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BRIEF COMMUNICATION

Cannibalism in non-native brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss* stream-dwelling populations

C. MUSSEAU*†‡#, S. VINCENZI§||, D. JESENŠEK¶ AND A. J. CRIVELLI**

**Department of Biology, Chemistry, Pharmacy, Institute of Biology, Freie Universität Berlin, Königin-Luise-Strasse 1–3, 14195 Berlin, Germany, †Berlin-Brandenburg Institute of Advanced Biodiversity Research (BBIB), Altensteinstrasse 34, 14195 Berlin, Germany, ‡Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Müggelseedamm 310, 12587 Berlin, Germany, §Institute of Marine Sciences, University of California, Santa Cruz, 110 Shaffer Road, Santa Cruz, CA 95060, U.S.A., ||Fisheries Ecology Division, Southwest Fisheries Science Center, National Marine Fisheries Service,110 Shaffer Road, Santa Cruz, CA 95060, U.S.A., ¶ Tolmin Angling Association, Modrej 26a, 65216 Most na Soci, Slovenia and ** Station Biologique de la Tour du Valat, Le Sambuc, 13200 Arles, France*

#Author to whom correspondence should be addressed. Tel.: +49 30 838-53959; email: musseau@igb-berlin.de

Running headline: CANNIBALISM IN INVASIVE *S. TRUTTA* POPULATIONS

Introduced and allopatric populations of brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss* were sampled in Slovenia for stable isotope analysis to assess dietary niche shifts through ontogeny and estimate the propensity for cannibalism. Both *S. trutta* and *O. mykiss* are cannibals, with higher average relative contribution of conspecific assimilated energy for *S. trutta* (27.9%) compared with *O. mykiss* (7.7%). The smallest cannibal was 166 mm in the *S. trutta* population and 247 mm in the *O. mykiss* population.

Key words: biological invasion; niche; ontogeny; piscivory; stable-isotope analysis.

Cannibalistic organisms kill and eat an individual belonging to the same species (Fox, 1975), have been found in a broad range of animal species (Smith & Reay, 1991; Pereira *et al.*, 2017) and are recognized as one of the main drivers of population dynamics. Theoretical and empirical studies have found that cannibalism can stabilize or induce population cycles, or lead to chaotic dynamics (Claessen *et al.*, 2004). Cannibalism is common in fishes (Smith & Reay, 1991; Pereira *et al.*, 2017), but most studies have focused on cannibalism in captive populations (Pereira *et al.*, 2017). In the context of biological invasions, it has been observed that introduced populations show a higher propensity for cannibalism than native populations (Tayeh *et al.*, 2014). While fish are among the most introduced organisms outside their native range, few studies have explored the rates and the effects of cannibalism in introduced populations of fish species (Gomiero & Braga, 2004).

Brown trout *Salmo trutta* L. 1758 and rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) are two of the most widely introduced invasive species in the world (Lowe *et al.*, 2000). Cannibalistic behaviour is common in lake-dwelling populations of salmonids (Grey *et al.*, 2002; Berg *et al.*, 2010; Florø-Larsen *et al.*, 2014), but is much less reported in stream-dwelling populations (Vik *et al.*, 2001). When living in streams in their native ranges, both *S. trutta* and *O. mykiss* feed mostly on invertebrates, with only a few observations of cannibalism on eggs (Aymes *et al.*, 2010) or juveniles (young-of-the-year; Vik *et al.,* 2001). Outside their native ranges, they often eat native fish and are a main threat to native populations (Lintermans, 2000; Young *et al.*, 2010; Meredith *et al.*, 2015; Stankovi *et al.*, 2015). It has been shown that the proportion of fish from other species in the diet of stream-dwelling *S. trutta* populations outside their native range is much higher than in the native European populations, especially at older ages (Budy *et al.*, 2013). Only a few studies have shown cannibalism in non-native and stream-dwelling populations of *S. trutta* and *O. mykiss*. Huryn (1996) reported a small consumption of young-of-the-year (YOY) by larger conspecifics in a population of *S. trutta* in New Zealand and two cases of predation upon YOY for *O. mykiss* have been observed in Hawaii (Kido *et al.*, 1999) and Italy (Candiotto *et al.*, 2011).

The goal of the present study was to estimate the rate of cannibalism in introduced *S. trutta* and *O. mykiss* populations living in allopatry in Slovenia, south-eastern Europe. The study of the diet of exotic *Salmo* spp. in these streams is crucial to understanding their roles as exotic top predators in food webs. The trophic ecology of one of the few European self-sustaining populations of *O. mykiss* and one of the introduced population of *S. trutta* of western Slovenia were examined and potential dietary shifts to cannibalism were assessed using stable-isotope analysis.

