Diet overlap of introduced atlamtc and native danubian lineages of brown trout (Salmo trutta) from inland waters

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DIET OVERLAP OF INTRODUCED ATLANTIC AND NATIVE DANUBIAN LINEAGES OF BROWN TROUT (Salmo trutta) FROM INLAND WATERS

MASTER'S THESIS

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I, **Luana Velagić** JMBAG 0178097908, born on 12 May 1994 in Pula, declare that I have independently written the thesis under the title of

DIET OVERLAP OF INTRODUCED ATLANTIC AND NATIVE DANUBIAN LINEAGES OF BROWN TROUT (*Salmo trutta*) FROM INLAND WATERS

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REPORT
ON EVALUATION AND MASTER'S THESIS DEFENSE

Master's thesis written by **Luana Velagić**, JMBAG 0178097908, under the title of
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SUMMARY

Of the master's thesis written by Luana Velagić, under the title of:

DIET OVERLAP OF INTRODUCED ATLANTIC AND NATIVE DANUBIAN LINEAGES OF BROWN TROUT (*Salmo trutta*) FROM INLAND WATERS

Brown trout has been widely introduced into suitable environments globally. In Croatia, the Danube (Da) lineage of brown trout is native. However, due to anglers' activities, brown trout of the Atlantic (At) lineage were stocked into Croatian streams and rivers. Brown trout of the At lineage have been determined as invasive, posing a threat to native Da populations. Until now, no research related to feeding competition between At and Da lineage of brown trout from inland waters has been performed. Thus, the purpose of this research is to examine the natural diet of brown trout of At and Da lineages and to compare their feeding overlapping. Feeding habits of both trout lineages are related to insects of both terrestrial and aquatic origin. High diet overlap between At and Da lineages were found. This result indicate that At lineage pose significant threat to native Da trout populations. Also, it seems that At lineage feed more by insects of terrestrial origin. Such feeding strategy could make the species more attractive for fly fishing and consequently more attractive for stocking which may complicate the implementation of conservation measures necessary to preserve the indigenous brown trout of the DA lineage.

Keywords: Trout, lineage, invasive, feeding habits, diet overlap, food sources, overstocking

1. INTORDUCTION

In recent years the concern over introduced (invasive) species and their effect on the environment has increased. Biological invasions are studied more closely, detailing impacts of invasions on whole ecosystems. Impacts are diverse and, depending on the variable, necessarily negative (David et al., 2017). Trout is a fish found all over the world, some are present naturally, and other have been introduced by human activities (some intentional for fishing, other by accident). For instance, in North America Atlantic salmon (*Salmo salar*) is a native species which evolved without the presence of European brown trout (*Salmo trutta*). The brown trout was introduced to Newfoundland in the 1880s and it rapidly spread all over the continent. It is considered invasive in much of its introduced range, i.e. in Newfoundland their year-round estuarine presence raises concerns for native salmonids (Warner et al., 2015). In New Zealand introduced brown trout shares characteristics with endemic trout species, it has been documented to disrupt natural ecosystem interactions, and in severe cases cause extinction. Juvenile brown trout feed primarily on insect larvae and small crustaceans. Later in the lifecycle, as they get bigger, they move to deeper waters and tend to be more carnivorous, feeding on larger prey, such as other fish (including cannibalism), and other unsuspecting smaller animals. This feeding behavior can result in competition between trout and other species found in the stream (Burrill, 2014). Nevertheless, terrestrial macro-invertebrates make up the biggest portion of natural feed. Their input is essential, especially during the summer months when the presence of aquatic benthic organisms is lessened; and as the temperature rises the nutritional requirements for the proper life-cycle is increased. Terrestrial invertebrates are important because they tend to fall into the streams during the day when the water level is lower, due to the fact that they remain floating on the surface, and due to their size, they provide easier and more nutritious pray than their aquatic counterparts. Today, most streams are in some way affected by humans. Use of insecticides in agriculture, and deforestation are largely to blame for the lower input of terrestrial invertebrates during summer months. Experiment in which a clear sheet was placed over the stream proved that trout growth and abundance follows the input of terrestrial organisms, in the experiment where that input was reduced trout number and size followed; proving the importance of ensuring the health of natural habitats (Eros et al., 2012). Diversity of Croatian brown trout is still debated, which results in several taxonomic differences and conservation problems, especially in protected areas. According to new genetics research, the difference between the Danube native trout and the invasive Atlantic trout has been confirmed. Systematically they have been separated in two species; Danube trout lineage (*Salmo labrax*) and the Atlantic lineage, brown trout (*Salmo trutta*) (Buj et al., 2019).

The Danube trout *Salmo labrax* is a native species in the Danube basin (Da haplotype) and non-native in Adriatic Basin of Croatia. Despite not being native to all the watercourses, it is widespread and found in almost any suitable stream all over the world. The reason for the intentional translocation/introduction of brown trout all over the world is mostly due to the ‘sport fishing’ and aquaculture. The main problem of this activities comes from the fact that most of the trout used in sport fishing and all trout found in fisheries (anglers’ brown trout stocking) belongs to the ‘alien’ strain of trout - belonging to the Atlantic lineage (At haplotype)

(Piria et al., 2019).
Until now, the differences between feeding habits of these two lineages (Da and At haplotypes) has not yet been investigated.
Thus, in this thesis the possibility of competition over food sources between the native, Danubian (Da), trout (*Salmo labrax*) and invasive Atlantic (At) trout (*Salmo trutta*) will be examined.

1.1 Hypothesis and Aims

The hypothesis of this research is that feeding habits of the invasive Atlantic lineages (At haplotype) of brown trout have an effect on the population of native Danubian lineage (Da haplotype) of brown trout.
To test this hypothesis following aims were specified:

1. to assess natural diet of trout from different streams
2. to divide At and Da feeding habits and to compare differences in feeding habits
3. to calculate feeding overlap between two lineages
4. to calculate length-weight relationship and condition of targeted species separated by different stream and by different lineages

2. LITERATURE REVIEW

2.1. Species description: *Salmo trutta* (Atlantic origin) and *Salmo labrax* (Danubian origin)

Trout is a highly adaptable euryhaline fish. The osmotic adaptation of the brown trout provides a possibility of adaptation to sea water of high salinity in the spring. Examined specimens even demonstrated an increased growth during warmer months when they were inspected in marine environment (Quillet et al. 1992). Trout species include purely freshwater populations (riverine ecotype) and migratory populations that spend most of their lifecycle in the oceans and return to fresh water only to spawn. Brown trout (*Salmo trutta*) is a native European species of *Salmonidae* fish that has today been widely distributed and established all over the world. Conservation Status of the brown trout in the ADW (Animal Diversity Web) is classified as a species of least concern (LC) by the ICUN (International Union for Conservation of Nature) regulations; however Croatian specification in the 'Red book of freshwater fish of Croatia' places it as a slightly vulnerable species (VU), mostly due to mixing with invasive salmons, and overfishing (Mrakovčić et al., 2006).

Brown trout is not threatened species, and is well managed and researched all over the world. Due to its frequency it is known that the brown trout can live longer and get larger in size than many other riverine fish. Body length parameters and creel limits of trout are established in an effort to manage its lake and river populations. Recently, native Danubian lineage of trout has been described as separated species and named black sea salmon (*Salmo labrax*) and only Atlantic lineage of trout is referred as brown trout (*Salmo trutta*), (Buj et al. 2019). However, *Salmo trutta* and *Salmo labrax* will in the future text be correspondently referred as At and Da lineages. When describing non-migratory trout in general, in future text, name trout only will be used.

2.1.1. Taxonomy

Trout belongs to the family *Salmonidae*. *Salmonidae*, together with related families of: *Esociformes*, *Osmeriformes*, and *Argentiniformes* comprise the superorder Protacanthopterygii. These families are known to inhibit both marine and freshwater environments. Genus name *Salmo* comes from scientific names of the arguably most familiar *Salmonidae* species: Atlantic salmon (*Salmo salar*), the brown trout (*Salmo trutta*), and the newly systhematic separation for the Croatian-Danube lineage of the brow trout (or black sea trout) – *Salmo labrax* (Table 1 and 2), (Buj et al., 2019).

Table 1: Taxonomic hierarchy of the Brown trout

Kingdom	Animalia
Subkingdom	Bilateria
Infrakingdom	Deuterostomia
Phylum	Chordata
Subphylum	Vertebrata
Infraphylum	Gnathostomata
Superclass	Actinopterygii
Class	Teleostei
Superorder	Protacanthopterygii
Order	Salmoniformes
Family	Salmonidae
Subfamily	Salmoninae
Genus	Salmo Linnaeus, 1758 – Atlantic salmon
Species	Salmo trutta Linnaeus, 1758 – brown trout, truite brune

Table 2: Taxonomic hierarchy of the Black sea salmon

Kingdom	Animalia
Subkingdom	Bilateria
Infrakingdom	Deuterostomia
Phylum	Chordata
Subphylum	Vertebrata
Infraphylum	Gnathostomata
Superclass	Actinopterygii
Class	Teleostei
Superorder	Protacanthopterygii
Order	Salmoniformes
Family	Salmonidae
Subfamily	Salmoninae
Genus	Salmo
Species	Salmo labrax Pallas 1814 – Black sea salmon

2.1.2. Distribution and habitat

Trout is the most common and well established Salmonidae species found in almost any European stream.
In Croatia it is found all over the country, its official Croatian name is 'Potočna pastrva' but due to its prevalence (Fig. 1) it is commonly known by many region specific names: baška, bistranjka, furela, pastrva crna, pastrva bijela, pastrva kamenjarka, struga (Mrakovčić et al. 2006).

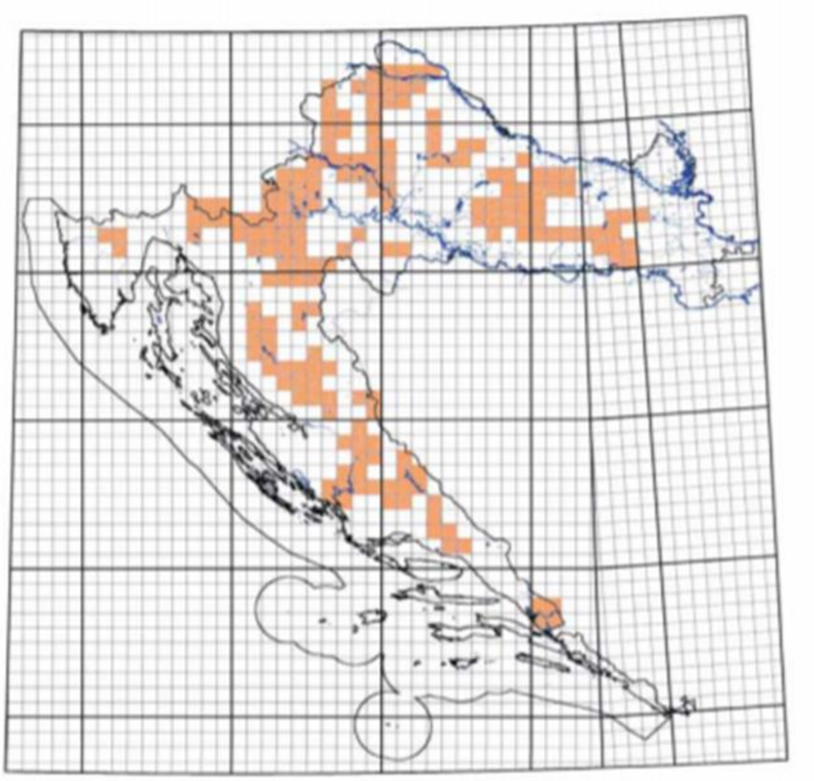

Figure 1. The distribution of brown trout (*Salmo trutta*) in Croatia
Picture source: red book of freshwater fish of Croatia. Mrakovčić M. et al (2006)

Preferred habitat of the trout are streams with moderate water currents at the upper or middle parts of streams, where water is relatively colder, and well oxygenized. Trout tolerates water temperatures up to 19°C, while the optimum temperature is between 8°C and 13°C. Younger specimens are usually found in shallower water, while adults inhibit the deeper parts of the streams (Burrill 2014). Brown trout's (At and Da lineages) native geographic range are: Europe, northern Africa, and western Asia; although today we can find them all over the world (excluding Antarctica). Some trout will spend most of their adult life in oceans, returning to freshwater streams only to spawn. In Croatia trout inhibit Black Sea river basin (Markovčić et al, 2006).

