

Text S1

Gut content DNA metabarcoding

Library preparation and sequencing were performed by Jonah Ventures (Boulder, Colorado, USA). We targeted the mitochondrial cytochrome *c* oxidase subunit I (COI) gene region, which performs well across metazoans, and the chloroplast 23S rRNA gene region, which performs well across autotrophs. For the COI markers, we used the m1COIintF forward primer (Leray et al. 2013) and the jgHCO2198 reverse primer (Geller et al. 2013). For the 23S markers, we used the p23SrV_f1 and Diam23Sr1 23S primers (Sherwood and Presting 2007, Hamsher et al. 2011, Cannon et al. 2016). We ran two-step PCR reactions at 25 μ L according to the Promega PCR Master Mix guidelines (Promega, Madison, Wisconsin, USA): 12.5 μ L Master Mix, 0.5 μ M of each primer, 1 μ L gDNA, and 10.5 μ L DNase/RNase-free water. We followed the following conditions for PCR amplification for the COI marker: denaturation at 94°C for 2 min, followed by 45 cycles of 15 s at 94°C, 30 s at 50°C, and 1 min at 72°C, then a final elongation at 72°C for 10 min. We used the following conditions for PCR amplification for the 23S marker: denaturation at 94°C for 3 min, followed by 40 cycles of 30 s at 94°C, 45 s at 55°C, and 1 min at 72°C, then a final elongation at 72°C for 10 min. Following the PCR amplifications, we visually inspected each reaction with a 2% agarose gel.

The following library preparation and sequencing protocols apply to both the COI and 23S gene. We performed clean-ups by incubating amplicons with Exo1/SAP for 30 min at 37°C, followed by inactivation at 95°C for 5 min. We then stored the products at -20°C. Afterwards, we ran a second indexing PCR to bind a unique 12-nucleotide index sequence, which included Promega Master mix, 0.5 μ M of each primer, and 2 μ L of template DNA under the following conditions: denaturation at 95°C for 3 min, followed by 8 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s. Each reaction was visually inspected with a 2% agarose gel to ensure successful amplification.

We cleaned and normalized 25 μ L of each indexed amplicon with the SequelPrep Normalization Kit (Life Technologies, Carlsbad, California, USA), pooled 5 μ L of each sample, and sent the library pools to the Genohub service provider (Austin, Texas, USA). Bead cleaning with Agencourt AMPure XP beads (Beckman Coulter, Brea, California, USA) was performed to remove < 200 bp amplicons, samples were quantified with a Qubit Fluorometer (Invitrogen, Carlsbad, California, USA), and amplicon average size was analyzed with an Agilent TapeStation 4200 (Agilent, Santa Clara, California, USA). Finally, sequencing was performed on an Illumina HiSeq using the HiSeq Rapid SBS Kit v2, 500-cycles (Illumina, San Diego, California, USA).

Sequence bioinformatics

We processed all raw COI and 23S sequences with the JAMP pipeline (<https://github.com/VascoElbrecht/JAMP>). After demultiplexing, forward and reverse reads were pair-end merged with USEARCH v11.0.667 (Edgar 2010), and primers were trimmed from both ends using Cutadapt v1.18 (Martin 2011). Quality filtering was performed with expected error filtering through USEARCH (Edgar and Flyvbjerg 2015), and all sequences affected by sequencing and PCR error were removed using the UNOISE algorithm (Edgar 2016). Exact sequence variants (ESVs) were then compiled into ESV tables, and taxonomy was assigned to

each ESV using the basic local alignment search tool (BLAST) hit when query coverage and percent identity exceeded 95% and 80%, respectively, or by mapping them against a 23S database from Silva (Yilmaz et al. 2014), specifying zero deviations to ensure mapping accuracy. Consensus taxonomy was generated from the hit tables, first considering 100% matches, then decreasing by 1% until hits were available for each ESV. Taxonomy that was present in at least 90% of the hits was reported; otherwise, an “NA” was assigned when several different taxa matched the ESV. For error reduction due to misidentified taxa, the bracket was increased to 2% when matches of 97% and higher were present, but no family-level or lower taxonomy was assigned. We then removed all self-hits from the COI dataset, which resulted from sequencing the host tissue, and omitted all ESVs that only had a single sequence read across the entire dataset. For the network analysis, we also removed all ESVs that were only used by a single individual to ensure convergence of the modularity metric. Further detail on the treatment of the assembled sequencing datasets is provided in the appended *R* script.

