**Quantifying among-individual behavioural and trophic variation in invasive marine fish**

(Or if Karr is separated, **Acute effects of PIT tagging on round goby behaviour**)

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**Short Running Title:** Quantifying behavioural and trophic variation

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**Abstract**

Behavioural differences among individuals of the same species are an major source of variation that influences an animal or population’s ecological interactions with their environment. Quantifying individual variation in behavioural traits is a often time- and resource-consuming process, requiring robust sample sizes, repeated trials and individual identification. Coupling this approach with methods to measure individual variation in their trophic state in the wild, such as stable isotope analysis, is challenging due the to need to remove tissue from individuals, and the potential for procedures to induce behavioural changes in individuals. Still, there is a clear need to quantify behavioural-trait effects on ecological interactions. For example individual behavioural variation (e.g. bold-exploratory traits) is linked to invasion processes, and is linked to trophic/food web interactions, therefore an individual approach is needed to properly understand the trophic impacts of a marine invasive species such as the round goby (*Neogobius melanostomus*). This study presents a novel methodological approach, combining field-based stable isotope analysis of food web interactions with laboratory-based personality trait analysis in an established invasive population of round gobies in the Southwest Baltic Sea. We find that substantial individual variation in trophic state and behavioural state in this species, ….. and that experimentally quantifying both is a viable approach to exploring the impacts of individual trait variation in ecosystems.

**Keywords**

**Introduction**

Within-species behavioural variation is closely linked to many ecological processes, including diet, predator-prey dynamics, social interactions and biological invasions (Réale et al., 2007; Wolf and Weissing, 2012; Moran et al., 2017, 2020). Among-individual differences in behaviour may be derived from underlying genetic or epigenetic variation among individuals, as well as individual differences in phenotypic plasticity (Nussey *et al.* 2007, Dingemanse *et al.* 2010). There is an interactive relationship between individual behavioural traits and their ‘state’, where state factors may include element of an individual's extrinsic or intrinsic environment that is strategically relevant to their future fitness (Wolf and Weissing 2010). Whiel behavioural phenotypes can determine the outcomes of interactions, interaction outcomes can in turn can influence behavioural phenotypes (i.e. state-behaviour feedbacks; Sih et al., 2015).

There is a lack of studies that quantify how behaviour trait variation (or ‘personality’ traits) translated to functional ecological variation in the wild. Therefore, the primary goal of this study is to trial and validate a methodology to concurrently quantify both trophic state variation in animals in the wild, and individual behavioural variation in the laboratory.

A particular challenge to analysing individual level trophic state and behavioural variation, is the need to remove animals from their environment and conduct minor, although invasive procedures (e.g., gut content analysis, isotope analysis)….

Specific aims of our analysis are as follows:

(1) Test whether individual tagging and tissue sampling procedures affect the measurement of round goby behavioural traits. We predict that PIT tagging and fin clip procedures will have no effect on behavioural traits.

(2) Quantify among individual variation in bold-exploratory behavioural traits and the trophic state in an established wild goby population. We predict that round gobies will show among individual variation in one or more behavioural variables in bold-exploratory assays, as well as distinct among-individual carbon and nitrogen isotope variation.

(3) Validate the use of fin clips to estimate the recent diet of round gobies.

**Methods**

Two distinct experimental groups were used in these. To test for effects of effects of tagging and fin clips on round goby behaviour in the lab, experimental fish were collected via local fisherman in October 2020 from Karrebæk Fjord (55.1923°, 11.67241°). Additionally, to quantify bold-exploratory traits and trophic variation in a well-established invasive population, fish were collected in June 2020 from a 1ha area (50 x 200 m) of a shallow brackish estuary (Guldborgsund, 54.69645°, 11.84067°). Finally, a longer term laboratory feeding trial was run using the remaining Guldborgsund fish, to measure the isotopic discrimination factor of fin and muscle tissue in relation to their laboratory diet.

Guldborgsund is one of the first Danish marine areas invaded by the round goby, first being observed ~2009, so can be considered a well-established population characterised by high population density and strong intraspecific competition (Azour et al., 2015). Karrebæk was invaded soon after (~2011), so the goby population is expected to show similar characteristics.

1. *Karrebæk Fjord Experiment* 
   1. *Tagging and finclip treatments*

Experimental fish were collected using fyke nets via a commercial fisherman, and transported to the laboratory on 1/10/2020.

Fish (n = 48) were subject to one of three tagging/sampling treatments: Control (no-PIT tag, not tissue sampling); Tagged (PIT tagged); Fin-clipped (PIT tagged and tissue sampled). Due to the low number of fish available, a non-factorial treatments design was used. Treatments were applied following an extended period of acclimation (40 days, 1/10/20 – 10/11/20), to minimise any confounding effects of laboratory acclimation on behavioural responses and survival.