2.2. Morphology

Trout is a medium sized fish that can usually grow up the length of 50 cm, lake varieties grow much faster and can reach the length of 1,4 m and up to 20kg in weight.
The body is spindle-shaped with: two pectoral fins (12-15 rays), two pelvic fins (9-10 rays), an anal fin (10-13 rays), a dorsal fin (14-16 rays), an adipose fin, and a caudal fin. The caudal fin can appear in various shapes (i.e. broad, forked, emarginated, truncated). Usually their color represents the surrounding environment (camouflage), for brown trout that means that naturally their back are olive colored with brown and black spots. Sides are lighter in color with reddish spots surrounded by weight or light blue hues (Figure 2.) They reach sexual maturity in the second or third year of life. Spawning time depends on water temperature, beginning in late autumn and lasting until February. Female makes a small dent on the gravel stream bottom, using her tail, where she lays her eggs. Incubation time lasts from 60 to 90 days depending on the water temperature (Mrakovčić M. et al. 2006).

Figure 2. The Brown trout (*Salmo trutta*)
Picture source: Duane Raver, U.S. Fish and Wildlife Service

Trout poses a large terminal mouth with the jaw extending to the back edge of the eyes. Breeding males develop a hook-like, upward-facing protrusion on the lower jaw called a kype. They have a short a short muscular esophagus which opens to the large portion of the stomach. Shorter, and more compact, cylindrical pyloric limb opens through a powerful pyloric sphincter into the intestine. The stomach is well developed, with four coats characteristically found in a vertebrate gut. The mucosa is distinguished by the presence of dense collagen, which forms a basis to the gut-wall (Figure 3.) (Burnstock, 1959).

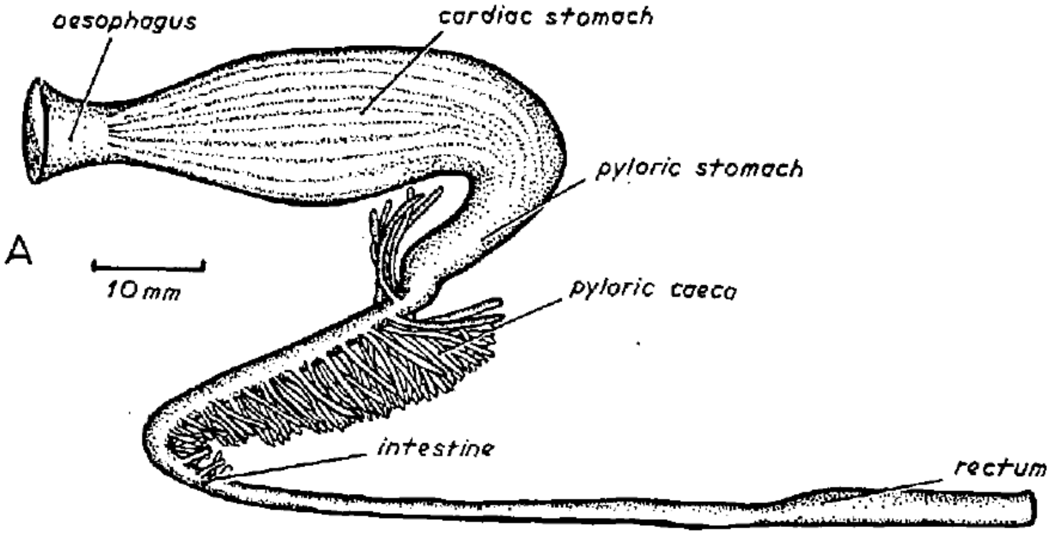

Figure 3. Brown trout gut morphology

Source: Burnstock (1959). The Morphology of the Gut of the Brown Trout (*Salmo trutta*)

2.3. Feeding habits

Knowledge of fish nutrition is important to understand fish biology and can help in fishery management practices. As previously noted trout are opportunistic feeders, mostly feeding on terrestrial and aquatic invertebrates, and crustaceans. Trout engulf their prey by approaching it opening their mouths and simply swallowing it whole, which makes the stomach content inspection easier; it is also one of the reason so many pebbles and other non-food items can easily be found in their guts. Trout often feed on locally available smaller fish so we consider them to be opportunistic piscivores, however insects make up the largest margin of their food supply through their lifecycle. Main factors that determine trout diet are: habitat, season, prey availability, ontogeny, and sex of the fish (Kara, 2003). They mostly feed during the day when the mosquitoes and other bugs are most active, best fishing times for trout are in the warmer seasons. During the summer in the early morning or late evening, in spring and fall during dusk. In regards to the feeding habits of trout not much research has been done in Croatia; despite trout inhibiting almost every stream found in the country.

In the USA the research done in Georgia came to the conclusion that trout mostly feed on aquatic and non-aquatic invertebrates found in the water. Mostly invertebrate from the class Diptera, Trichoptera, Plecoptera, Hymenoptera (i.e. Formicidae), Blattodea (Termitidae), Annelidae, and Malacostraca (Isopoda) (O’Ruke, 2014). Another research done in USA, on the stream Douglas in Wyoming; shows that trout feed on similar classes of invertebra, mostly Diptera, Plecoptera, Ephemeroptera, and Tricoptera (Hubert et al., 1993).

In Sweden, research showed that trout mostly feed on invertebra belonging to the class Diptera, Arachnida, Coleoptera, Hymenoptera, Chironomidae and Simulimae (Eros et al., 2012).

In Iceland, when research was done on river Laxa it showed trout feed on invertebra belonging to the order diptera; mostly belonging to class: Simuliidae, and Chironomidae. Besides insects, some gastropoda were found, species like *Lymnaea peregra* were common (Steingrimsson and Gislason, 2001).

In India research done on river Kashmir showed that trout feed on classes: Trichoptera, Ephemeroptera, Diptera, Plecoptera, Coleoptera, and Malacostraca (Amphipoda) (Rasool et al., 2012).

Results were similar when the research was done on the river Una, mostly insect belonging to the classes Ephemeroptera, Trichoptera, Diptera, Coleoptera and Hymenoptera were found. Besides insects various kinds of other animals belonging to the class od Gastropoda, Malacostraca, and some Vertebrata, fish like *Cottus gobio*. (Trožić-Borovac, 2002).

During the research of trout feed mostly similar methods are used. Research is conducted to get the data about the abundance, number, and mass of prey items. Mostly used is the method that shows the frequency of occurrence, percentage of occurrence and gravimetrical methods. (Treer and Piria., 2019).

3. MATERIALS AND METHODS

3.1. Research area

For the purpose of this thesis the streams at ‘Gorski Kotar’ and ‘Žumberak’ regions were sampled. Results come from the sampling during the April and May of 2017, and samplings conducted in November 2009.

‘Gorski Kotar’ or Croatian ‘Mountain District’ is a mountainous region of Croatia placed between the city of Karlovac in the North-East and the city of Rijeka in the South-West. 63% of the region is heavily forested and intertwined with many streams, for the purpose of this thesis the streams belonging to the ‘Black Sea’ basin were examined.

‘Žumberak’ is a Nature Park in Samobor highland region of Croatia, it covers around 333 km². Examined streams were all marked by appropriate lettering for easier distinction, their names, markings, time of the examination, and geographical longitude/latitude is shown in the table below (Table 3.).

Table 3. Research sites, date of samplings and coordinates

Watercourse ‘Gorski Kotar’	Markings	Date	Latitude	Longitude
Bresni Potok	BP	04.05.2017	45.5414525	14.6489864
Mala Lešnica	LE	02.05.2017	45.440747	14.850466
Watercourse ‘Žumberak’				
Ribnjaci Vrbac	KČ (numbers)	04.04.2017.	45.750800	15.413465
Curak Zeleni Vir	CZ	02.05.2017.	45.4260697	14.8925195
Slapnica	SL	04.04.2017	45.778166	15.613476
Gradna	L4	02.12.2009	45.823626	15.731345
Rude	L5	02.12.2009	45.767510	15.674319
Kupčina	KČ (letters)	04.04.2017	45.540556	15.810369
	L2	25.11.2009	45.540556	15.810369

3.2. Sample collection

All specimens were collected during the daylight hours, the exact time was specified. Samples were acquired using the electrofishing method, using the backpack model of ‘Hans Grassel GmbH 1,3 kw’ aggregate. From each stream trout specimens were collected, immediately stored at -20°C, and brought to the University Of Zagreb Faculty Of Agriculture Department Of Fisheries, Apicculture, Wildlife management and spec. zoology. Trout stomach and guts were taken out for further examination. For intestines preservation guts were stored in 50 ml volume plastic bottles containing 96% ethanol solution. Each bottle was marked with the initials of the stream and the number depicted by which order they were gathered. Each specimen haplotype data was taken from the Kanjuh et al. (2018) to determine the Danube or Atlantic lineage.

3.3. Sample processing

Sample processing was done in the laboratory of Department of Fisheries, Apiculture, Wildlife Management and spec. zoology Zagreb, during March and April of 2019. In the laboratory

each specimen was measured for total length (TL) and weight (W). Trout length was measured on millimeter paper; weight determined on the 'Kern scale'.
The stomachs and guts were removed from the fish, measured for total length on the millimeter paper (Figure 4.), and weighted on the 'kern scale'. During the weighting of intestines, swimming bladder, and any other parts that might have been accidentally taken with the intestines had to be removed to avoid disruption of the measurements. Intestines were then again stored in 96% ethanol solution until further examination could take place.

Figure 4. Process of measuring the length of the digestive system on millimeter paper
Picture source: Ivana Lisica

To get the prey items and measure them separately the guts were taken out of the containers and opened on a tray using simple laboratory scissors. Stomach contest was separated and put on a petri dish using tweezers and histological needle, it was weighted to the second decimal number on the 'kern scale'. The percentage of stomach fulfilment was determined approximately, without special measurements or calculations. Prey items were stored in plastic containers containing 96% ethanol solution. After removing each specimens guts, weighting them, removing the prey items, and weighting them separately the examination of specific prey items could take place.
Determination of prey items was done by taking the sample out of the plastic container, putting the content on petri dish and closely examining it. The aim was to determine each specimen found in the guts to the lowest systematic category possible, count them, and determine their percentage and frequency. Assessment of the specific invertebrate species was made more difficult due to the fact that some specimens found in the guts were already half digested and found in mixed fragments. Stomach contest was examined under a laboratory magnifying glass. Some specimens were too small or too fragmented to be recognizable under a magnifying glass, hence they were further studied under a 'light microscope'.
To properly determine the stomach contest a manual by Kerovec (1986) were used. Using simple schemes manual goes through all the invertebrate classes found in our streams, to help determine different classes or even species of invertebrate present in the stomach content.
When the prey items couldn't be distinguished using the manual other methods were applied. English published manual describing the invertebrates found all over Europe was often in use, combined with the knowledge of our professors working at the department of 'Fisheries, Apiculture, Wildlife Management and spec. Zoology' in Zagreb.

During the prey species examination all found determined specimens were written down, counted (N), and the percentage of their representation in each vial was written down (%).