Text S2

Data processing and statistical analysis

We performed the analyses using the software *R* (R Core Team 2019) and the following packages: *drake* (Landau 2018), *tidyverse* (Wickham et al. 2019), *rstan* (Stan Development Team 2016), *brms* (Bürkner 2017), *bipartite* (Dormann et al. 2009), *iNEXT* (Hsieh et al. 2016), (Pollock et al. 2014), and *fishualize* (Schiettekatte et al. 2019). All code and data necessary to fully reproduce the results presented in this paper are available on the lead author’s GitHub (https://github.com/simonjbrandl/gobies_metabolism) page and Figshare ([10.6084/m9.figshare.19157876](https://doi.org/10.6084/m9.figshare.19157876)). A README.md document that provides guidance on how to reproduce the results is included. The supplementary tables below are output from the analyses and can also be found as separate documents in the “outputs” folder.

Table S1: Model summary for standard metabolic rate. The model was run for 5,000 iterations using the default brms priors.

<i>Predictors</i>	log 10(SMR)	
	<i>Estimates</i>	<i>CI (95%)</i>
Intercept	-2.69	-4.15 – -1.24
log10W	0.62	0.48 – 0.77
SpeciesGnatholepiscauerensis	0.07	0.01 – 0.14
Temp C	0.06	0.01 – 0.11
log10W.SpeciesGnatholepiscauerensis	-0.04	-0.21 – 0.13
Observations	30	
R ² Bayes	0.922	

Table S2: Model summary for maximum metabolic rate. The model was run for 5,000 iterations using the default brms priors.

<i>Predictors</i>	log 10(Max MR)	
	<i>Estimates</i>	<i>CI (95%)</i>
Intercept	-3.88	-6.25 – -1.49
log10W	0.59	0.36 – 0.83
SpeciesGnatholepiscauerensis	0.20	0.10 – 0.30
Temp C	0.12	0.03 – 0.20
log10W.SpeciesGnatholepiscauerensis	0.04	-0.23 – 0.31
Observations	30	
R ² Bayes	0.850	

Table S3: Model summary for horizontal gape. The model was run for 2,000 iterations using the default brms priors.

H gape		
<i>Predictors</i>	<i>Estimates</i>	<i>CI (95%)</i>
Intercept	-0.31	-1.32 – 0.71
SL	0.16	0.13 – 0.20
SpeciesGnatholepiscauerensis	0.01	-0.27 – 0.30
Observations	33	
R ² Bayes	0.765	

Table S4: Model summary for vertical gape. The model was run for 2,000 iterations using the default brms priors.

V gape		
<i>Predictors</i>	<i>Estimates</i>	<i>CI (95%)</i>
Intercept	-1.16	-2.25 – -0.05
SL	0.20	0.16 – 0.24
SpeciesGnatholepiscauerensis	-0.03	-0.35 – 0.29
Observations	33	
R ² Bayes	0.809	

Table S5: Model summary for girth. The model was run for 2,000 iterations using the default brms priors.

Girth		
<i>Predictors</i>	<i>Estimates</i>	<i>CI (95%)</i>
Intercept	-1.02	-1.78 – -0.25
SL	0.20	0.18 – 0.23
SpeciesGnatholepiscauerensis	-0.27	-0.48 – -0.05
Observations	33	
R ² Bayes	0.908	

Table S6: Model summary for gastrointestinal tract. The model was run for 2,000 iterations using the default brms priors.

GIT		
<i>Predictors</i>	<i>Estimates</i>	<i>CI (95%)</i>
Intercept	-7.16	-29.48 – 15.18
SL	0.76	0.12 – 1.38
SpeciesGnatholepiscaurensis	18.36	12.17 – 24.60
Observations	22	
R ² Bayes	0.690	

Table S7: Model summary for number of bites. The model was run for 2,000 iterations using the default brms priors and a negative binomial error distribution with a log link-function.