Trout were sampled in June 2011 in the headwaters of the upper Volaja and Godiča Rivers for *S. trutta* and *O. mykiss*, respectively. Both the upper Volaja and Godiča catchments are covered mainly by deciduous forests with neither legal fishing nor poaching. Mean summer water temperature (July–August) in the upper Volaja was 11.10 ± 0.32° C (± S.D.; n = 12 years) and in Godiča 14.40 ± 0.87° C (n = 6 years). Trout were sampled using a petrol-powered portable backpack electrofishing unit. Each individual was anaesthetized with phenoxy-ethanol and was measured and weighed to the nearest mm and g. Age was determined by reading scales. In June 2011, pectoral fin samples for the stableiisotope analysis to evaluate potential ontogenetic diet shift were collected from 77 fish: 40 from *S. trutta* and 37 from *O. mykiss*. Fin clips are a good non-lethal surrogate for muscle tissue in salmonids and are thus appropriate for stable-isotope analysis (Hanisch *et al.*, 2010).

Benthic and terrestrial invertebrates of different trophic groups (decomposers, grazers, predators) were collected for stable-isotope analyses (Supporting Information Methods).

Ratios of ^{15}N and ^{13}C isotopes provide information on the trophic position and the origin of resources, respectively (Fry, 2006; Layman *et al.*, 2012; Supporting Information Methods).

In the following analysis, trout were grouped according to their size: < 100 mm, 100–49 mm, 150–199 mm, 200–249 mm and > 250 mm (Supporting Information Fig. S1). Since *S. trutta* individuals < 100 mm and > 250 mm were not present among the sampled fish, those categories were not included in the niche analysis for *S. trutta*. To evaluate dietary niche shift, a probabilistic method for quantifying multi-dimensional niches was used to estimate niche region for each size group and intraspecific diet overlap among size groups in both species (Swanson *et al.*, 2015; package *nicheROVER*). The estimated niches based on ′ ^{15}N and ′ ^{13}C were bi-dimensional and the niche region of each size group was estimated from randomly chosen individuals from a given size group within species (*n* = 1000). Niche overlap between two groups was estimated as the probability of an individual from group A being found in the niche region of group B; overlap metrics were estimated from Monte Carlo simulations (*n* = 1000). The niche region is the bi-dimensional space in which the individual has a probability ± = 0.95 of being found (Supporting Information Methods, Fig. S1 and Table SI). Differences in ′ ^{15}N and ′ ^{13}C density distributions between size groups were tested using Kolmogorov-Smirnov tests (± = 0.05).

Mixing models for stable-isotope data were used to estimate the relative dietary contribution (%) of each food resource assimilated by each individual (Parnell & Jackson, 2013, package SIAR). For each individual, a mixing model was run with three groups of prey (500 000 iterations): freshwater invertebrates, terrestrial invertebrates and fish (Supporting Information Methods). Since mixing models are sensitive to trophic enrichment factors (TEF), equations from Caut *et al.* (2009) were used. TEF for ′ ^{15}N used in this study were 4.22 ± 0.23 ‰ (*O. mykiss)* and 3.61 ± 0.18 ‰ (*S. trutta*). For ′ ^{13}C, TEF were 2.34 ± 0.23 ‰ (*O. mykiss)* and 2.55 ± 0.12 ‰ (*S. trutta*). Generalized linear models (GLM, *quasibinomial* distribution with *logit* as link function) were used to test the effects of the total length (L_T) of consumer and the species on the relative contribution of the different food categories to the diet. R 3.3.0 was used for all statistical analyses (www.r-project.org).

For *S. trutta*, the ´ ^{15}N density distribution was more depleted in ^{15}N in the 100–149 mm than in the 150–199 mm and 200–249 mm trout (Fig. 1 and Table I). Density distributions of ´ ^{15}N for the 150–199 mm and 200–249 mm categories were not significantly different (Table I). ´ ^{13}C density distributions of the three size categories were similar (Table I). The probability of an individual *S. trutta* being in the niche region of a different size category was low for the 100–149 mm group and high for the 150–199 mm and 200–249 mm groups (Table II). These results indicate a trophic niche shift to higher trophic positions in *S. trutta* $L_T > 150$ mm. The trophic shift is consistent with the results of Bayesian mixing models which revealed that conspecifics were a main part of *S. trutta* diet. Relative contribution of conspecifics in *S. trutta* diet increased with predator size. The smallest cannibal was aged 3+ years and 166 mm *S. trutta* with 36.6% of assimilated energy coming from cannibalism. In the *S. trutta* population, the average relative contribution of conspecifics to trout assimilated energy was 27.9% (± 16.6% S.D.), ranging from 0.0% to 43.7% (Fig. 2).