3.4. Statistical analysis

Determined data was written in the excel program, arranged in columns depending on the prey specimen, appropriate mark representing the stream where the fish was captured, and the number (or letter) in order by which they were taken out of the stream, and stored for examination. Using these numbers the calculation of the mass of every group of prey specimens was calculated in excel. Sum and Count Formulas were used to determine the representative population numbers, and frequency of occurrence for all the examined trout specimens.

To analyse specific categories of prey specimens the following formulas were used (Hyslop, 1980; Piria, 2003; Danilović, 2010; Becer i Ozvarol, 2011; Treer i Piria,2019) :

- Frequency of occurrence (F%):

$$F\% = \frac{fi}{\sum f} \times 100$$

Where: fi – represents the frequency of 1 prey category
∑f – represents the total frequency of all prey categories

- Number percentage (N%):

$$N\% = \frac{ni}{\sum n} \times 100$$

Where: ni – represents the total number of specific feed categories
∑n – represents the total number of prey items consumed

- Mass percentage (W%):

$$W = \frac{Wi}{\sum W}$$

Where: Wi – represents total mass of a single prey item
∑W – represents mass of all prey items

- Coefficient of absolute meaning IAI

$$IAI\alpha = F\% + N\% + W\%$$

- The index of relative importance IRI was calculated for each prey category:

$$IRI = 100 \times \frac{IAI\alpha}{\sum_{\alpha=1}^{n} IAI\alpha}$$

Where: α-specific prey category
n-number of different prey categories

- Diet overlap was calculated using the index proposed by Schoener (1970) based on IRI

$$\alpha = 1 - 0.5(\sum_{i=1}^{n} |PVxi - PVyi|)$$

Where: n- represents the number of all prey categories
PVxi – represents the proportion of consumed prey category *i* for species *x*
PVyi- represents the proportion of consumed prey category *i* for species *y*

- Length weight relationship (LWR)

$$\boldsymbol{W = a \times TL^{b}}$$

Where: W -represents the weight in grams
TL -represents the total length in cm
a and *b* are constants used from Ricker research conducted in 1975

- Condition factor (CF)

$$CF = W \times TL^{-3} \times 100$$

4. RESULTS

4.1. Qualitative and quantitative description of prey categories and descriptive analysis of all examined trout specimens

For the purpose of this research 107 specimens were examined, out of 107 specimens 24 were determined to be Atlantic haplotype, 13 which were gathered from the streams at random, and all 11 specimens gathered at 'Vrbac Fishery' (KČ1...KČ10). Some had completely empty intestines, thus they were removed from further examination. For instance, all the specimens gathered on the 'Ribnjaci Vrbac' – 'Vrbac fishery' had empty intestines because they were fed with pellets which tend to degrade rapidly. The fish intestines were empty because there was no need for additional feed.
Qualitative analysis of all specimens was examined. A table showing a systematic overview of all identified items, prey specimens will be shown below. It was determined that examined trout specimens from Samobor and Gorski Kotar region feed on 9 different classes of organisms; further separated in 15 different orders. Different orders mostly belong to the insecta class of invertebrate. Prey specimens were identified to the lowest systematic category possible (Table 4), unfortunately detailed determination of family and species was not possible. In further study feeding habits of trout from each region and stream will be presented. and separate diet of Atlantic and Danube haplotype specimens will be calculated.

Table 4. Qualitative analysis of all prey specimens found in Croatian streams, distinguished to the lowest category possible

CLASS	ORDER	Family	*Species*
BIVALVIA			
GASTROPODA	PULMONATA	Planorbidae	*Anisus vorticulus*
CLITELLATA	HAPLOTAXIDA		
	RHYNCHOBDELLIDA	Piscicolidae	
HIRUDINEA			
AMPHIPODA			
ARACHNIDA			
MALACOSTRACA	AMPHIPODA		
	DECAPODA	Astacidae	*Austropotamobius torrentium*
INSECTA	COLEOPTERA	Cerambycidae: Subfamily-Lamiinae	
	DIPTERA	Muscidae	
		Simuliidae	
		Psychodidae	
	EPHEMEROPTERA		
	HEMIPTERA	Corixidae: Subfamily-Micronectinae	
		Subfamily- Corixinae	
	HYMENOPTERA	Formicidae	
		Vespidae	
	ODONATA	Anisoptera	
	ORTHOPTERA	Caelifera	
	PLECOPTERA	Capniidae	
	TRICHOPTERA	Hydropsychidae	
		Philopotamidae	
ACTINOPTERYGII	SCORPAENIFORMES	Cottidae	*Cottus gobio*

Each specimen was measured for total body length (TL), the largest examined specimen was 32 cm (code: L2-2), correspondently the longest intestines belonged to the same specimen, measured at 33.3cm. The smallest specimen measured for TL was 7.1cm (code: L2-14); which correspondently had the shortest intestines, 5.2 cm. Average trout length was measured as 18.07cm, and the intestines length as 15.28 cm.

Largest total mass was found in an Atlantic haplotype specimen measured at 323.52 g (code: LE3), it also had the largest intestines mass, 27.14 g. Interesting to note that this specimen, when measured for length, was smaller than the L2-14 specimen (non-Atlantic haplotype).

Smallest specimen was only 3.1 g in mass, correspondently its intestines were measured as 0.15g (empty at the time). Average mass was calculated as 89.17g.; and average intestines mass as 4.73g. This shows that the length and mass of a specimen are (usually) in positive correlation.

It has to be noted that while the Atlantic haplotype (LE3) had the largest mass, which would not correspond to the fact that length and mass are positively correlated, its intestines fulfillment was measured as 100%, and weighted 13.85g; while the largest L2-2 specimen mass of stomach content weighted only 2.01g. For some specimens measurement and analysis of intestines content was impossible because the samples got ruined during the storage, those specimens were noted as ruined in the spreadsheets and removed from further canalization.

4.2. Gorski Kotar watercourse

4.2.1. Bresni Potok

On ‘Bresni Potok’ (Bresni Stream) 10 specimens were gathered during the research conducted on May 4th 2017. Genetic evaluation determined that 8 out of 10 specimens belonged to the At haplotype (all besides specimens under codes: BP8 and BP9).
Longest specimen measured for TL was 24.7 cm long, average length was calculated as 18.48 cm. The same specimen also had the highest mass, measured as 155.92 g. on the Kern scale. Average mass was calculated as 79.09 g. Value *b* of length weight relationship show isometric growth of trout analysed at Bresni potok location (b=2.922), (Figure 5; Table 5).

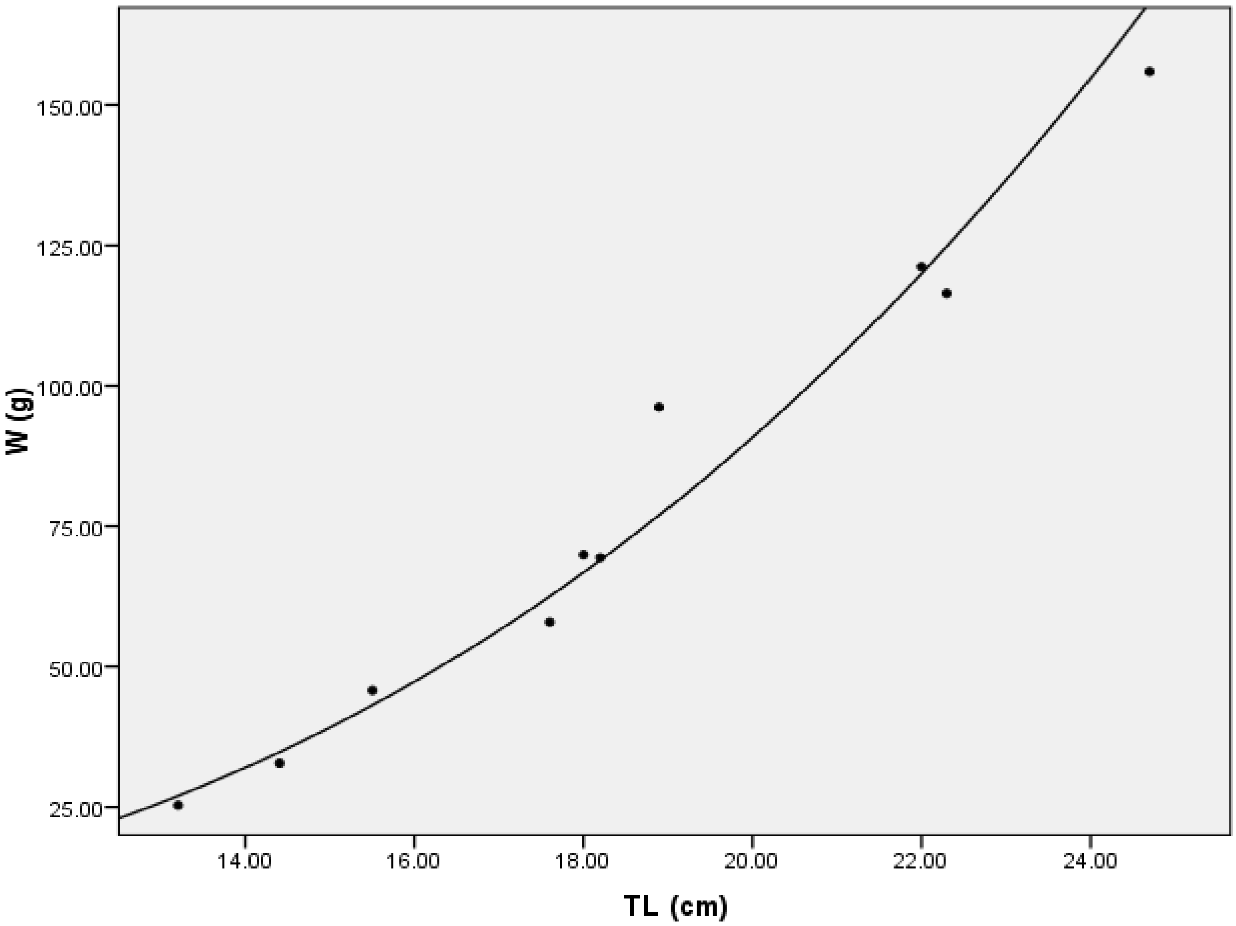

Figure 5. Length/weight relationship of all trout specimens gathered on the location: Bresni Potok

Table 5. Anova and power regression values of length weight relationship of specimens from the Bresni potok

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
ln(dv_tl)	2.922	.167	.987	17.497	.000
(Constant)	.014	.007		2.061	.073

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Regression	3.047	1	3.047	306.137	.000
Residual	.080	8	.010		
Total	3.127	9			

The most important prey items for both lineages were Trichoptera. Only At lineages fed with crayfish *Austropotamobius torrentium* and terrestrial insects from Formicidae group. Secondary prey for Da lineages were aquatic Amphipoda (Table 6, 7 and 8).