<i>Predictors</i>	feed	
	<i>Incidence Rate Ratios</i>	<i>CI (95%)</i>
Intercept	17.94	10.26 – 34.87
species: Gnatholepis cauerensis	17.93	7.55 – 46.38
monovsmixmono	0.06	0.02 – 0.17
speciesGnatholepis_cauerensis.monovsmixmono	10.05	2.41 – 40.68
Observations	36	
R ² Bayes	0.679	

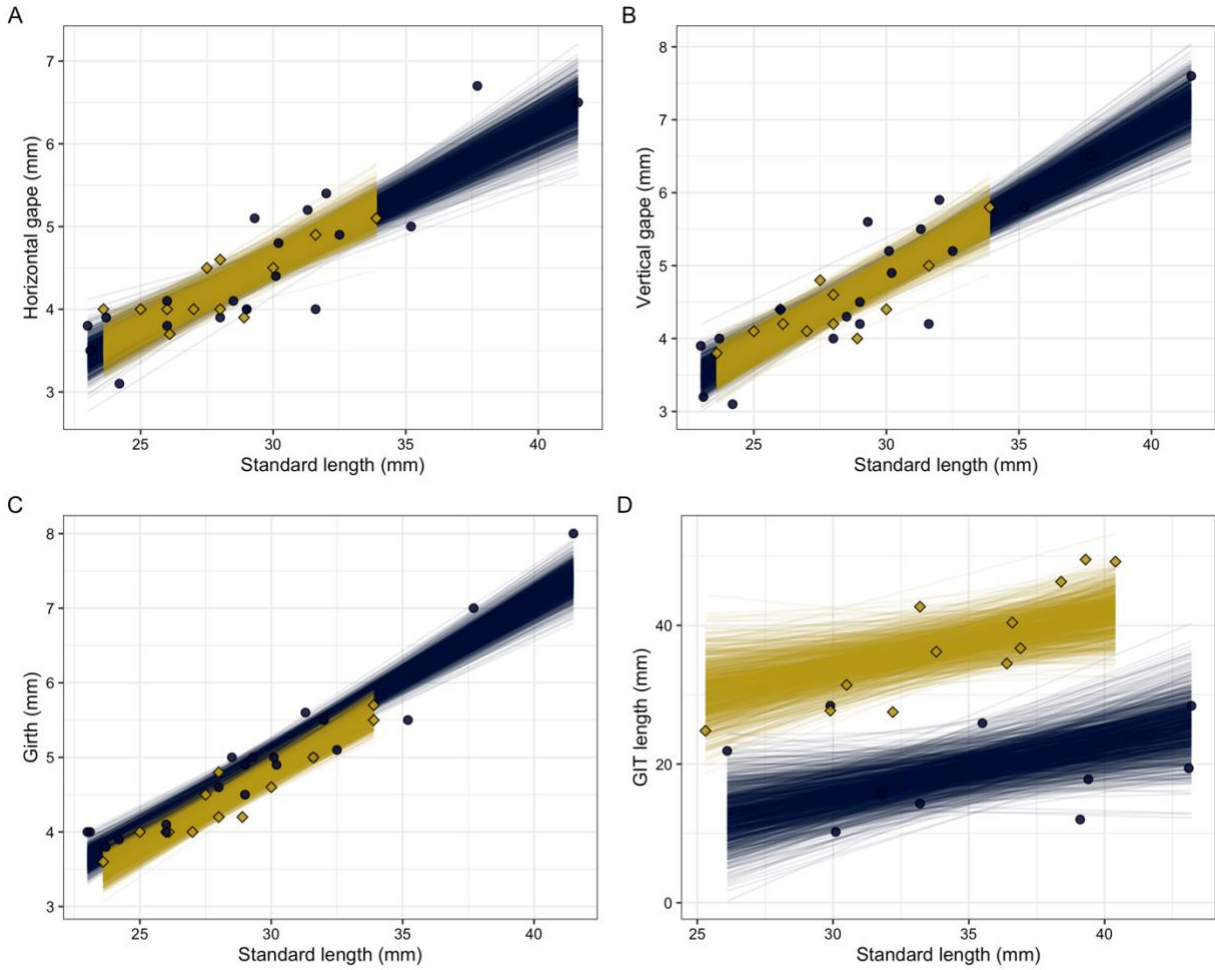


Fig. S1. Relationship between standard length (SL) and three morphological traits for *F. neophytus* (blue circles) and *G. cauerensis* (gold diamonds): (A) vertical gape, (B) horizontal gape, (C) girth, and (D) length of the gastrointestinal tract (GIT). Symbols represent the raw data, while lines represent 1,000 draws from the posterior of the respective Bayesian regression models.