In *O. mykiss*, pair-wise comparisons of ´ ^{15}N density distributions showed non-significant differences between 100–149 mm and 150–199 mm, 150–199 mm and 200–249 mm fish (Fig. 1 and Table I). Other pairwise comparisons were significantly different (Table I). *Oncorhynchus mykiss* 100–149 mm showed more depleted ´ ^{13}C density distributions than 150–199 mm, 200–249 mm and > 250 mm fish (Table I). Other pairwise comparisons showed no significant differences (Table I). The smallest *O. mykiss* cannibal was of age 2+ years and 247 mm in size. Energy assimilated through cannibalism in *O. mykiss* increased with total length of the consumer and ranged from 0.0 to 50.5% in *O. mykiss* (average: 7.7% ± 16.4% S.D.).

Salmo trutta had a higher propensity for cannibalism than *O. mykiss* ($t = –5.7$, $P < 0.001$). The proportion of cannibalism increased through ontogeny in both species (slope estimate = 0.86, $t = 9.8$, $P < 0.001$). Relative contributions of benthic and terrestrial invertebrates to diet decreased with L_T (slope estimate = –0.21, $t = –5.1$, $P < 0.001$ and slope estimate = –0.35, $t = –12.6$, $P < 0.001$; Fig. 2).

In this study, *S. trutta* was more cannibalistic than *O. mykiss*, which is consistent with previous findings on piscivory in introduced populations in Patagonian lakes (Macchi *et al.*, 1999). The few reports of cannibalistic behaviour in introduced *S. trutta* and *O. mykiss* populations have focused on predation upon YOY. In New Zealand, cannibalism on small individuals was estimated to be a small part of large *S. trutta* diet (Huryn, 1996). Two examples of cannibalism in *O. mykiss* have been reported; the first one in Hawaii, regarding a 262 mm *O. mykiss* eating a juvenile (Kido *et al.*, 1999) and the second in Italy, where a 244 mm *O. mykiss* was found with the remains of two small fish in its gut (Candiotto *et al.*, 2011). In this study, *S. trutta* and *O. mykiss* populations were sampled in mid-June, before the emergence of YOY; thus, it was possible to estimate rates of cannibalism only on age 1+ year and older trout. For *S. trutta*, Vik *et al.* (2001) found in a stream dwelling population in Norway that the smallest cannibalistic trout had a size of 170 mm, which is similar to the size of the smallest cannibalistic *S. trutta* found in the upper Volaja. This size threshold is much smaller than the size at which *S. trutta* starts cannibalism in lake-living populations, between 200 and 250 mm (Grey *et al.*, 2002) and > 300 mm (Jensen *et al.*, 2012), but similar to the size at which the species shifts to fish feeding on other salmonids in northern European lakes

(Jonsson *et al.*, 1999). Despite the variation in the size at which *S. trutta* becomes piscivorous, *c*. 160 mm may be the threshold size at which the species can handle small salmonids as prey.

In the present study, the overall proportions of cannibals among the sampled individuals were 75.0% and 18.9% in *S. trutta* and *O. mykiss* populations, respectively. This study showed that cannibalism is a sizeable part of the diet of *S. trutta* and *O. mykiss* in introduced populations. Owing to its effects on mortality rates, cannibalism can have large effects on population dynamics (Vik *et al.*, 2001) and thus be a crucial determinant of invasion success and of persistence and expansion of species already established outside their native ranges. Further study will aim at estimating the number of trout eaten using a bioenergetic model and at developing a model of population dynamics for these two non-native species living in a system of great conservation interest.

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Supporting Information

Supporting Information may be found in the online version of this paper:

S.I. Methods: field methods, stable-isotope analysis, quantifying niche overlaps, mixing model.

TABLE SI. Analysis of sensitivity of overlap probability: posterior means and 95% C.I. of the probability of overlap (%) for niche region with ± ranging from ± = 0.80, 0.90, 0.95 and 0.99 for each pairwise comparison.

FIG. S1. Relationship between age and total length (L_T) of trout (a) and (b) and distribution of analysed trout's L_T in the different size-categories considered (c) and (d) for *Salmo trutta* (a) and (c) and *Oncorhynchus mykiss* (b) and (d).

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CORRESPONDING AUTHOR INFORMATION	

Title	Dr.
First Name	Camille
Middle Name	
Last Name	Musseau
Position	Post Doctoral Research
Department	
Institution	IGB - FU Berlin
Address Line 1	Königin-Luise-Str. 1-3
Address Line 2	Institut für Biologie
Address Line 3	AG Jeschke
Address Line 4	
City	Berlin-Dahlem
State	
Zip	14195
Country	GERMANY
Primary Phone Number	0049 30 838-53959
Fax Number	
E-mail Address	musseau@igb-berlin.de
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