Table 6. Prey items found in both AT and DA trout lineages on 'Bresni Potok' location (n=10)

Prey class	F%	N%	W%
Amphipoda	3.7	2.53	2.38
Anisus	3.7	1.27	0.25
Austropotamobius torrentium	3.7	1.27	31.53
Coleoptera	3.7	1.27	1.15
Coleoptera larvae	3.7	1.27	0.63
Diptera	7.41	5.06	0.30
Ephemeroptera	3.7	2.53	0.33
Formicidae	14.81	12.66	4.79
Hydropsychidae	3.7	1.27	0.59
Odonata	3.7	1.27	0.72
Plecoptera	14.81	6.33	0.43
Trichoptera case makers	25.93	60.76	56.47
Trichoptera	7.41	2.53	0.42
TOTAL	100%	100%	100%

Table 7. Prey items found in At trout lineage on ‘Bresni Potok’ location (n=8)

Class	F%	N%	W%
Anisus	4.55	1.45	0.25
Austropotamobius torrentium	4.55	1.45	30.64
Coleoptera larvae	4.55	1.45	0.70
Diptera	4.55	4.35	8.92
Ephemeroptera	4.55	2.9	0.32
Formicidae	18.18	14.49	4.73
Hydropsychidae	4.55	1.45	0.58
Odonata	4.55	1.45	0.70
Plecoptera	13.64	5.8	4.57
Trichoptera case makers	27.27	62.32	48.18
Trichoptera	9.09	2.9	0.41
TOTAL	100%	100%	100%

Table 8. Prey items found in Da trout lineage on ‘Bresni Potok’ location (n=2)

CLASS	F%	N%	W%
Amphipoda	20	20	22.82
Coleoptera	20	10	4.13
Diptera	20	10	2.85
Plecoptera	20	10	4.13
Trichoptera case makers	20	50	66.07
TOTAL	100%	100%	100%

4.2.2. Mala Lešnica

On location 'Mala Lešnica' 10 specimens were gathered during the research conducted on the May 2nd 2017. 2 of which were determined as At haplotype: LE3, LE4. From 10 examined trout specimens 2 had empty intestines, specimens LE1 and LE4. Specimen LE3 was the heaviest of all the researched ones, and had a completely full intestines (100%). Average intestines fulfillment for all the researched specimens gathered at the 'Mala Lešnica' location was calculated as 28.6%. Value b of length weight relationship show positive allometric growth of trout analysed at Bresni potok location (b=3.110), (Figure 6; Table 9).

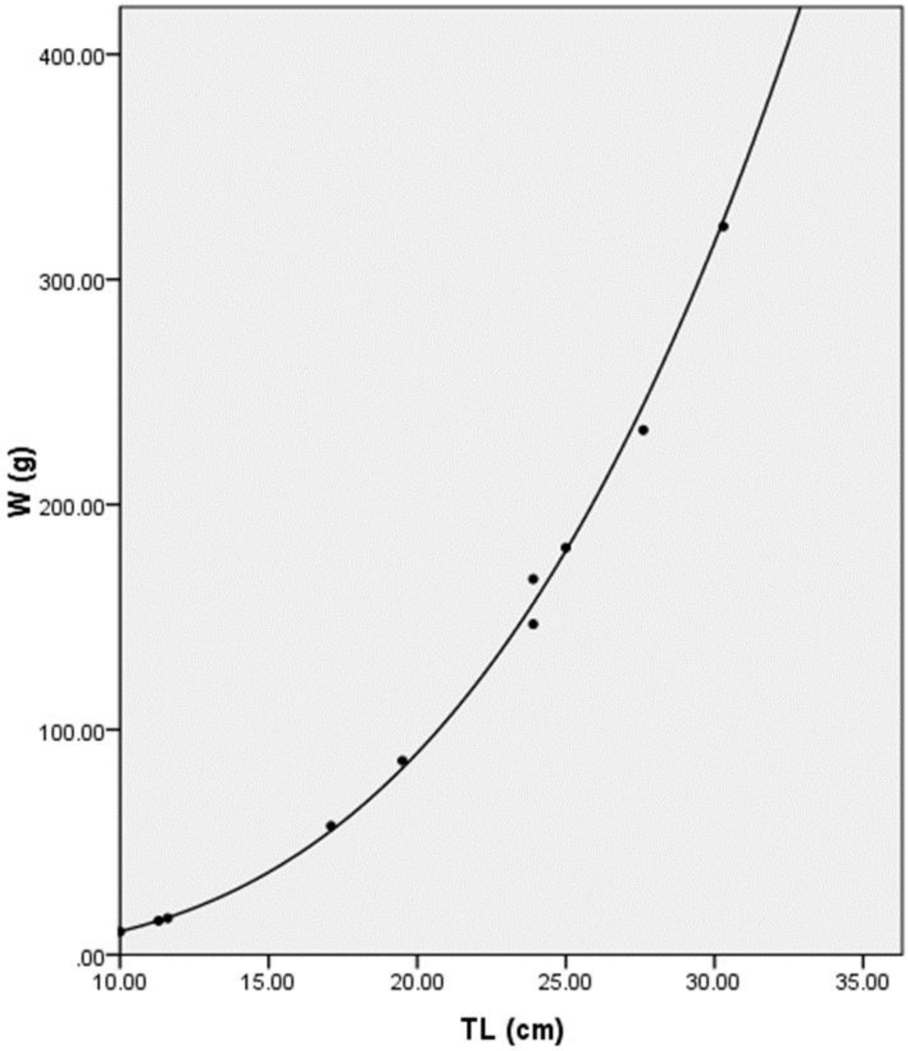

Figure 6. Length/weight relationship of all trout specimens gathered on the location: Mala Lešnica

Table 9. Anova and power regression values of length weight relationship of specimens from the Mala Lešnica

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
ln(dv_tl)	3.110	.035	1.000	89.893	.000
(Constant)	.008	.001		9.786	.000

The dependent variable is ln(dv_w).

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Regression	14.245	1	14.245	8080.768	.000
Residual	.014	8	.002		
Total	14.259	9			

The independent variable is dv_tl.

The most important prey items for both lineages were Trichoptera. Only At lineages fed with fish (*Cottus gobio*) and Coleoptera. Secondary prey for Da lineages were aquatic Plecoptera and Amphipoda (Table 10, 11 and 12).

Table 10. Prey items found in Da and At trout lineages on 'Mala Lešnica' location (n=10)

PREY CLASS	F (%)	N (%)	W%
Amphipoda	6.25	25	1.41
Arachnida	6.25	5.56	0.11
Bivalvia	6.25	2.78	0.09
Coleoptera	12.5	5.56	4.42
Corixinae	6.25	2.78	0.09
Cottus gobio	6.25	8.33	49.73
Diptera	6.25	5.56	0.91
Plecoptera	12.5	5.56	5.51
Trichoptera case makers	31.25	36.11	37.27
Trichoptera	6.25	2.78	0.46
TOTAL	100%	100%	100%

Table 11. Prey items found in Da trout lineage on ‘Mala Lešnica’ location (n=8)

CLASS	F%	N%	W%
Amphipoda	7.69	31.3	2.92
Arachnida	7.69	6.9	0.23
Bivalvia	7.69	3.45	0.18
Coleoptera	7.69	3.45	0.58
Corixidae	7.69	3.45	0.18
Diptra	7.69	6.9	1.89
Plecoptera	15.38	6.9	11.41
Trichoptera case makers	30.77	34.48	25.74
Trichoptera	7.69	3.45	0.95
TOTAL	100%	100%	100%

Table 12. Prey items found in At trout lineage on ‘Mala Lešnica’ location (n=2)

CLASS	F%	N%	W%
Coleoptera	33.3	14.29	5.26
Trichoptera case makers	33.3	42.86	31.58
Cottus gobio	33.3	42.86	63.16
TOTAL	100%	100%	100%

4.3 Žumberak watercourse

4.3.1. Curak - Zeleni vir

‘Curak Zeleni Vir’ belongs to the ‘Žumberak’ watercourse. On the research done on the 2nd of May 2017 eight trout specimens was gathered. 2 were determined as At haplotype (CZ3, CZ5). Two specimens had empty intestines (CZ2, CZ3), and one (CZ8) had to be removed from further evaluation because it got ruined during storage. Therefore, the research on ‘Zeleni Vir’ location was done on 5 specimens, 4 Da and 1 At. Average intestines fulfillment was determined as 40%, one specimen had 100%. Longest measured specimen (Atlantic CZ3) had TL of 27.1 (average was 22.57. The same specimen had the biggest mass of 241.01 (average 147.45). Value b of length weight relationship show positive allometric growth of trout analysed at Curak Zeleni Vir location (b=3.175), (Figure 7; Table 13).

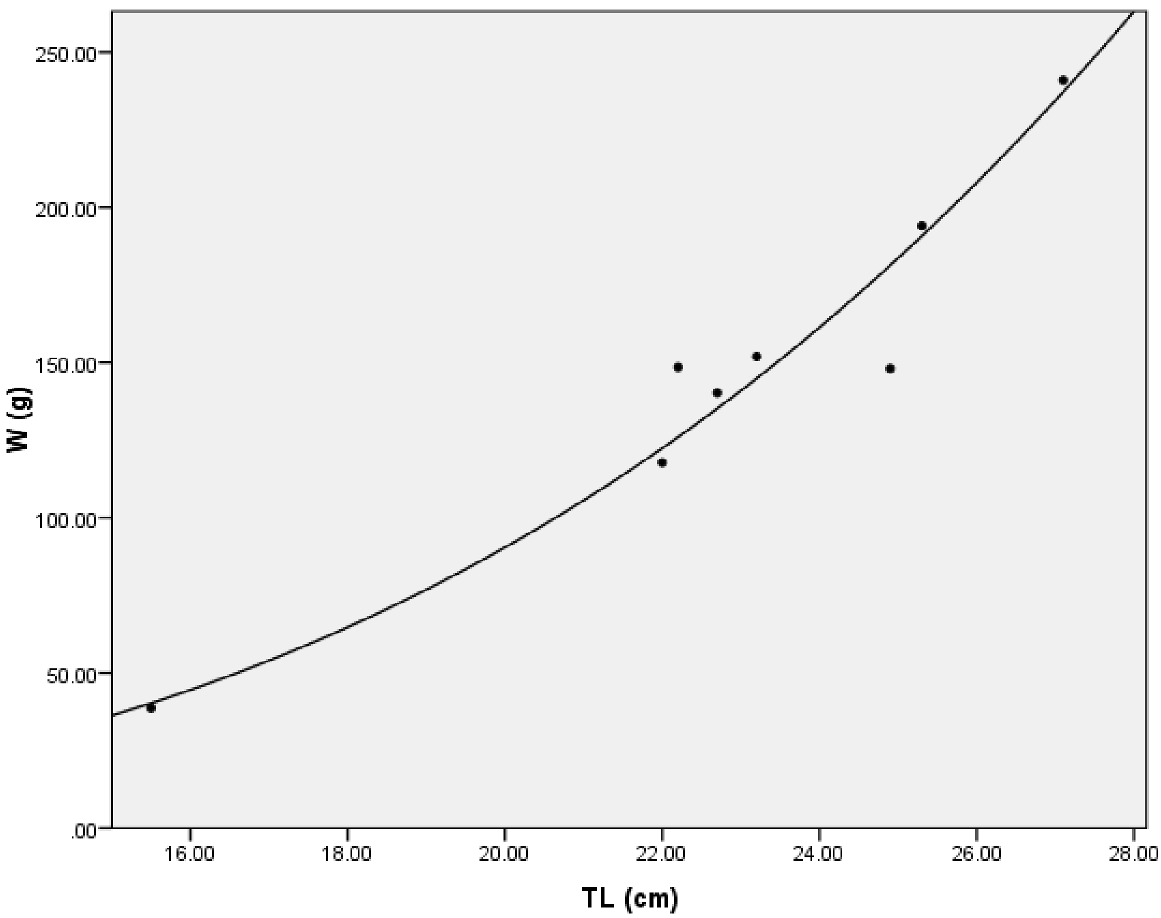

Figure 7. Length/weight relationship of all trout specimens gathered on the location: Curak Zeleni Vir

Table 13. Anova and power regression values of length weight relationship of specimens from Curak Zeleni Vir

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
ln(dv_tl)	3.175	.251	.982	12.625	.000
(Constant)	.007	.005		1.274	.250

The dependent variable is ln(dv_w).

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Regression	2.006	1	2.006	159.388	.000
Residual	.076	6	.013		
Total	2.082	7			

The independent variable is dv_tl.