* 1. *Behavioural experiment*

A common open field/ novel environment assay was used to measure activity-exploratory behavioural variables.

* 1. *Survival and growth response*
  2. *Data analysis*

Continuous moderators were z-transformed to aid interpretation (Schielzeth, 2010).

1. *Guldborgsund Experiment* 
   1. *Fish and community sampling*

Our approach required both *community sampling* focused on collecting potential prey items of the round goby (i.e. benthic invertebrates, small fish) and basal food web sources. Following this, *individual goby sampling* (date) focused on collecting live fish for individual isotopic and behavioural analysis. This goby sampling was conducted second, as tissue isotopes reflect assimilation of prey isotope ratios over a period of weeks to months [(Thomas and Crowther 2015)](https://www.zotero.org/google-docs/?TDryEa), so goby tissue samples were likely to involve prey consumed during the period of community prey sampling. Gobies tend to show relatively high site affinity during spring/summer breeding periods, where they tend to occupy shallower, near-shore environments like the one sampled (REF), so it is assumed that isotopic variation reflects their diet within the sampled area.

*Individual goby sampling* used a combination of passive (...), and active netting (...), to select an unbiased mixture of behavioural types [REF]. Behavioural traits in gobies may in influenced by parasite infection (specifically reducing anti-predator behaviour, although boldness and shelter use were unaffected; as per [(Rodriguez et al. 2018)](https://www.zotero.org/google-docs/?pL2p7z), so eyes were inspected for cataracts indicating eye fluke infection, and ectoparasites.

Fish were transported to DTU Aqua fish stable facility (Lyngby, Denmark) and maintained under in a ... day:night cycle, at 10 ± 1 °C and 16 ± 1 ppt salinity, and fed to satiation three times per week with commercial high-nutrition pellet fish feed (???). Laboratory salinity is within the natural range of the source location [(Feistel et al. 2010)](https://www.zotero.org/google-docs/?T3Op7a), and although it was slightly above salinity at collection (11.45 ppt), it is well within the osmoregulatory tolerance of the species [(Behrens, Deurs, and Christensen 2017)](https://www.zotero.org/google-docs/?PMzkql).

* 1. *Stable isotope analysis*

Invertebrates were identified to family level where possible, based on historical records of the invertebrate community in the region.

* 1. *Behavioural experiments*

Prior two behavioural experiment, all fish were tagged

* 1. *Data analysis*

Sex, weighing

Treatment groups were held in 3 tanks (enriched), with tank ID recorded as a potential covariate to account for subtle tank effects.

1. *Isotope processing*

Fin clips, tail fin taken from the outer 5mm of the fin, generally showed no sign of infection.

* Any sections of the fins with visible parasite infections were removed under a dissecting microscope. Help in 2ml eppendorfs.
* Rinsed thoroughly with deionised water to remove any surface contamination.
* Drying: 60 degrees, 48 hours
* Sections composed of both fin ray and soft tissue, so sections ground into homogenous in tube using steel rods.

Invertebrates

* Grouped to relatively course taxonomic groups
* 3 replicates per group
* *Behavioural assays*

Two types of behavioural experiments were used to characterise individual behavioural variation, an *Activity* assay and an *Exploration* assay. To avoid effects of sociality, all assays were run with single individuals alone (REF). Similarly, water was entirely replaced between trials in the *Activity* to avoid carryover effects between trials due to odour signals, while as the *Exploration* assay was run in a continually flowing system in an input of uncontaminated water, which was flushed through with water between trials to minimise carryover odour effects between trials.

In the *Activity* trial, individuals were placed by hand into a 32.5 x 50 cm open field arena. Eight arenas were used in a 2 x 4 grid, to run multiple trials simultaneously. Following a 5 min acclimation period, individuals were filmed for 20 mins.

Fish movement behaviour was tracked using Toxtrac (v2.90, [(Rodriguez et al. 2018)](https://www.zotero.org/google-docs/?NPY2zs)). To account for potential among-arena sampling error due to parallax distortions, pixel/mm ratios for each arena were calculated for manual .

The following variables were extracted for further analysis: **average speed (mm/s), proportion of time spent moving (%), total distance travelled (mm) and edge use (time, s, spent > 10 cm from the outer edge of the arena,).**

The order of the assays.

**Results**

**Discussion**

**Acknowledgements**

**Authorship**

**Data** **Accessibility**

All data and code used (including data processing, preparation, analysis and presentation) are available at the Open Science Framework (https://osf.io/rnz7q/, doi: 10.17605/OSF.IO/RNZ7Q).

**References**

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