**Quantifying among-individual behavioural and trophic variation in the invasive round goby**

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**Short Running Title:** Individual 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**

Intraspecific 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). Behavioural differences among individuals may be derived from underlying genetic or epigenetic variation, as well as via 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). Specifically, behavioural phenotypes can determine the outcomes of ecological interactions, while 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) To test effects of individual tagging and tissue sampling procedures on round goby behavioural traits. We predicted that PIT tagging and fin clip procedures will have no effect on behavioural traits over a short (2 day), and medium term (10 day) period. Additionally, we tested for growth and survival effects over a longer (10 week) period, and predicted no treatment effects

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

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

In addition to the specific aims above, we also conducted an exploratory analysis to explore the role that behavioural variation may have in round gobies impacts on prey communities, by testing for correlations/covariation between personality traits and trophic/diet variation.

**Methods**

Two populations were used for these experiments. To test for effects of effects of tagging and fin clips on round goby behaviour in the laboratory, round gobies were collected from a local fisherman on 1 October 2020 from Karrebæk Fjord (55.1923°, 11.67241°). Separately, to quantify bold-exploratory traits and trophic variation in a well-established invasive population, both round gobies and their potential prey community were sampled over 16-17 June 2020 from a shallow brackish estuary (Guldborgsund, 54.69645°, 11.84067°). Finally, to measure the isotopic discrimination factor of fin and muscle tissue in relation to their laboratory diet, a longer term laboratory feeding trial used the remaining Guldborgsund fish. Guldborgsund is one of the first Danish marine areas invaded by the round goby, first being observed ~2009, while Karrebæk was invaded soon after (~2011), so both can be considered well-established populations characterised by high population density and strong intraspecific competition (Azour et al., 2015).

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

. Any lethargic fish or individuals with visible wounds were excluded.

Transport and Holding conditions.

Treatments were applied immediately after fish undertook their first behavioural assay (*day 0*), 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. 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.

* 1. *Behavioural experiment*

Three behavioural trials were conducted to measure short and medium-term behavioural effects of tagging and sampling; one pre-treatment (*day 0*) and two post-treatment (*day 2* and *day 10*). Previous studies have shown little to no physiological effects over …….. 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*

Sampling focused on both collecting round goby individuals, and their potential prey items (i.e. benthic invertebrates, small fish), over a 2 ha (100 x 200 m) area of the near coast habitat. Gobies tend to show high site affinity during spring/summer breeding periods, where they tend to occupy shallower, near-shore environments (REF). As tissue isotopes reflect assimilation of prey isotope ratios over a period of weeks to months (Thomas and Crowther, 2015), 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).

Prey fauna were sampled using a combination of methods to ensure a cross-section of the mobile and sessile fauna community were represented. This included:

1. *Behavioural experiments*

Prior two behavioural experiment, all fish were tagged

1. *Feeding experiments*
2. *Stable isotope analysis*

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

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**

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**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**

Azour, F., Deurs, M. van, Behrens, J., Carl, H., Hüssy, K., Greisen, K., et al. (2015). Invasion rate and population characteristics of the round goby Neogobius melanostomus: effects of density and invasion history. *Aquatic Biology* 24, 41–52. doi:10.3354/ab00634.

Moran, N. P., Sánchez‐Tójar, A., Schielzeth, H., and Reinhold, K. (2020). Poor nutritional condition promotes high-risk behaviours: a systematic review and meta-analysis. *Biological Reviews* n/a. doi:https://doi.org/10.1111/brv.12655.

Moran, N. P., Wong, B. B. M., and Thompson, R. M. (2017). Weaving animal temperament into food webs: implications for biodiversity. *Oikos* 126, 917–930. doi:10.1111/oik.03642.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., and Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews* 82, 291–318. doi:10.1111/j.1469-185X.2007.00010.x.

Sih, A., Mathot, K. J., Moirón, M., Montiglio, P.-O., Wolf, M., and Dingemanse, N. J. (2015). Animal personality and state–behaviour feedbacks: a review and guide for empiricists. *Trends in Ecology & Evolution* 30, 50–60. doi:10.1016/j.tree.2014.11.004.

Thomas, S. M., and Crowther, T. W. (2015). Predicting rates of isotopic turnover across the animal kingdom: a synthesis of existing data. *Journal of Animal Ecology* 84, 861–870. doi:10.1111/1365-2656.12326.

Wolf, M., and Weissing, F. J. (2012). Animal personalities: consequences for ecology and evolution. *Trends in Ecology & Evolution* 27, 452–461. doi:10.1016/j.tree.2012.05.001.

**Tables**

**Figure Legends**

**Figures**