The most important prey items for both lineages were Trichoptera. Secondary prey for At lineages were Diptera and odonatan and for Da lineages Formicidae and Corixidae (Table 14, 15 and 16).

Table 14. Prey items found in Da and At trout lineages on 'Curak Zeleni Vir' location (n=10)

CLASS	F%	N%	W%
Arachnida	4.76	2.17	2.72
Coleoptera	4.76	2.90	2.42
Coleoptera larvae	4.76	0.72	0.22
Corixidae	9.52	9.42	10.85
Diptera	9.52	16.67	12.78
Diptera larvae	4.76	2.90	1.48
Formicidae	9.52	15.22	16.40
Odonata	9.52	14.49	5.40
Pisciocolidae	4.76	2.17	2.07
Plecoptera	4.76	2.90	1.46
Trichoptera	14.29	5.80	4.71
Trichoptera case makers	19.05	24.64	39.49
TOTAL	100%	100%	100%

Table 15. Prey items found in At trout lineage on 'Curak Zeleni Vir' location (n=2)

Class	F%	N%	W%
Arachnida	16.67	8.82	3.01
Diptera	16.67	23.53	40.09
Odonata	16.67	29.41	25.06
Piscicolidae	16.67	8.82	5.01
Plecoptera	16.67	11.76	6.80
Trichoptera case makers	16.67	17.65	20.04
TOTAL	100%	100%	100%

Table 16. Prey items found in Da trout lineage on 'Curak Zeleni Vir' location (n=8)

CLASS	F%	N%	W%
Coleoptera	6.67	4.12	3.36
Coleoptera larvae	6.67	1.03	0.31
Corixidae	13.33	13.4	13.6
Diptera	6.67	8.25	5.77
Diptera larvae	6.67	4.12	2.07
Formicidae	13.33	21.65	22.82
Odonata	6.67	10.31	7.51
Trichoptera	20	8.25	6.56
Trichoptera case makers	20	28.87	38.01
TOTAL	100%	100%	100%

4.3.2. Slapnica

On location 'Slapnica' 10 specimens were gathered during the research conducted on the April 4th 2017. During the genetic evaluation none of the specimens caught at 'Slapnica' were determined to be At haplotype. From 10 examined trout specimens 1 (SL1) was taken out of the future examination due to the fact that it's intestines got ruined during the storage, so any future examination was impossible; 2 specimens had their intestines fulfilment determined at 100% (SL4, SL6).

When measured for TL the longest specimen was measured as 19.9 cm (SL4), average TL was calculated as 14.2 cm. SL4 also had the highest mass: 85.05 g.

Average intestines fulfillment for all the researched specimens gathered at the 'Slapnica' location was calculated as 75.56%, highest being 100%, and lowest (excluding the ruined one) as 40%. Value b of length weight relationship show negative allometric growth of trout analysed at Slapnica location (b=2.919), (Figure 8; Table 9).

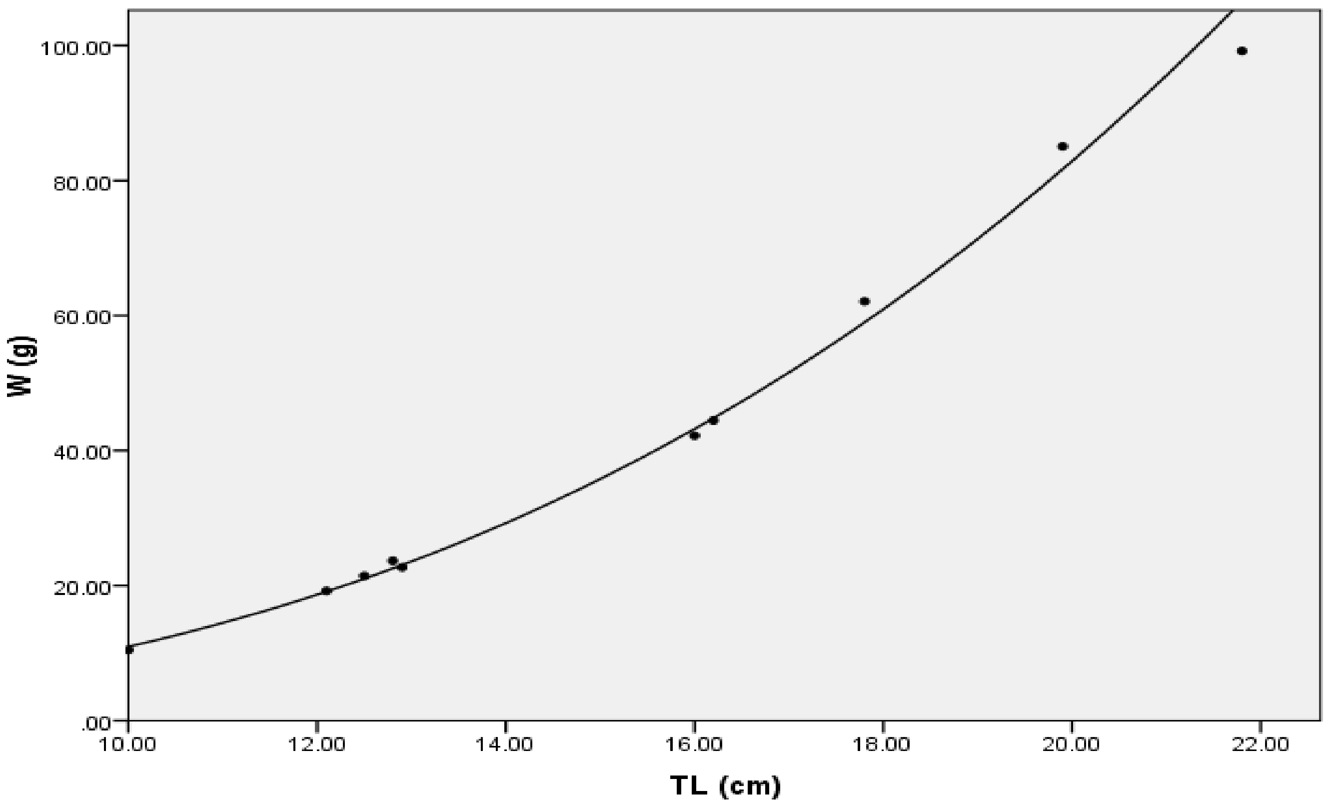

Figure 8. Length/weight relationship of all trout specimens gathered on the location: Slapnica

Table 17. Anova and power regression values of length weight relationship of specimens from the Slapnica stream

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
ln(l)	2.919	.059	.998	49.258	.000
(Constant)	.013	.002		6.242	.000

The dependent variable is ln(w).

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Regression	4.674	1	4.674	2426.327	.000
Residual	.015	8	.002		
Total	4.689	9			

The independent variable is l.

The most important prey at Slapnica stream for Da lineages were Trichoptera, Vespidae and Bivalvia (Table 18).

Table 18. Prey items found in stomach of Da trout lineage at 'Slapnica' location (n=10)

PREY CLASS	F%	N%	W%
Amphipoda	3.23	0.5	17.55
Bivalvia	3.23	10.05	17.09
Coleoptera	12.9	4.52	5.17
Corixidae	9.68	13.07	8.61
Diptera larvae	6.45	1.01	3.34
Ephemeroptera	6.45	1.01	2.62
Formicidae	9.68	3.52	2.40
Hirudidae	3.23	0.5	2.89
Odonata	6.45	1.51	1.89
Plecoptera	6.45	1.51	1.46
Trichoptera case makers	16.13	54.27	18.78
Vespidae	16.13	8.54	18.18
TOTAL	100%	100%	100%

4.3.3. Gradna

On location ‘Gradna' 17 specimens were gathered during the research on February 2nd 2009. The sampling to determine the genetic haplotype was not done in 2009, therefor only the prey items will be shown. Out of 17 specimens gathered that day only 1 had eaten something previously (table below), all others were completely empty.
Maximum TL was 17.6 cm, average was calculated as 15.6 cm. Average mass was 45.9, the highest being 199.6 g. Value b of length weight relationship show isometric growth of trout analysed at Gradna location (b=3.012), (Figure 9; Table 19).

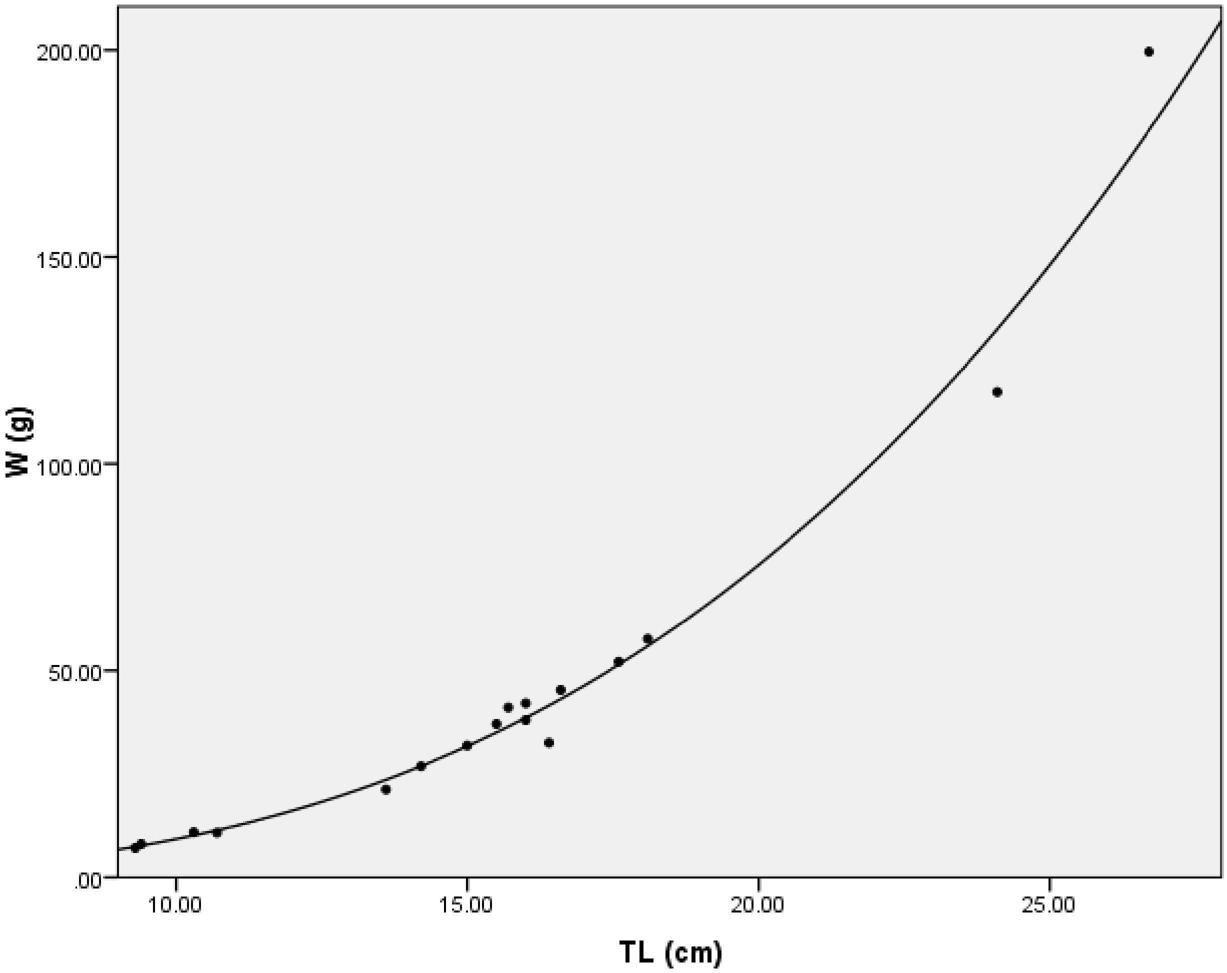

Figure 9. Length/weight relationship of all trout specimens gathered on the location: Gradna

Table 19. Anova and power regression values of length weight relationship of specimens from the Gradna stream

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
ln(dv_tl)	3.012	.082	.994	36.774	.000
(Constant)	.009	.002		4.485	.000

The dependent variable is ln(DV_w).

