream populations of *B. strumicae* and *A. strymonicus* for the morphometric data.

	B. strumicae	*A. strymonicus*
	Root 1	Root 1
D1	4,387	0,036
D2	4,502	0,034
D3	1,062	0,303
D4	0,904	0,342
D5	0,048	0,827
D6	1,186	0,276
D7	0,186	0,666
D8	0,047	0,829
D9	0,587	0,444
D10	1,174	0,279
D11	0,005	0,942
D12	0,163	0,686
D13	0,528	0,467
D14	1,022	0,312
D15	0,375	0,540
D16	1,031	0,310
D18	0,277	0,599
D19	0,651	0,420
D20	0,065	0,798
D21	0,363	0,547
D22	0,571	0,450
D23	0,002	0,963
D24	6,024	0,014
D25	0,337	0,561
D26	0,491	0,483
D27	0,046	0,830
D28	1,452	0,228
D29	1,729	0,188
D30	0,465	0,495
D31	1,484	0,223
D32	5,564	0,018
D33	1,334	0,248
D34	1,875	0,171
D36	1,912	0,167
D37	0,328	0,567
D38	3,180	0,075
D39	0,307	0,579
	Stat.	Stat./Df
Deviance	18,383	0,227
Scaled Deviance	120,000	1,481
Pearson Chi^2	18,383	0,227
Scaled P. Chi^2	120,000	1,481
Loglikelihood	-57,708	

Table I.3. The results of MANOVA for the morphometric data of *B. strumicae*. The variables that discriminate the two populations are indicated with bold fonts ($p < 0.05$).

Distance	**Wilks' λ**	**F-remove (1,42)**	***p*-level**	**Distance**	**Wilks' λ**	**F-remove (1,42)**	***p*-level**
D1	**0.423**	**2.755**	**0.0437**	D21	0.398	0.091	0.7638
D2	0.397	0.018	0.8940	D22	0.404	0.787	0.3802
D3	0.401	0.440	0.5105	D23	0.406	1.000	0.3230
D4	0.397	0.045	0.8339	D24	0.397	0.001	0.9745
D5	**0.424**	**2.858**	**0.0498**	D25	0.397	0.057	0.8125
D6	**0.432**	**3.747**	**0.0460**	D26	0.397	0.004	0.9519
D7	**0.433**	**3.880**	**0.0355**	D27	0.401	0.441	0.5104
D8	**0.439**	**4.464**	**0.0406**	**D28**	**0.407**	**1.092**	**0.0302**
D9	**0.450**	**5.674**	**0.0218**	D29	0.402	0.536	0.4680
D10	0.418	2.235	0.1424	**D30**	**0.406**	**1.010**	**0.0321**
D11	0.398	0.092	0.7629	D31	0.397	0.003	0.9589
D12	0.402	0.522	0.4740	D32	0.399	0.188	0.6667
D13	0.409	1.256	0.2688	D33	0.397	0.005	0.9461
D14	0.397	0.026	0.8729	D34	0.423	2.751	0.1046
D15	0.399	0.208	0.6505	D36	0.397	0.014	0.9050
D16	0.413	1.671	0.2032	D37	0.397	0.043	0.8369
D18	0.397	0.001	0.9793	**D38**	**0.480**	**8.836**	**0.0049**
D19	0.433	3.843	0.0566	D39	0.400	0.291	0.5926
D20	**0.437**	**4.261**	**0.0452**				