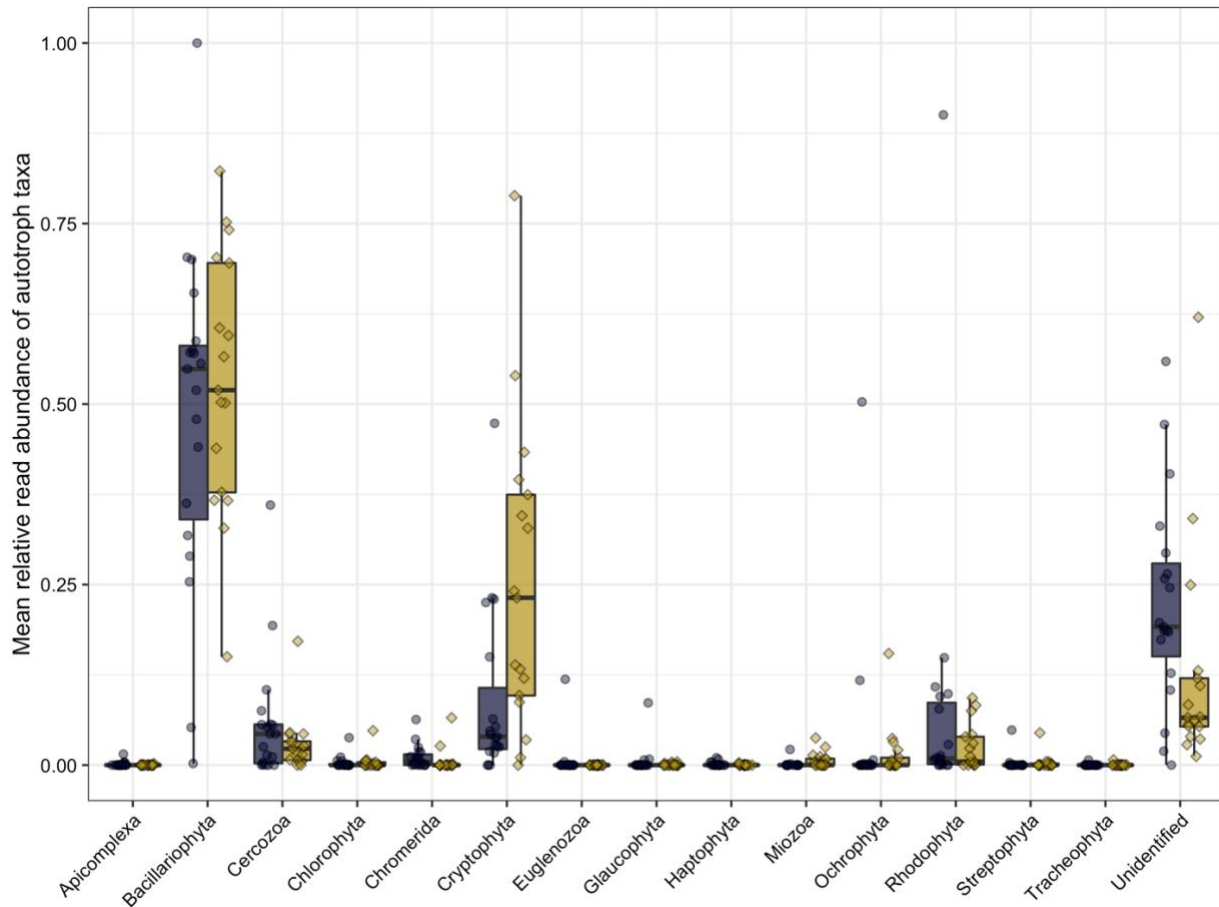


Fig. S2. Relative read abundance (RRA) of autotroph taxa in the guts of the two goby species, averaged across individuals (blue = *Fusigobius neophytus*; gold = *Gnatholepis cauerensis*).

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