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Regression	12.364	1	12.364	1352.363	.000
Residual	.137	15	.009		
Total	12.501	16			

The independent variable is dv_tl.

The most important prey of trouts at Gradna stream were Trichoptera and *Gammarus* sp. (Table 20).

Table 20. Prey items found in trout on research conducted in 2009 on ‘Gradna’ location (n=2)

CLASS	F%	N%	W
Gammarus sp.	50	50	17.21
Trichoptera	50	50	85.29
TOTAL	100%	100%	100%

4.3.4. Rude

On location ‘Rude’ 16 specimens were gathered during the research on February 2nd 2009. As with the other research done in 2009 genetic haplotype determination was not determined. Out of 17 specimens 4 had empty intestines and thus were removed from further analysis. Maximum TL was 27 cm (L5-2) average was calculated as 18.58 cm. Average mass was 71.45g the highest being 173.6 g (L5-2). Value b of length weight relationship show negative allometric growth of trout analysed at Rude location (b=2.903), (Figure 10; Table 21).

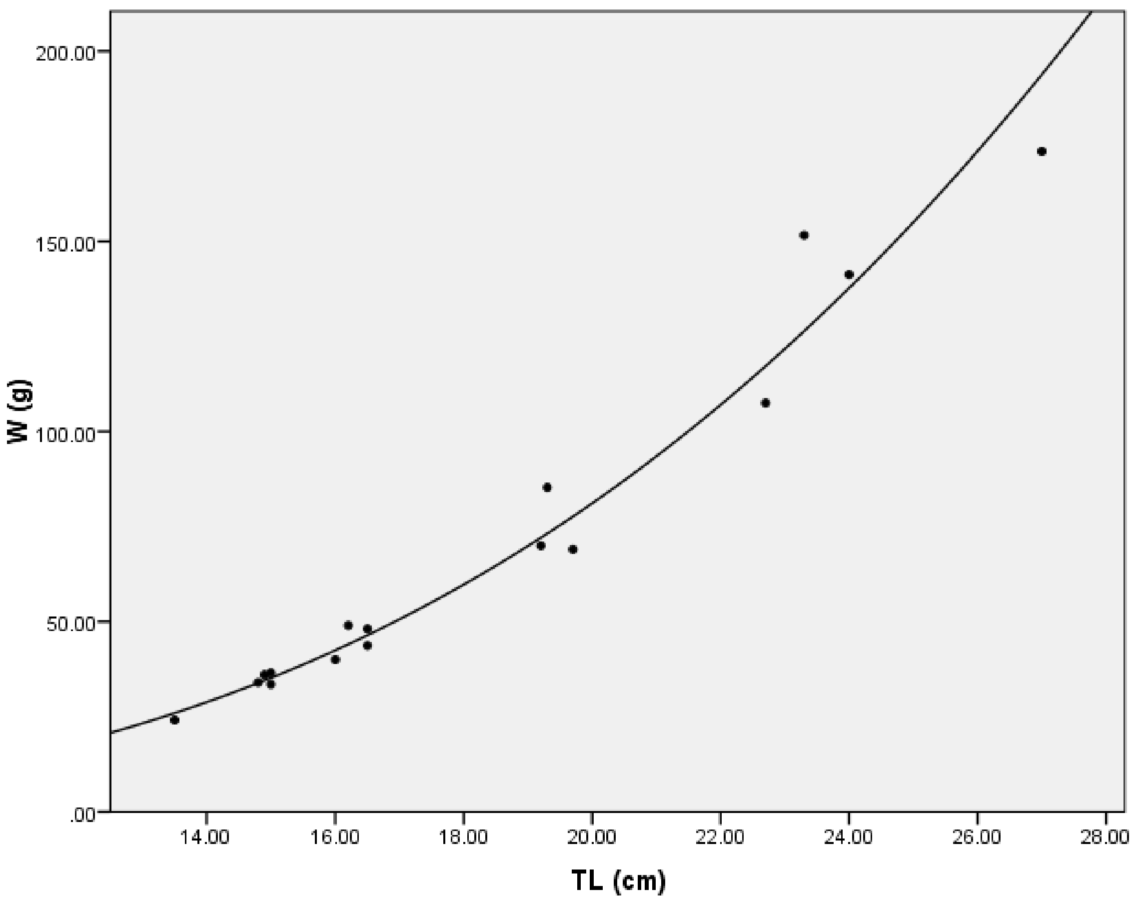

Figure 10. Length/weight relationship of all trout specimens gathered on the location: Rude

Table 21. Anova and power regression values of length weight relationship of specimens from the Rude stream

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
ln(dv_tl)	2.903	.116	.989	24.979	.000
(Constant)	.014	.005		2.972	.010

The dependent variable is ln(dv_w).

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Regression	5.461	1	5.461	623.961	.000
Residual	.123	14	.009		
Total	5.583	15			

The independent variable is dv_tl.

The most important prey of trout specimens at Rude stream were Amphipoda (*Gammarus* sp.) and Plecoptera (Table 22).

Table 22. Prey items found in trout on research conducted in 2009 on ‘Rude’ location (n=13)

CLASS	F%	N%	W%
Ephemeroptera	29.41	9.24	3.03
Gammarus	29.41	73.95	83.39
Lumbricidae	5.88	0.84	2.71
Plecoptera	17.65	12.61	3.56
Pulmonata	5.88	0.84	0.16
Trichoptera	11.76	2.52	7.15
TOTAL	100%	100%	100%

4.3.5. Kupčina

2009

On 'Kupčina' two research have been done. One in 2009 and another in 2017. The results from 25th of November 2009 will be presented first. As with previous no genetic haplotype evaluation has been done. 14 specimens have been extracted, 2 of them had empty intestines and have been removed from further research. Longest specimen measured for TL was 32 cm, average was 18.67 cm. Largest mass was determined on 'kern' scale as 244.5 g, average as 82.29 g. Value b of length weight relationship show positive allometric growth of trout analysed at Kupčina location (b=3.002), (Figure 11; Table 23).

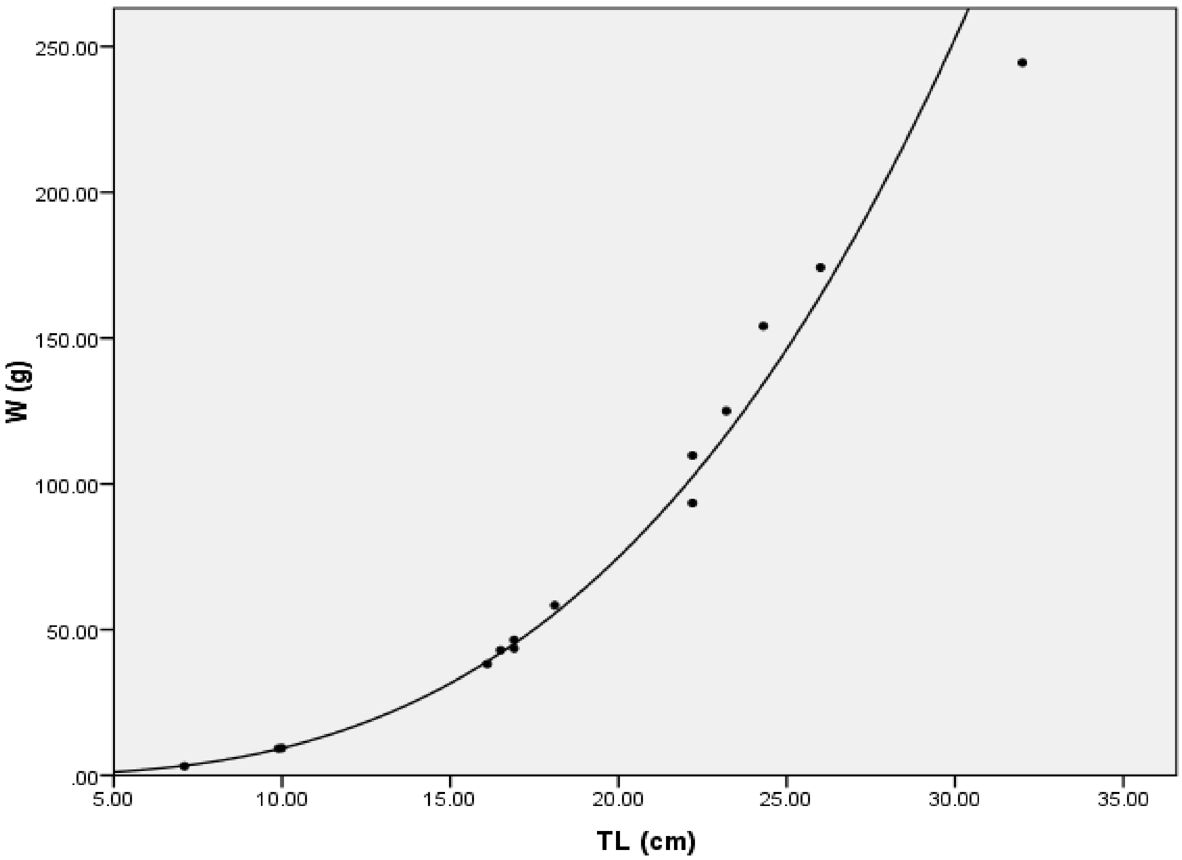

Figure 11. Length/weight relationship of all trout specimens gathered on the location: Kupčina (2009)

Table 23. Anova and power regression values of length weight relationship of specimens from the Kupčina (2009) stream

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
ln(dv_tl)	3.002	.061	.998	48.937	.000
(Constant)	.009	.002		5.656	.000

The dependent variable is ln(dv_w).

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Regression	20.601	1	20.601	2394.795	.000
Residual	.103	12	.009		
Total	20.704	13			

The independent variable is dv_tl.

Table 24. Prey items found in trout on research conducted in 2009 on 'Kupčina' location (n=15)

CLASS	F%	N%	W%
Cottus gobio	3.03	1.37	29.46
Ephemeroptera	15.15	6.85	5.13
Gammarus	30.30	27.40	4.19
Gastropoda	3.03	1.37	0.21
Insecta	3.03	1.37	0.21
Oligocheta	3.03	1.37	0.05
Plecoptera	12.12	6.85	19.21
Pulmonata	3.03	1.37	0.55
Trichoptera	9.09	28.77	30.82
Trichoptera case makers	18.18	23.29	10.16
TOTAL	100%	100%	100%

<u>2017</u>

On the research conducted on the 4th of April 2017 on 'Kupčina' stream 10 trout specimens have been collected. Out of 10 specimens one was later determined as At haplotype (KČH). Maximum TL was 21.5 cm (KČA), and the average 18.5; while the maximum mass was 98.32 g (KČA), and average 68.97. Value b of length weight relationship show negative allometric growth of trout analysed at Kupčina location (b=2.869), (Figure 12; Table 25).

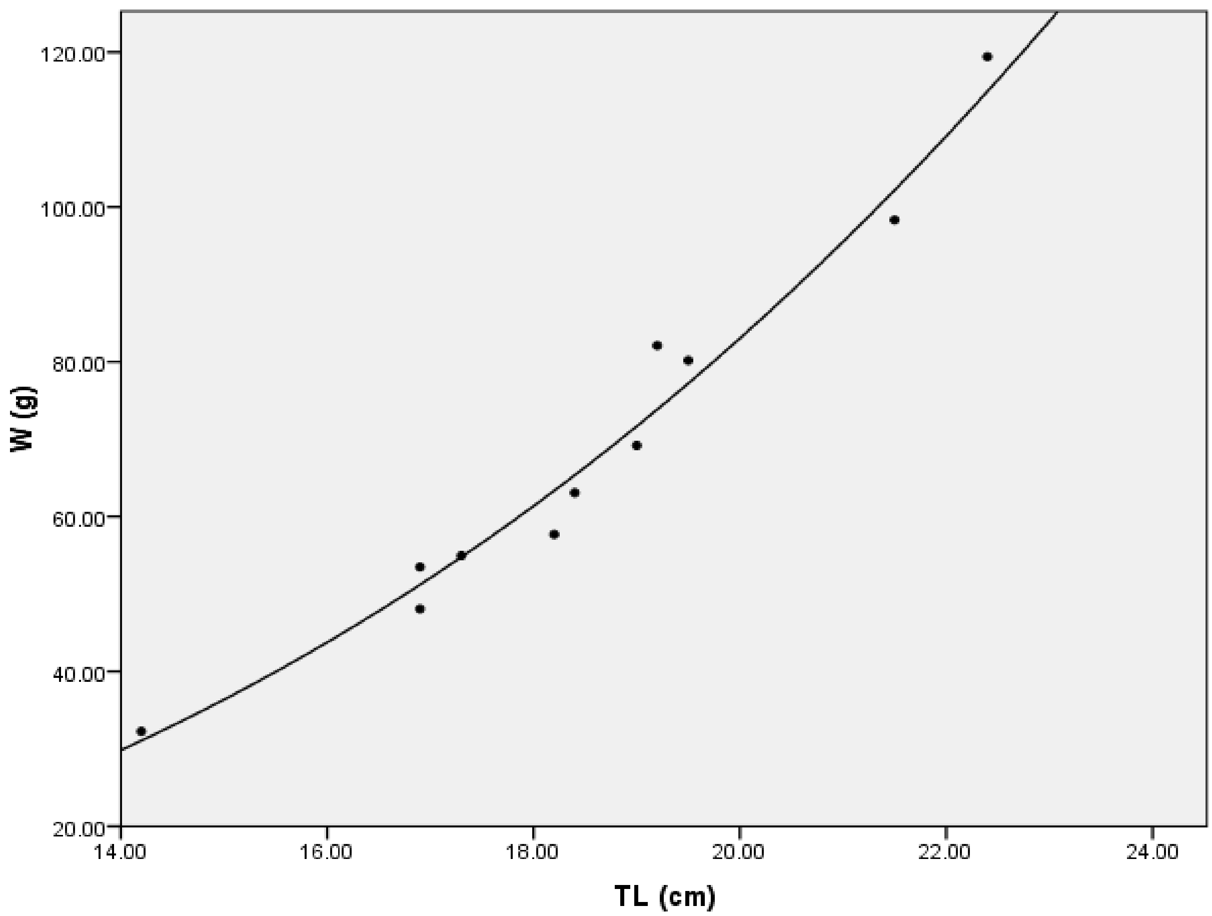

Figure 12. Length/weight relationship of all trout specimens gathered on the location: Kupčina (2017)

Table 25. Anova and power regression values of length weight relationship of specimens from the Kupčina (2017) stream

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
ln(dv_tl)	2.869	.156	.987	18.347	.000
(Constant)	.015	.007		2.195	.056

The dependent variable is ln(dv_w).

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Regression	1.273	1	1.273	336.598	.000
Residual	.034	9	.004		
Total	1.307	10			

The independent variable is dv_tl.

Table 26. Prey items found in Da and At trout lineages on location: ‘Kupčina' (n=10)

CLASS	F%	N%	W%
Amphipoda	6.12	4.38	4.89
Anisoptera	4.08	10.68	6.67
Arachnida	2.04	0.27	0.26
Caelifera	4.08	1.1	1.41
Coleoptera	4.08	1.1	1.47
Coleoptera larvae	2.04	0.27	0.35
Diptera	4.08	17.81	8.59
Diptera larvae	4.08	1.1	0.52
Ephemeroptera	4.08	0.55	0.8
Formicidae	4.08	0.55	0.53
Hydropsychidae	4.08	1.64	2.76
Hymenoptera	2.04	0.27	0.53
Muscidae	2.04	0.55	2.4
Odonata	4.08	3.56	3.76
Plecoptera	6.12	4.3	7.25
Psychodidae	8.16	19.45	14.77
Trichoptera	22.45	12.24	24.62
Trichoptera case makers	12.24	28.22	18.43
TOTAL	100%	100%	100%

The most important prey items for Da lineage were Trichoptera and Diptera and for At lineage Odonata and Anisoptera (Table 26, 27 and 28).

Table 27. Prey items found in Da trout lineage on location: ‘Kupčina' (n=9)

CLASS	F%	N%	W%
Amphipoda	7.14	4.53	5.13
Anisoptera	2.38	10.76	4.81
Arachnida	2.38	0.28	0.28
Caelifera	2.38	0.28	1.48
Coleoptera	2.38	0.85	1
Coleoptera larvae	2.38	0.28	0.36
Diptera	4.76	18.41	9.01
Diptera larvae	2.38	0.85	1.64
Ephemeroptera	4.76	0.57	0.84
Formicidae	2.38	0.57	0.55
Hydropsychidae	4.76	2.27	2.89
Hymenoptera	2.38	0.57	0.55
Muscidae	2.3	0.85	2.52
Odonata	2.38	2.27	1.21
Plecoptera	7.14	4.53	7.6
Psychodidae	9.52	20.11	15.5
Trichoptera	23.81	4.25	19.34
Trichoptera case makers	14.29	28.9	25.29
TOTAL	100%	100%	100%

Table 28. Prey items found in At trout lineage on location: Kupčina (n=1)

CLASS	F%	N%	W%
Anisoptera	16.67	8.33	26.67
Caelifera	16.67	25	20
Coleoptera	16.67	8.33	6.67
Diptera larvae	16.67	8.33	6.67
Odonata	16.67	41.67	33.33
Trichoptera	16.67	8.33	6.67
TOTAL	100%	100%	100%

4.4. Comparison of the feeding habits of At and Da haplotype of all specimens

To properly conclude is there a difference in feeding habits of Atlantc invasive trout and non-invasive Danube trout tables containing all the examined specimens will be shown. Tables below will be conducted only showing the difference in feeding habits with no regards from which streams the specimen's came from. Only the results from 2017 will be shown due to the fact that only on these specimens the proper genetic evaluation has been done. Specimens from 'Vrabac fish farm will also be excluded, all of them have been determined as At haplotype, but due to the fact that all of them have been fed with pellets their stomach content was empty and excluded from feeding evaluation. Value b of length weight relationship show negative allometric growth for Da specimen haplotype (b=1.625) and isometric growth for At specimen haplotype (b=3.003), (Figure 13, 14; Table 29, 31).

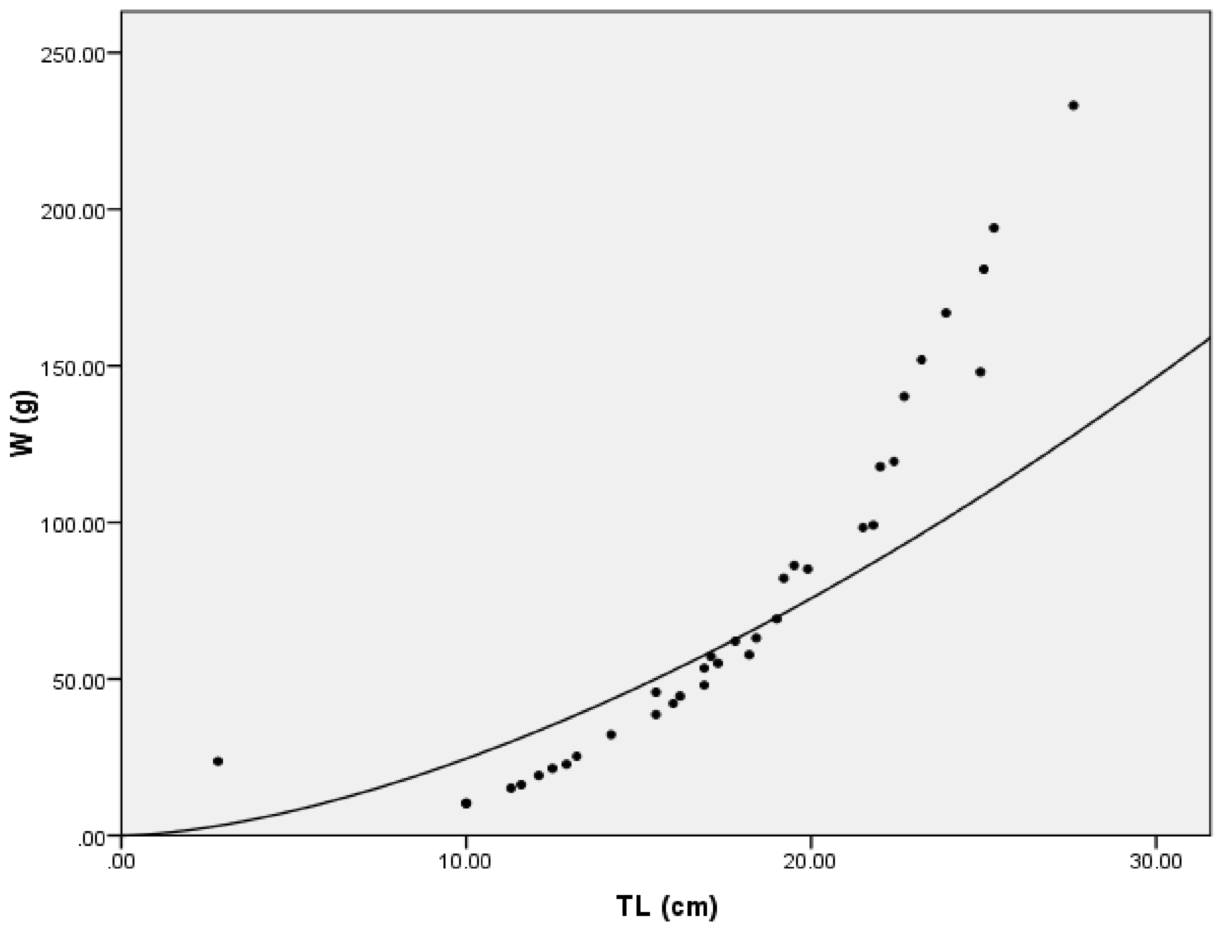

Figure 13. Length/weight relationship of all trout specimens determined as Da haplotype

Table 29. Anova and power regression values of length weight relationship of Da specimens haplotype

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
ln(dv_tl)	1.625	.221	.783	7.337	.000
(Constant)	.583	.366		1.593	.120

The dependent variable is ln(dv_w).

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Regression	15.404	1	15.404	53.834	.000
Residual	9.729	34	.286		
Total	25.134	35			

The independent variable is dv_tl.

Table 30. Prey items found in all trout specimens determined as Da lineage (n=36)

CLASS	F%	N%	W%
Amphipoda	4.46	3.86	3.74
Anisoptera	0.89	5.44	1.82
Arachnida	2.68	0.57	1.09
Bivalvia	1.79	3.00	4.15
Caelifera	0.89	0.14	0.56
Coleoptera	7.14	2.58	3.11
Coleoptera larvae	1.79	0.29	0.24
Corixidae	6.25	5.72	5.75
Diptera	4.46	10.87	5.96
Diptera larvae	3.57	1.29	2.75
Ephemeroptera	3.57	0.57	1.85
Formicidae	5.36	4.29	5.32
Hirudidae	0.89	0.14	0.69
Hydropsychidae	2.68	1.00	1.10
Hymenoptera	0.89	0.14	0.21
Muscidae	0.89	0.29	0.96
Odonata	3.57	3.00	0.91
Plecoptera	7.14	3.15	7.14
Psychodidae	4.46	10.30	5.88
Trichoptera	15.18	23.75	12.27
Trichoptera case makers	16.96	17.17	30.16
Vespidae	4.46	2.43	4.36
TOTAL	100%	100%	100%

The most important prey for both lineages were Trichoptera (Table 30 and 32), but according to IRI of prey importance seems that At lineages feed more with terrestrial prey that falls into the water (Caelifera, Formiciidae, Diptera imago) than Da lineages. Also in stomach content of At lineage specimens larger prey (fish, crayfish) were found (Figure 15).

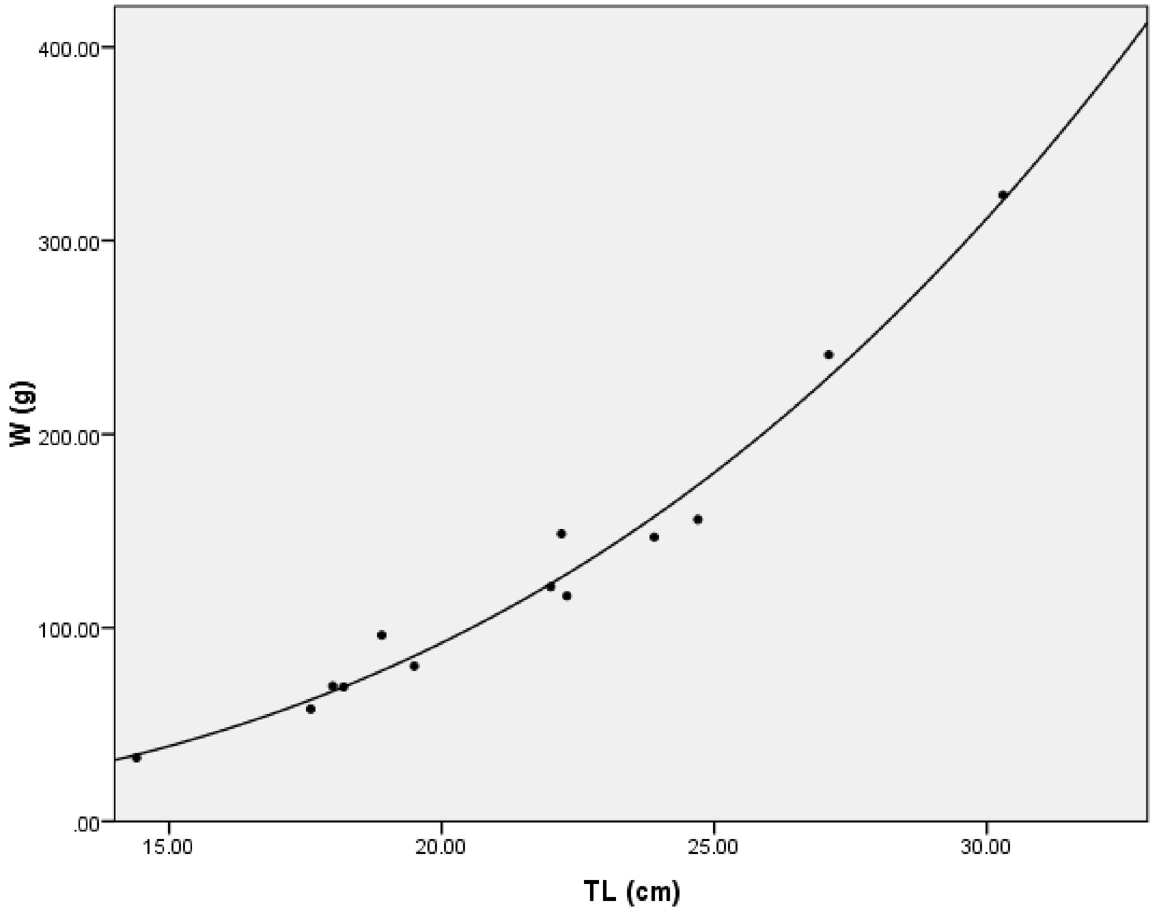

Figure 14. Length/weight relationship of all trout specimens determined as At haplotype

Table 31. Anova and power regression values of length weight relationship of At specimens haplotype

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
ln(dv_tl)	3.003	.146	.987	20.552	.000
(Constant)	.011	.005		2.241	.047

The dependent variable is ln(dv_w).

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Regression	4.380	1	4.380	422.364	.000
Residual	.114	11	.010		
Total	4.494	12			

The independent variable is dv_tl.

Table 32. Prey items found in all trout specimens determined as At haplotype (n=13)

CLASS	F%	N%	W%
Anisoptera	2.70	0.78	0.79
Anisus	2.70	0.78	0.08
Arachnida	2.70	2.33	0.27
Austropotamobius torrentium	2.70	0.78	10.39
Caelifera	2.70	2.33	3.12
Coleoptera	5.41	1.55	2.87
Coleoptera larvae	2.70	0.78	0.24
Cottus gobio	2.70	2.33	32.06
Diptera	5.41	13.95	4.72
Diptera larvae	2.70	0.78	0.20
Ephemeroptera	2.70	1.55	0.11
Formicidae	10.81	7.75	2.68
Hydropsychidae	2.70	0.78	0.20
Odonata	8.11	12.40	3.45
Pisciocolidae	2.70	2.33	0.45
Plecoptera	10.81	6.20	3.83
Trichoptera	8.11	2.33	0.40
Trichoptera case makers	21.62	40.31	34.15
TOTAL	100%	100%	100%

IRI coefficient is presented in the graph showing the importance of different pray categories depending on the trout lineage (Figure 15.).

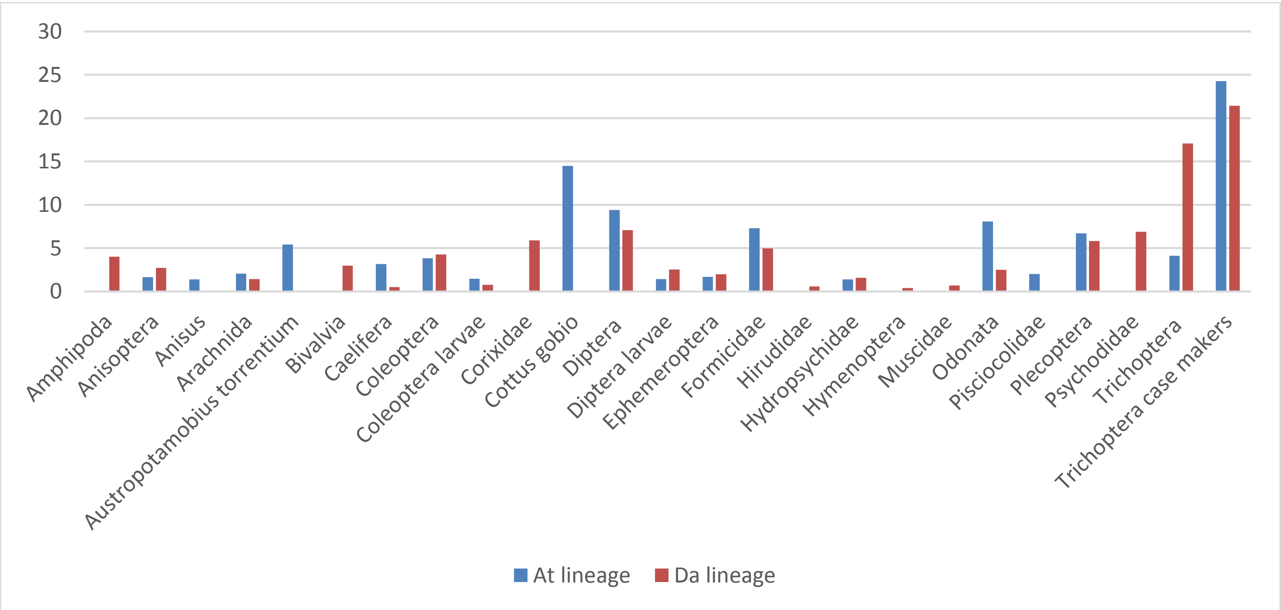

Figure 15: IRI prey importance for AT and DA lineages of brown trout from ‘Gorski kotar’ and ‘Žumberak’ regions

5. DISCUSSION

Trout specimens of both Da and At lineage feed mostly on insects of both terrestrial and aquatic origin. The identification of prey items found in stomach of all the examined trout specimens from all the streams during 2009 and 2017 conducted that the most common are aquatic invertebrate. Prey items most frequently found in stomach contest of both lineage were aquatic invertebrate identified as: Trichoptera (with and without the case), Plecoptera, Coleoptera, and Diptera (including the larvae). Most frequent terrestrial invertebrate were Formicidae which were found in both lineages. In the Da lineage Vespidae was also frequent.
Trichoptera was the most abundant prey item of both lineages, it was also found in all examined streams, both in 2009 and in 2017. Such prey composition matches the results from research on river Una (Trožić-Borovac, 2002). There, it was determined that 91.66% fed on the Trichoptera specimens. Besides Trichoptera trout there also fed on Plecoptera, Ephemeroptera, Diptera, Coleoptera and Hymenoptera, which were all abundant in this research.

Besides the invertebrates, trout fed on *Cottus gobio,* particularly At haplotype. *Cottus gobio* is the only vertebrate prey determined in this research. It found only in At haplotype trout on the location 'Mala Lešnica'. These results seem common in many researches all over the world. Similar prey composition was determined in Kashmir valley (India) (Rasool et al., 2012), Sweden (Eros et al., 2012), River Laxa (Iceland) (Steingrimsson and Gislason, 2001), Georga and Wyoming (USA) (O'Ruke, 2014; Hubert et al., 1993).
In this research it was determined that At trout lineage usually grew larger in size and correspondently ate larger prey items. Prey like *Cottus gobio* and *Austropotamobius torrentium* was only determined in At haplotype. At haplotype proved to feed on Odonata and Caelifera more frequently.

The length/weight relationships were similar in all locations, measured around 3. However, during the separate analysis of Da and At haplotypes the results were different. Da haplotype had a extremely low b value (b=1.625), while the At specimens possess isometric growth value (b=3.003). These results indicate that At lineage compete with native Da lineage for habitat and food resources.

At trout lineage is much less abundant (only 13 specimens found for this research), however according to Schoener index a significant diet overlap has been determined (S = 0.9989). High index confirmed that both At and Da trout lineages feed on similar prey items, and thus compete in food resources. However, it seems that the At and Da trout lineages don't have the same feeding strategy. At trout lineage proved to have a better hunting ability (for larger prey) and the better ability to catch terrestrial invertebrates. The Da trout lineage mostly feed on available aquatic prey. This kind of difference may prove to be troubling do to the fact that At trout haplotype could be more attractive for fly fishing, and therefore might be intentionally overstocked. In that case more conservation measures should be implemented for the preservation of the indigenous Da trout lineage.

6. CONCLUSION

Based on this research it could be concluded that there is a significant diet overlap of introduced Atlantic and native Danube lineages of brown trout. Value b of length weight relationship of Da and At lineages also indicate possible competition due the better growth of At lineage. Trout feed on all prey available in the stream, so when they share space such overlap is bound to happen. It seems that different trout lineages have different hunting strategies; At lineage of trout feed more with prey (insects) of terrestrial origin (Formiciidae, Caelifera) and Da with prey (insects) of aquatic origin. This result indicate that such feeding strategy could be more attractive anglers and stocking which may complicate the implementation of conservation measures necessary to preserve the indigenous brown trout of the DA lineage.

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